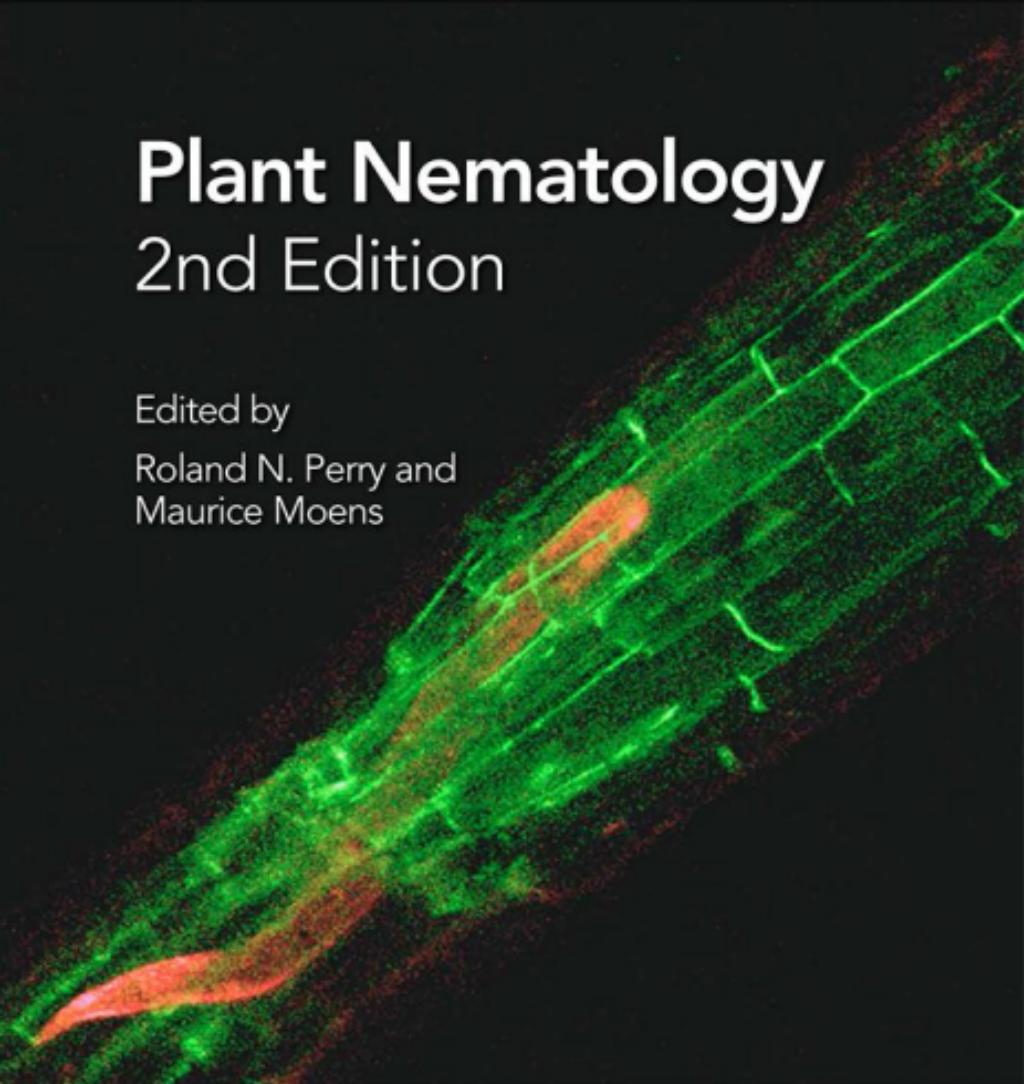


Plant Nematology

2nd Edition

Edited by

Roland N. Perry and
Maurice Moens



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2nd Edition

To Clare and Monique, with thanks for their patience
and support during the preparation of this second edition.

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The Editors

Roland N. Perry

Professor Roland Perry is based at Rothamsted Research, UK. He graduated with a BSc (Hons) in zoology from Newcastle University, UK, where he also obtained a PhD in zoology on physiological aspects of desiccation survival of *Ditylenchus* spp. After a year's postdoctoral research at Newcastle, he moved to Keele University, UK, where he taught parasitology; after 3 years at Keele, he was appointed to Rothamsted Research. His research interests have centred primarily on plant-parasitic nematodes, especially focusing on nematode hatching, sensory perception, behaviour and survival physiology, and several of his past PhD and postdoctoral students are currently involved in nematology research.

He co-edited *The Physiology and Biochemistry of Free-living and Plant-parasitic Nematodes* (1998), *Root-knot Nematodes* (2009), *Molecular and Physiological Basis*



of *Nematode Survival* (2011) and the first edition of this textbook, *Plant Nematology* (2006). He is author or co-author of over 40 book chapters and refereed reviews and over 100 refereed research papers. He is co-Editor-in-Chief of *Nematology* and Chief Editor of the *Russian Journal of Nematology*. He co-edits the book series *Nematology Monographs and Perspectives*. In 2001, he was elected Fellow of the Society of Nematologists (USA) in recognition of his research achievements; in 2008 he was elected Fellow of the European Society of Nematologists for outstanding contribution to the science of nematology; and in 2011 he was elected Honorary Member of the Russian Society of Nematologists. He is a Visiting Professor at Ghent University, Belgium, where he lectures on nematode biology, focusing on physiology and behaviour.

Maurice Moens

Professor Maurice Moens is Honorary Director of Research at the Institute for Agriculture and Fisheries Research (ILVO) at Merelbeke, Belgium, and honorary professor at Ghent University, Belgium, where he gave a lecture course on agro-nematology at the Faculty of Bioscience Engineering. He is a past director of the



postgraduate international nematology course (MSc Nematology) and coordinator of the Erasmus Mundus – European Master of Science in Nematology, where he gave five lecture courses on plant nematology. The MSc course is organized in the Faculty of Sciences of Ghent University.

He graduated as an agricultural engineer from Ghent University and obtained a PhD at the same university on the spread of plant-parasitic nematodes and their management in hydroponic cropping systems. Within the framework of the Belgian Cooperation, he worked from 1972 to 1985 as a researcher in crop protection, including nematology, at two research stations in Tunisia. Upon his return to Belgium, he was appointed as senior nematologist at the Agricultural Research Centre (now ILVO). There, he expanded the research in plant nematology over various areas covering molecular characterization, biology of host–parasite relationships, biological control, resistance and other forms of non-chemical control. He was appointed head of the Crop Protection Department in 2000 and became Director of Research in 2006. He retired from both ILVO and Ghent University in 2012 but continues to supervise PhD students. In 2010, he was elected Fellow of the Society of Nematologists (USA) for outstanding contributions to nematology; in the same year he was elected Fellow of the European Society of Nematologists for outstanding contribution to the science of nematology; and in 2012 he was elected Honorary Fellow of the Chinese Society for Plant Nematology. He supervised 23 PhD students, who now are active in nematology all over the world. Currently, he is the president of the European Society of Nematologists. He co-edited *Root-knot Nematodes* (2009) and the first edition of this textbook, *Plant Nematology* (2006). He is author or co-author of ten book chapters and refereed reviews and over 150 refereed research papers. He is a member of the editorial board of *Nematology*, *Annals of Applied Biology* and the *Russian Journal of Nematology*.

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Preface to the First Edition

Plant-parasitic nematodes are of considerable importance worldwide and their devastating effects on crops have major economic and social impacts. Control of these plant pests is imperative and with the banning or limitation of the use of many nematicides, alternative control strategies are required that, in turn, will have to be based on a sound knowledge of nematode taxonomy and biology. Such information is also a basic necessity for effective formulation and implementation of quarantine regulations. Molecular approaches to all aspects of nematology have already made a major contribution to taxonomy and to our understanding of host–parasite interactions, and will undoubtedly become increasingly important.

There have been several excellent specialized texts on plant-parasitic nematodes, aimed primarily at research scientists. However, there is no book on plant-parasitic nematodes aimed at a broader readership, especially one including students specializing in the subject at undergraduate and postgraduate levels. The driving force for this book was the need for a text to support the MSc course in nematology run by Ghent University, Belgium. The students on this course come from a wide spectrum of scientific backgrounds and from many different countries and, after obtaining their degree, will return to their own country to undertake various jobs, including advisory work, statutory and quarantine work, PhD degrees and teaching posts. Many of these students will return to countries where facilities for plant nematology work are basic. Thus, the book needed to provide a wide range of information on plant-parasitic nematodes and needed to be inclusive, appealing to workers from developing and developed countries. An excellent book, edited by John Southey and entitled *Plant Nematology*, provided this type of information but is now very dated and has long been out of print. We have used the general format of Southey's book as a template for the present volume. We hope that, as well as being informative, this book will stimulate interest in plant-parasitic nematodes.

Research on taxonomy, biology, plant–nematode interactions and control has generated an extensive volume of literature. In this book, we have deliberately limited the number of references, although key research papers have been included where these are of major significance. Important book chapters and reviews are cited so that a reader interested in a specific aspect can access these to obtain source references.

We are grateful to all the authors of the chapters for their time and effort in compiling their contributions. In addition, we wish to thank David Hunt (CABI, UK), John Jones (SCRI, UK) and Brian Kerry (Rothamsted Research, UK) for their advice and comments on various chapters, and Bram Moens (Wetteren, Belgium) for preparing some of the figures. This book is primarily for students and the impetus for it came from students; we would like to thank all those whose enthusiasm and interest in plant nematology made this book possible.

Roland Perry and Maurice Moens
May 2005

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Preface to the Second Edition

We were delighted with the positive response to the first edition of *Plant Nematology*. Initially aimed at the MSc course in nematology at Ghent University, Belgium, the book has proved popular with students and staff worldwide. The first edition was written in 2005 and published in April the following year. Several chapters now need updating to meet the requirements of current nematology students.

In producing this second edition, we have taken the opportunity to revise all chapters, especially those, such as the molecular chapters, where a wealth of new information has accumulated over the intervening years since the first edition. We are grateful to the authors who have prepared the revised chapters; their time and effort are greatly appreciated. Also, we would like to acknowledge the constructive comments from users of the first edition; in particular, we thank Axel Elling (Washington State University, USA), Rick Davis (North Carolina State University, USA), David Hunt (CABI, UK), John Jones (The James Hutton Institute, UK), Charlie Opperman (North Carolina State University, USA) and Nicole Viaene (ILVO, Merelbeke, Belgium) for their important and useful comments.

The book is aimed at students, to introduce them to the delights and challenges of plant nematology, and the immense economic and social damage done by plant-parasitic nematodes. The need for young, enthusiastic nematologists to tackle the immense problems caused worldwide by plant-parasitic nematodes is paramount. We hope that the enthusiasm of the editors and chapter authors is contagious!

Roland Perry and Maurice Moens
December 2012

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Part I Taxonomy, Systematics and Principal Genera

Knowledge of nematode morphology and life-cycle biology underpins all aspects of research, advisory work, implementation of quarantine legislation and selection of control strategies. Traditional, descriptive taxonomy is now routinely accompanied by molecular diagnostics, with the two approaches complementing each other. Molecular techniques may even supplant traditional microscopic identification, sometimes because of the paucity of expert classical taxonomists. Where precision and rapid, reliable diagnostics are required, molecular techniques are replacing classical identification; this is particularly true for root-knot and cyst nematodes, and increasingly for many other groups, as classical techniques lack the necessary precision to separate cryptic species groupings. Whilst the endoparasitic root-knot and cyst nematodes are the most devastating plant-parasitic groups worldwide, several species in the migratory endoparasitic and ectoparasitic groups of nematodes are also of considerable economic and social importance.

The chapters in Part I are intended to reflect all these aspects by presenting the basic structures of nematodes, followed by a chapter on molecular taxonomy, systematics and phylogeny. The subsequent four chapters focus on the major groups of plant-parasitic nematodes, presenting information on their morphology, taxonomy, basic biology and management.

Throughout this book, the systematic scheme follows the higher classification (i.e. family level and above) of De Ley and Blaxter (2002).

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1

Structure and Classification*

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1.1. Introduction

Nematodes are pseudocoelomate, unsegmented worm-like animals, commonly described as filiform or thread-like, a characteristic reflected by the taxon name *nema* (Greek, *nema* = thread) and its nominative plural *nemata*. Zoologically speaking, the

* A revision of Decraemer, W. and Hunt, D.J. (2006) Structure and classification. In: Perry, R.N. and Moens, M. (eds) *Plant Nematology*, 1st edn. CAB International, Wallingford, UK.

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vernacular word ‘nematode’ is a corruption for the order name Nematoidea, one of the five historical orders of the class Helminthia, which embraced all thread-like forms or roundworms (gordians and nematodes). At present, nematodes are generally regarded as a separate phylum, the Nematoda or Nemata (De Ley and Blaxter, 2002). The systematic scheme presented is based on the higher classification proposed by De Ley and Blaxter (2002) and has been updated where appropriate to reflect new taxa proposals. However, recent molecular phylogenetic analyses recognize 12 clades within the Nematoda, with plant-parasitic taxa located in the basic clade I (Trichodoridae) and clade II (Longidoridae) and in the more advanced clade 12 with the Tylenchomorpha (Holterman *et al.*, 2006).

Nematodes are the most numerous Metazoa on earth. They are either free-living or parasites of plants and animals and although they occur in almost every habitat, they are essentially aquatic animals. Nematodes depend on moisture for their locomotion and active life and therefore soil moisture, relative humidity and other environmental factors directly affect nematode survival. However, many nematodes can survive in an anhydrobiotic state (see Chapter 7). Soil structure is influential as pore size affects the ease with which nematodes can move through the soil interstices (see Chapter 8). In general, sandy soils provide the best environment for terrestrial nematodes but saturated clay soils can be colonized successfully by certain specialized nematodes, including *Hirschmanniella* and some *Paralongidorus*. Soil pH may affect nematodes, but local variations in soil temperature are rarely a particularly important factor (see Chapter 8).

This review of the anatomy of plant-parasitic nematodes will also include mention of some free-living and animal-parasitic species for comparative purposes. Although currently only about 4000 species of plant-parasitic nematodes have been described (i.e. 15% of the total number of nematode species known), their impact on humans by inflicting heavy losses in agriculture is substantial. The maxim that ‘where a plant is able to live, a nematode is able to attack it’ is a good one. Nematodes are even able to attack the aerial parts of plants provided that the humidity is high enough to facilitate movement. Such conditions are provided in flooded rice fields where foliar species, such as *Aphelenchoides besseyi* and *Ditylenchus angustus* can be devastating. Some *Bursaphelenchus* species, vectored by wood-boring insects, directly attack the trunk of coconut palm or pines. Other nematodes, such as some *Hirschmanniella* and *Halenchus* spp., attack algae and can live in seawater. It has been estimated that a single acre of soil from arable land may contain as many as 3,000,000,000 nematodes and a single wheat gall formed by *Anguina tritici* typically contains approximately 11,000–18,000 nematodes, although as many as 90,000 have been recorded.

In order to constrain or even banish this limiting factor in agricultural production, it is vital to identify accurately the nematode pests and to understand their biology. At present, many nematode identifications still rely upon morphological characters, but an integrated approach is becoming increasingly common, including isozyme patterns and DNA sequences, and has become essential for some taxa such as root-knot nematodes (see Chapter 2).

Despite their great diversity in lifestyle, nematodes display a relatively conserved body plan. The body consists of an external cylinder (the body wall) and an internal cylinder (the digestive system) separated by a pseudocoelomic cavity filled with fluid under pressure and containing a number of cells and other organs such as the reproductive system. About 99% of all known nematodes have a long, thin cylindrical

body shape, which is round in cross section and tapered towards both ends, although usually more so towards the posterior or tail end. The tail may be short or long and varies in form from broadly rounded to filiform. The tail may also differ in shape between developmental stages or between sexes.

Nematodes usually crawl or swim with undulating movements in a dorsoventral plane (see Chapter 8). Aberrant body shapes, for example a swollen female body, may indicate either a loss of locomotion, as in cyst nematodes, or be related to an atypical locomotory pattern. On a solid surface a nematode crawls on its lateral surface except, for example, in the free-living marine families Draconematidae and Epsilonematidae, which move on their ventral surface. Nematodes travel fastest in soil when pore space is about 0.3 times their body length. In plant-parasitic nematodes, all migratory ectoparasites of roots, including all Trichodoridae and Longidoridae and many Tylenchomorpha, are vermiform throughout their life cycle (Figs 1.1 and 1.2). Other, more highly evolved, Tylenchomorpha have a sedentary endoparasitic lifestyle, one or more stages

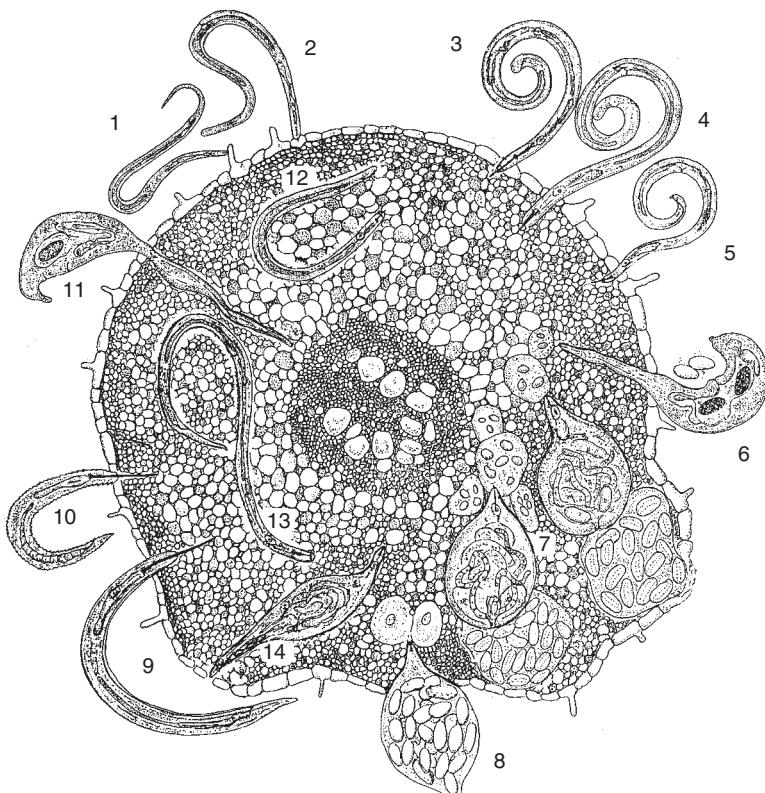


Fig. 1.1. Root in transverse section showing the diverse appearance and feeding modes of plant-parasitic nematodes. 1: *Cephalenchus*. 2: *Tylenchorhynchus*. 3: *Rotylenchus*. 4: *Hoplolaimus*. 5: *Helicotylenchus*. 6: *Rotylenchulus*. 7: *Meloidogyne*. 8: *Heterodera*. 9: *Hemicyclophora*. 10: *Criconemoides*. 11: *Tylenchulus*. 12: *Pratylenchus*. 13: *Hirschmanniella*. 14: *Nacobbus*. Adapted from Siddiqi (1986).

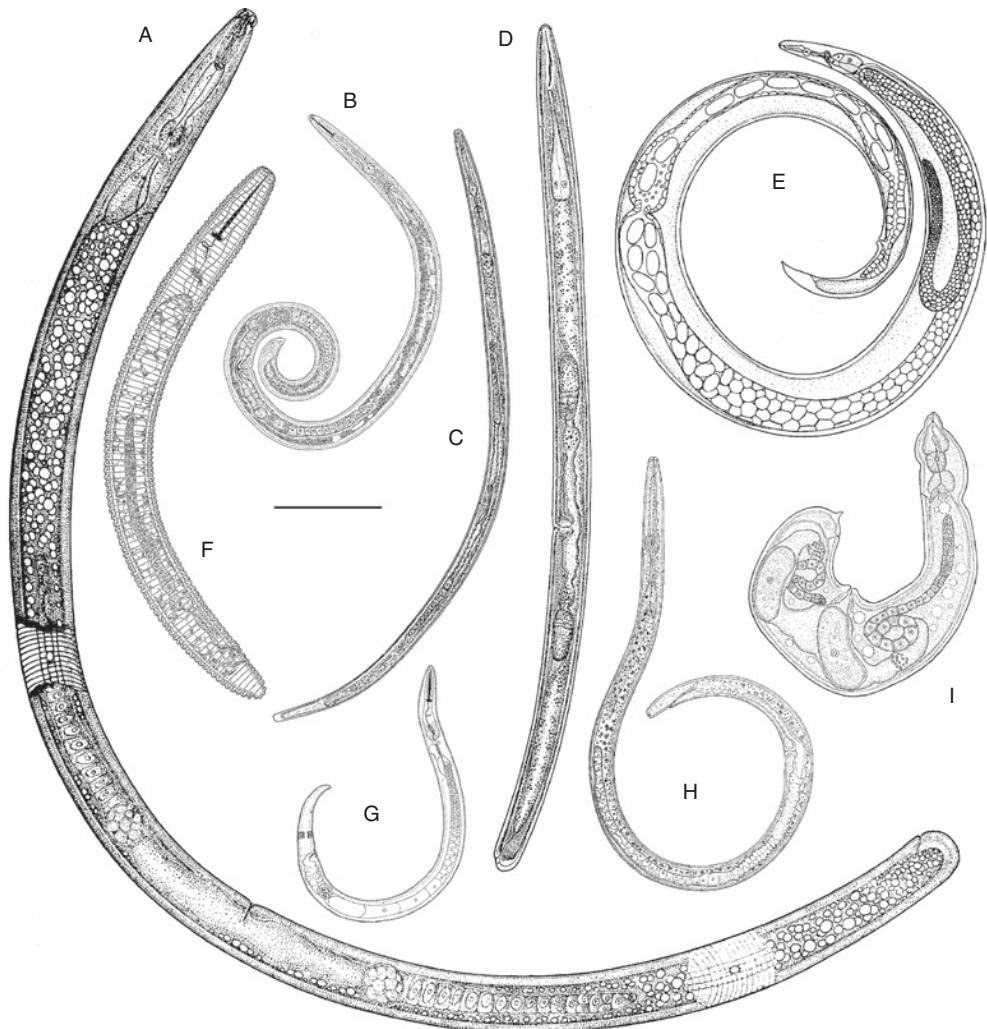


Fig. 1.2. Relative body size and range in form of mature females of some common plant-parasitic nematodes. A: *Hoplolaimus galeatus*. B: *Helicotylenchus dihystera*. C: *Tylenchorhynchus annulatus*. D: *Trichodorus primitivus*. E: *Anguina tritici* (note that, because of its length of 3–5 mm, this nematode is shown at a different scale to the others). F: *Criconemoides xenoplax*. G: *Paratylenchus bukowinensis*. H: *Aphelenchus avenae*. I: *Rotylenchulus reniformis*. Being typically rather long and often extremely slender, no members of the Longidoridae are figured due to problems of scale, but note that the smallest *Xiphinema* is about the same length as the *Hoplolaimus* and that the longest *Paralongidorus* is about six times as long. Figure digitally composed from drawings taken from CIH Descriptions of Plant-Parasitic Nematodes. (Scale bar: A–D, F–I = 100 µm; E = 250 µm).

inciting specific feeding cells or feeding structures within the root tissue and becoming obese (Fig. 1.1). This type of life cycle is seen in the root-knot and cyst nematodes where the mature female becomes pyriform, globose or lemon-shaped in form.

The body shape, or habitus, assumed by nematodes on relaxation varies from straight through ventrally curved to spiral and this can be a useful diagnostic character, particularly under the stereomicroscope. Free-living and plant-parasitic nematodes are mostly less than 1 mm in length (Fig. 1.2), although some species, particularly in the Longidoridae, may greatly exceed this, *Paralongidorus epimikis*, for example, attaining a maximum length of over 12 mm. Animal-parasitic nematodes can be substantially longer and often achieve lengths of many centimetres, exceptionally even metres. Externally, the body shows little differentiation into sections apart from the tail region. The nematode body can be divided into a dorsal, a ventral and two lateral sectors. The secretory-excretory (S-E) pore, vulva and anus in the female or the cloacal opening in the male are all located ventrally whereas the lateral regions may be identified by the apertures of the amphids (few exceptions), deirids and phasmids, when present. The mouth opening is usually located terminally at the anterior end. The body displays a bilateral symmetry although the anterior end also shows a radial symmetry. The body wall consists of the body cuticle, epidermis and somatic musculature.

1.2. General Morphology

The variation in size and body form of a selection of typical plant-parasitic nematodes is shown in Fig. 1.2, while the general morphology of a typical tylenchomorph nematode is shown in Fig. 1.3, major organ systems being depicted in relation to one another. Organ systems are usually tubular in form and are suspended within the pseudocoelomic cavity. The following sections deal with the various structures in more detail and also compare and contrast organ systems between tylenchomorph, longidorid and trichodorid nematodes, thereby facilitating diagnostics of the major groups of plant-parasitic nematodes.

1.2.1. Body cuticle

Most nematodes possess a cuticle, although some, such as *Fergusobia*, lack a cuticle in the adult insect-parasitic female. Cuticle structure may be extremely variable (Fig. 1.4; Box 1.1), not only between different taxa, but also intraspecifically between sexes and developmental stages or between different body regions of an individual (Decraemer *et al.*, 2003). The nematode cuticle varies from being simple and thin (Fig. 1.4H) to highly complex and multilayered (Fig. 1.4L). The body cuticle invaginates at the mouth opening, amphids, phasmids, S-E pore, vulva and anus or cloacal opening, forming the lining to the cheilostom, amphidial fovea (canal), terminal canal, part of the terminal duct of the S-E system, *pars distalis vaginae*, cloaca and rectum. As nematodes lack both a skeleton and a circular muscle system, the cuticle functions as an antagonistic system that prevents radial deformation of the body when the longitudinal muscles contract during undulatory locomotion. Initially, the cuticle plays a role in maintaining body shape after elongation of the embryo. The cuticle, together with the epidermis, also functions as a barrier to harmful elements in the environment and, being semipermeable, plays a role in secretion-excretion or in the uptake of various substances.

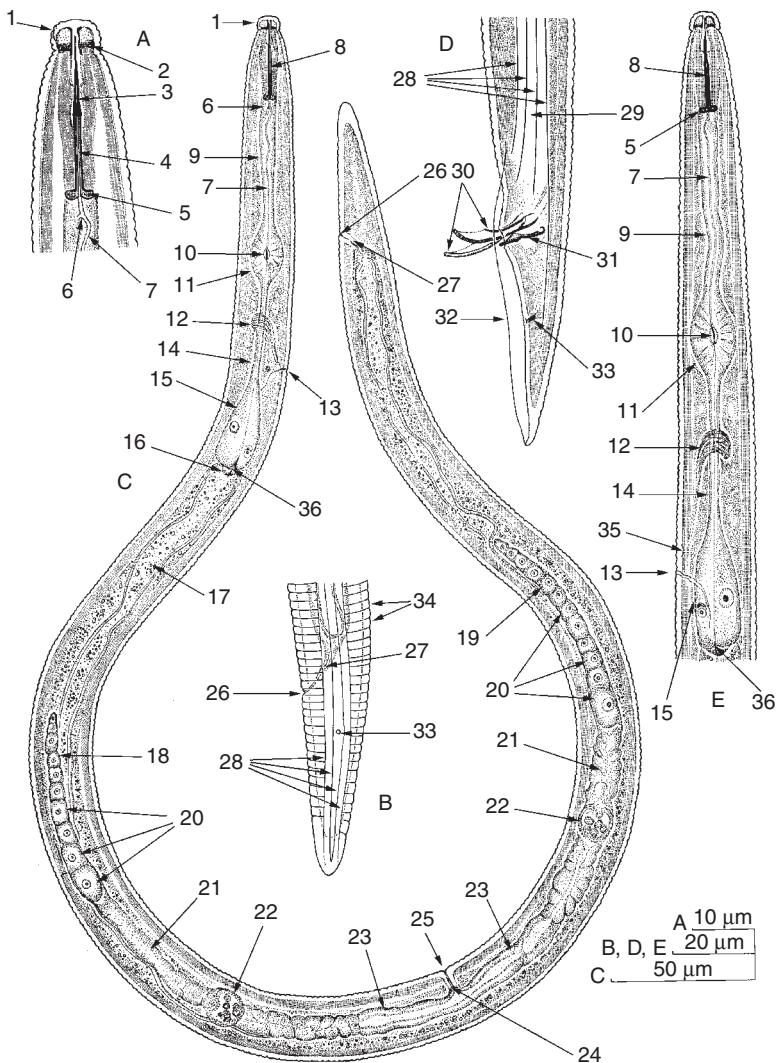


Fig. 1.3. Overview of the morphological structures in a female and male plant-parasitic nematode (*Tylenchorhynchus cylindricus*). A: Female anterior body region. B: Female posterior body region. C: Entire female. D: Male posterior region. E: Neck region.
 1: Cephalic region. 2: Cephalic framework. 3: Stylet cone. 4: Stylet shaft. 5: Stylet knobs. 6: Outlet of dorsal pharyngeal gland. 7: Pharyngeal lumen. 8: Stylet protractor muscles. 9: Procorpus. 10: Valve. 11: Metacorpus. 12: Nerve ring. 13: Secretory-excretory pore. 14: Isthmus. 15: Pharyngeal bulb with gland nuclei. 16: Pharyngeal bulb (abutting intestine, not overlapping). 17: Intestine. 18: Ovary of anterior genital branch. 19: Ovary of posterior genital branch. 20: Developing oocytes in ovaries. 21: Oviduct. 22: Spermatheca with sperm inside. 23: Uterus. 24: Vagina. 25: Vulva. 26: Anus. 27: Rectum. 28: Four lines or incisures in lateral field. 29: Lateral field. 30: Spicules. 31: Gubernaculum. 32: Bursa or caudal alae. 33: Phasmid. 35: Hemizonid. 36: Cardia. Adapted from Siddiqi (1972).

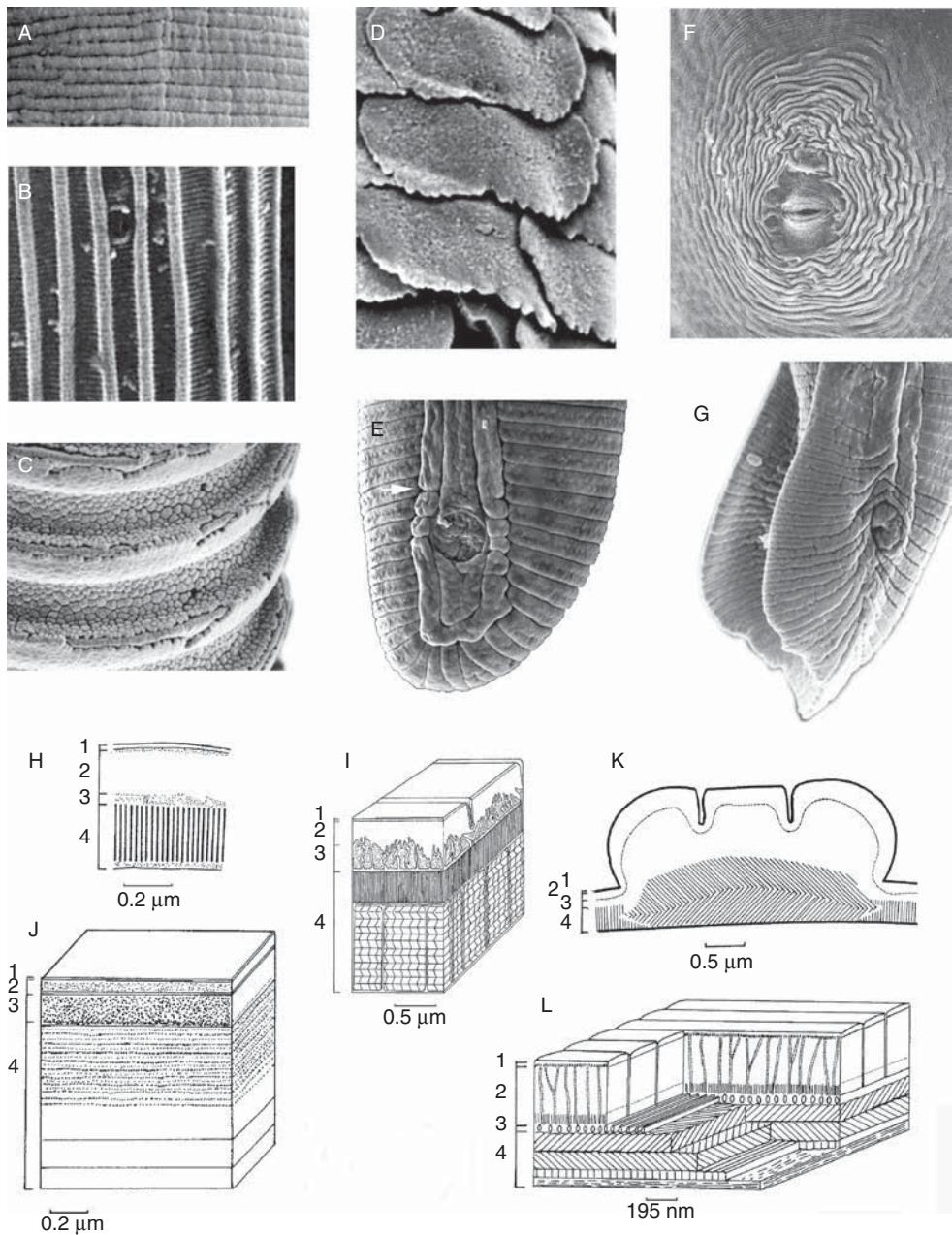
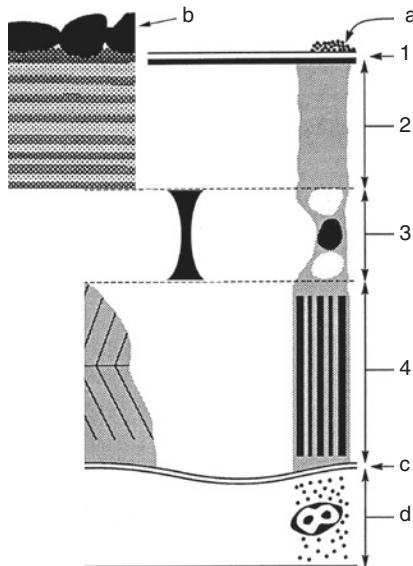


Fig. 1.4. Surface structure of the body cuticle. A: Transverse striae, SEM (Trichodoridae). B: Longitudinal ridges (Actinolaimidae, Dorylaimida) (Vinciguerra and Claus, 2000. Courtesy Universidad de Jaén). C, D: *Criocerina paradoxiger*. C: External cuticular layer in female. D: Scales in juvenile (Decraemer and Geraert, 1992. Courtesy Brill). E: Lateral field with longitudinal ridges and areolation (arrow) in *Scutellonema*. F: Perineal pattern in *Meloidogyne* (Siddiqi, 1986. Courtesy CABI). G: Caudal alae in *Scutellonema* male. H–L: Diverse ultrastructure of body cuticle of plant-parasitic nematodes. H: *Pratylenchus*. I: *Rotylenchus*. J: *Trichodorus*, adult (Trichodoridae). K: *Tylenchorhynchus*, lateral field. L: *Xiphidorus*, adult (Longidoridae). H, I, K: Tylenchomorpha, females. (Decraemer *et al.*, 2003. Courtesy Cambridge Philosophical Society (Biological Reviews)). See Box 1.1 for details of layers 1–4 in H–L.

Box 1.1. Ultrastructure of nematode body cuticle.



Scheme ultrastructure. 1: epicuticle, 2: cortical zone, 3: median zone, 4: basal zone, a: surface coat, b: extra- or non-cuticular material in criconematids, c: basal lamina, d: epidermis (based on Bird and Bird, 1991).

Epicuticle: Trilaminar outermost part of the cuticle, first layer to be laid down during moulting.

Cortical zone: Zone beneath the epicuticle, may be more or less uniform in structure, amorphous or with radial striae, or may show a subdivision into an outer amorphous part and an inner, radially striated layer, or be multilayered in criconematids. Cortical radial striae are unknown from plant parasites.

Median zone: Internal to the cortical zone, variable in structure: homogeneous or layered, with or without granules, globules, struts, striated material or fibres. The median zone may be absent.

Basal zone: Innermost zone of the cuticle, usually with the most complex structure of the three main zones; comprises outer sub-layers of spiral fibres and inner layer with or without other fibres, laminae or radial striae.

Radial striae: Either cortical or basal in position, consist of longitudinal and transverse circumferential interwoven laminae which, at high magnification, appear as osmiophilic rods separated by electron-light material; the spacing and periodicity of these rods may vary between species, but in transverse sections of tylenchids it is about 19 nm.

Radial elements: Superficially resembling radial striae (in Longidoridae).

Basal radial striae: In basal cuticle zone, always interrupted at level of lateral chords and usually replaced by oblique fibre layers; mainly found in free-living terrestrial stages and most plant-parasitic Tylenchina.

Cortical radial striae: In cortical zone, not interrupted at level of lateral chords; characteristic of a free-living aquatic lifestyle.

Basal spiral fibre layers: Helically arranged fibre layers (angle $54^{\circ}44'$ or more), play a role in maintaining the internal turgor pressure.

Struts: Column-like supporting elements in the median zone (common in free-living aquatic nematodes and some animal parasites).

1.2.1.1. Outer cuticle structure and ornamentation

The body cuticle is often marked by transverse and/or longitudinal striae (Fig. 1.4). The transverse striae range from being very fine, superficial (i.e. restricted to the cortical zone) and close together, to deeper and wider apart, in which case they delimit the annuli. Transverse striae are present in Longidoridae and Trichodoridae but are only visible by electron microscopy (Fig. 1.4A and L), being indistinct or difficult to observe by light microscopy. When both transverse and longitudinal striae are present all over the body, the cuticle has a tessellate or chequered appearance. Apart from striae, longitudinal elevations or ridges may also be present (with or without an internal support). Alae are thickened wing-like extensions of cuticle which are often found laterally or sublaterally on the body, but may also be localized in the caudal region of the male where they form the bursa or caudal alae (Fig. 1.4G). Other cuticular outgrowths, such as transverse or longitudinal flaps, occur at the female genital opening where they may cover or guard the vulva. More elaborate cuticular ornamentation may also occur (spines, setae, papillae, tubercles, warts, bands, plates, rugae and pores). In plant-parasitic nematodes, cuticular ornamentations are important diagnostic features, especially in Criconematidae, and there may be an extra cuticular layer in *Criconema* (*Amphisbaenema*) and *Nothocriconema* (Decraemer *et al.*, 1996) (Box 1.1; Fig. 1.4C).

In many nematodes, the lateral body cuticle is modified to form the lateral fields (Fig. 1.4E and K). In Tylenchomorpha, the lateral fields are marked by longitudinal incisures and may be elevated above the body contour to form longitudinal ridges or bands. These ridges may be intersected by the transverse striae, in which case the lateral field, which now has a block-like appearance, is described as being areolated (Fig. 1.4E). The number of longitudinal lines or incisures is of taxonomic importance but, as their number decreases towards the extremities, the number of lines should be counted in the mid-body region. It is important to differentiate between longitudinal ridges and lines or incisures, there being one more line or incisure than there are ridges. Sometimes, the lateral field shows anastomoses and occasionally lateral cuticle differentiation may be absent, as in obese females of *Heterodera* or all Longidoridae and Trichodoridae. Cuticular differentiations may also occur at or around the vulva and anus, as in the perineal patterns of mature females of root-knot nematodes (Fig. 1.4F).

1.2.1.2. Cuticle ultrastructure

The cuticle is secreted in layers and essentially consists of four parts: (i) a thin epicuticle at the external surface, which is provided with a surface coat of glycoproteins and other surface-associated proteins or, more rarely, with an additional sheath formed from cuticle or extra cuticular particles; (ii) a cortical zone; (iii) a median zone; and (iv) a basal zone. Certain zones may be absent. For example, in Tylenchomorpha, the body cuticle changes in structure at the base of the cephalic region, i.e. the median and radially striated basal zones disappear. The latter appears to continue as the electron-dense zone of the cephalic capsule. The trilaminar epicuticle acts as a hydrophobic barrier and is composed of non-collagenous proteins, cuticles and lipids. In cyst nematodes, quinones and polyphenols in the epicuticle

result, upon the action of phenoloxidase, in the tanning of the female cuticle to form a resistant cyst wall. The surface coat is a highly dynamic layer secreted by the epidermis and is part of the immune system (Davies *et al.*, 2008). In *Meloidogyne incognita*, *in vivo* root exudates triggered an increase in surface coat lipophilicity and allowed the root-knot nematodes to adapt to survive plant defence processes (Davies and Curtis, 2011).

The most important structural elements of the cuticle morphology are the presence/absence of: (i) cortical radial striae; (ii) basal radial striae; (iii) spiral fibre layers in the basal zone; and (iv) supporting elements, e.g. struts, in the fluid matrix of the median zone (Box 1.1). All these features are thought to be responsible for the radial strength of the cuticle. At the level of the lateral chords, the cuticle may not only show an external differentiation in ornamentation or cuticular outgrowths, such as lateral alae, but also displays ultrastructural differences when compared to the dorsal and ventral regions of the body (Fig. 1.4K). Intracuticular canals have been observed in many species (e.g. Trichodoridae, Hoplolaimidae) and may be involved in transport of material from the epidermis to the other layers of the body cuticle. In Longidoridae, adults and juveniles have an identical cuticle structure composed of three main zones: (i) the cortical zone with radial filaments and radial elements at the inner base; (ii) the median zone with a layer of median thick longitudinal fibres; and (iii) the basal zone with two spiral fibre layers and either a layered or a homogenous inner region (Fig. 1.4L). Trichodoridae have a cortical zone without radial striae and a homogenous median zone, but with a basal zone characterized by concentric layers, an apparent synapomorphy for the family (Fig. 1.4J). The absence of radial striae in the cortical or basal zone, as well as the absence of spiral fibre layers, may be related to the low internal pressure in trichodorids (trichodorids do not ‘explode’ when punctured), as well as to their slow locomotion. In Tylenchomorpha, the cuticle structure is more diverse but cortical radial striae are always absent (Fig. 1.4H, I and K). The cortical zone is rarely subdivided but in females of the Criconematidae it is multilayered except in the cephalic region. The median zone in the majority of Tylenchomorpha is vacuolated, either with or without granular material or ovoid to globular structures, but may be absent resulting in the cortical zone abutting the radially striated basal zone (e.g. *Aphelenchus avenae*). In the majority of Tylenchomorpha, all developmental stages have the basal zone characterized by a radially striated layer; additional internal sublayers as part of the basal zone may be present in Hoplolaimidae and Heteroderidae females. In globose females of the Heteroderidae, the radially striated layer is discontinuous. Basal radial striae appear to induce some physical constraints, e.g. to growth, which may explain their absence under certain conditions or their breaking up into small patches in obese endoparasitic females of the Heteroderinae. In Tylenchomorpha, the cuticle at the level of the lateral field is differently structured compared to the rest of the body, resulting in replacement of the basal radial striae by fibre layers, an apparent functional requirement to accommodate small changes in body diameter. Basal radial striae also appear to be involved in locomotion because they disappear in second-stage juveniles (J2) of *Meloidogyne* shortly after the juvenile becomes a sedentary endoparasite.

Most juveniles of plant-parasitic Tylenchomorpha have a similar cuticle structure to the adults. Cuticular changes other than during the moulting process occur when changing lifestyle and in sedentary stages. Upon invasion of plant roots, the conspicuous

radially striated basal zone of the cuticle of the pre-parasitic J2 of *Meloidogyne* is modified in the parasitic J2 into a thicker cuticle lacking basal radial striae (Jones *et al.*, 1993).

All stages of the plant-parasite *Hemicyclophora arenaria* possess an additional sheath covering the normal cuticle (Johnson *et al.*, 1970). This sheath is composed of a trilaminar outer layer and either four (female) or two (male) inner layers of cuticle.

Most nematodes moult four times (exceptionally three times, e.g. as in some Longidoridae) during their development. At each moult the cuticle is reconstructed. During moulting, the cuticle can either be shed completely (Trichodoridae) or partially resorbed (*Meloidogyne*). New cuticle formation is characterized by the occurrence of epidermal folds or plicae over which the new cuticle becomes highly convoluted (Bird and Bird, 1991). The epicuticle is the first layer to be laid down and is connected to the epidermis by hemidesmosomes.

In addition to the anterior cuticular sense organs, such as labial and cephalic sensilla and the amphids, there are also somatic sense organs that terminate in setae or in pores, phasmids or deirids (see Section 1.2.5.3).

1.2.2. Epidermis

The epidermis secretes the cuticle and is responsible for the overall architecture, including elongation of the embryonic tadpole stage (Costa *et al.*, 1997). The epidermis is probably the limiting structure in homeostatic regulation. The epidermis consists of a thin layer and four main internal bulges that form the longitudinal chords, one dorsal, one ventral and two lateral, dividing the somatic muscles into four fields (Box 1.2). Anteriorly, it pervades the region of the cephalic framework and is responsible for its formation. The epidermis can be cellular, partly cellular or syncytial (Tylenchomorpha). The cellular condition is a primitive one occurring in free-living species and some parasitic species plus juveniles of parasites that possess a syncytial epidermis in adults. In some species there are no cell boundaries between the chords but cell walls exist within the chords, especially the lateral chords. The cell nuclei are usually located in the chords, although the dorsal chord only has nuclei in the pharyngeal region. The structure of the epidermis may show pronounced changes during development. For example, in the insect-parasitic stage of *Fergusobia* the cuticle and feeding apparatus are degenerate and the epidermis is convoluted into the numerous microvilli responsible for uptake of nutrients (Giblin-Davis *et al.*, 2001). The epidermis contains various specialized structures such as epidermal glands, caudal glands and ventral gland(s) of the S-E system. In some aquatic nematodes, such as *Geomonhystera disjuncta*, vacuoles in the epidermal chord may act as a compartmentalized hydrostatic skeleton (Van De Velde and Coomans, 1989).

1.2.3. Somatic musculature

Only a single layer of obliquely orientated and longitudinal aligned somatic muscle cells lies beneath the epidermis. The number of rows per quadrant between the chords varies from a few (up to five cells), known as the meromyarian condition,

Box 1.2. Body wall and pseudocoel.

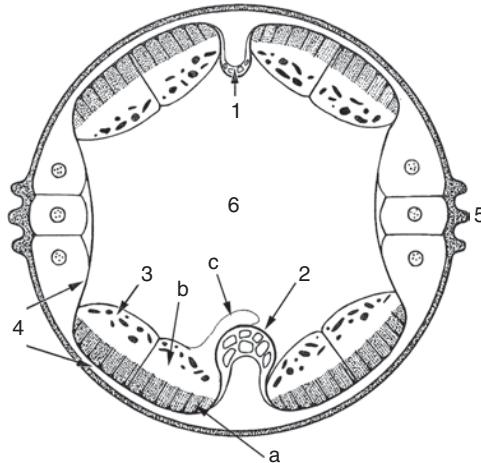


Diagram of transverse section depicting the epidermal chords, the somatic musculature with detail of muscle cell and pseudocoel. Internal organs have been omitted. 1: dorsal epidermal chord, 2: ventral chord with ventral nerve, 3: muscle cell, 4: basal lamina, 5: lateral alae, 6: pseudocoel, a: contractile part of muscle cell, b: non-contractile part, c: process of muscle cell (based on Bird and Bird, 1991).

Somatic muscle cell: Mainly spindle-shaped, consists of (a) a contractile portion of the cell towards the epidermis, (b) a non-contractile portion towards the body cavity and (c) an arm or process that extends from the non-contractile portion of the cell toward the dorsal or the ventral nerve; muscle cells anterior to the nerve ring send processes directly into the nerve ring.

Platymyarian muscle cell: The whole contractile part of the muscle cell is flat and broad and borders the epidermis; common in small species.

Coelomyarian muscle cell: Spindle-shaped muscle cell; laterally flattened so that the contractile elements are arranged not only along the epidermis, but along the sides of the flattened spindle as well; coelomyarian muscle cells bulge into the pseudocoel; common in large species.

Circomyarian muscle cell: Muscle cell in which the sarcoplasm is completely surrounded by contractile elements.

Meromyarian musculature: Few (five or six) rows of muscle cells are present per quadrant.

Polymyarian musculature: More than six rows of muscle cells present per quadrant; spindle-shaped muscle cells laterally flattened.

to many rows, the polymyarian condition. The general sinusoidal movement of nematodes is brought about by alternate contraction of the ventral and dorsal musculature, thereby giving rise to waves in a dorsoventral plane (see Chapter 8). In Criconematidae with strongly developed transverse cuticular annuli, contraction of the somatic muscles shortens and relaxation extends the body, resulting in a creeping movement comparable to that of earthworms. A typical characteristic

of a nematode muscle cell, a feature found in only a few other invertebrate taxa (e.g. some Gastrotricha), is that instead of the nerve process running towards the muscle, a process of the non-contractile portion of the muscle cell extends towards the dorsal or ventral nerve in the corresponding epidermal chord. The arrangement of the contractile portion groups the muscle cells into three types: (i) platymyarian (flat contractile part bordering the epidermis); (ii) coelomyarian (muscle cell bulging into the pseudocoelom, contractile part not completely bordering the epidermis); and (iii) the circomyarian type (contractile elements surrounding the central sarcoplasm) (Box 1.2). The platy-meromyarian type is more common in small species such as plant-parasitic nematodes. Specialized muscle cells are associated with the digestive system and the male and female reproductive systems.

1.2.4. Pseudocoelom

The pseudocoelom or body cavity is a secondary structure lacking mesentery and is lined by the somatic muscles and the basal lamina that covers the epidermal chords. This fluid-filled cavity bathes the internal organs and contains some large amoeboid cells called pseudocoelomocytes. These vary in number, size and shape and their function includes osmoregulation, secretion and transport of material. The pseudocoelomic fluid acts as part of the turgor-pressure system, but also has some circulatory function.

1.2.5. Cephalic region, sense organs and nervous system

1.2.5.1. Cephalic region and anterior sensilla

Nematodes lack a true head region, but in this chapter the term ‘cephalic region’, together with its derivatives, will be used. In the literature, note that the cephalic region is also referred to as the labial or lip region. The basic pattern in nematodes is for there to be six lips around the mouth opening (two sub-dorsal, two sub-ventral and two lateral) (Fig. 1.5A). The lips can be fused, for example two by two resulting in three lips, one dorsal and two ventrosublateral (*Ascaris*), or the lateral lips may be reduced or absent (*Pseudoacrobeles (Bonobus) pulcher*). The lips are either clearly separated or partially to completely fused (Longidoridae, Trichodoridae) (Fig. 1.5C). In Tylenchomorpha, the anterior end shows an amalgamated, usually hexagonal, lip region and so lip-like differentiations, when present, are better referred to as lip sectors or lip areas (Fig. 1.5B), e.g. there are six lip sectors in aphelenchs, but only four in *Belonolaimus* as the two lateral sectors have been reduced. Loof and De Grisse (1974) introduced the term ‘pseudolips’ for the six areas around the oral opening of some Criconematidae (Fig. 1.5E). The area around the oral opening may be differentiated into an oral and/or labial disc. In many groups of Tylenchomorpha there are two consecutive openings due to invagination of the cephalic cuticle, the outermost being the pre-stomatal opening and the innermost, the stomatal opening. The region between the openings, or anterior to the stoma opening when the prestoma opening is wide, is referred to as the prestoma.

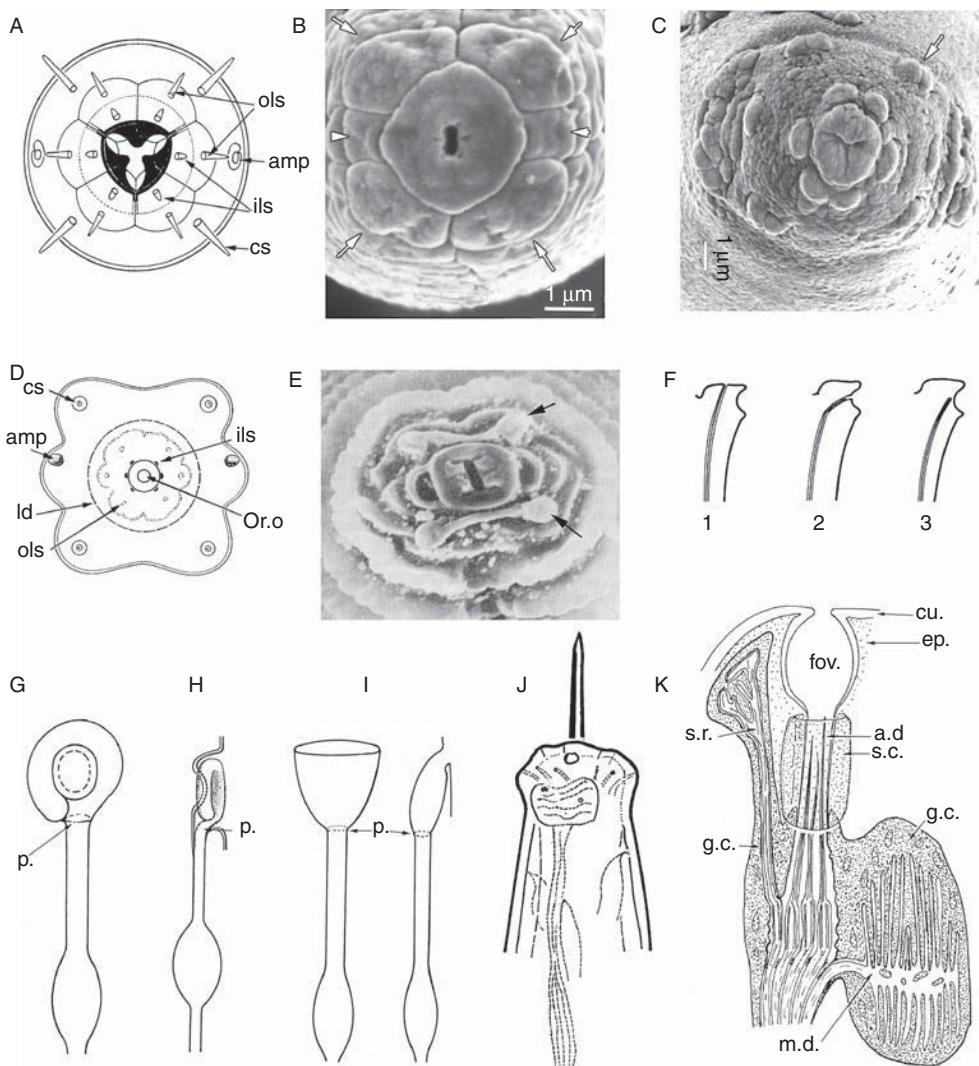


Fig. 1.5. Cephalic region and anterior sensilla. A: Basic scheme (de Coninck, 1965). B: *Scutellonema* with six lip areas (arrows); *en face* view. C: *Paratrichodorus* in *en face* view with outer labial and cephalic papillae in a single circlet (arrow). D: Arrangement in *Aphelenchoïdes*: amp: amphid, cs: cephalic sensilla, ils: inner labial sensilla, ld: labial disc, ols: outer labial sensilla, Or.o: oral opening. E: *Criconemoides* (= *Criconemella*), *en face* view showing pseudolips (arrows) (Van Den Berg and De Waele, 1989). F: Inner labial sensilla: 1: *Ditylenchus*. 2: *Merlinius*. 3: *Hemicyclophora* (after De Grisse, 1977). Amphid structure: G, H: Spiral amphid, lateral and ventral view; p. pore. I: Pocket-like amphidial fovea in lateral (left) and ventral (right) view. J: *Xiphidorus*, pouch-like amphidial fovea with pore-like opening. K: Ultrastructure of amphid: a.d.: amphidial duct, cu: cuticle, ep: epidermis, fov: fovea, g.c.: gland or sheath cell, m.d.: multivillous dendrite, s.c.: socket cell (after Coomans, 1979).

In Tylenchomorpha, the cephalic region is internally supported by a variously developed cephalic cuticular framework that may be well developed and heavily sclerotized. The lip region can be continuous with the body contour, as in *Trophurus*, or more or less offset from the rest of the body, either by a depression or constriction (e.g. *Belonolaimus*, *Hoplolaimus*), or be broader than the adjoining body and therefore expanded (e.g. *Paralongidorus* spp. in the *Siddiqia* group; some *Xiphinema* spp.). In Tylenchomorpha, the true cephalic height is not always easy to establish as the cephalic region may be offset at a different level to the basal cephalic framework. The cephalic region may be smooth (e.g. *Trophurus*) or bear transverse striae (many genera), the annuli so formed sometimes being divided into blocks by longitudinal striae (e.g. *Hoplolaimus*, *Rotylenchus robustus*).

The lip region carries a concentration of anterior sensilla, each composed of a neuronal and non-neuronal section formed by two epidermal cells, the socket cell and the sheath cell (Coomans, 1979). In nematodes, there are primitively 12 labial sensilla and four cephalic sensilla arranged in three circlets to form six inner labial sensilla, six outer labial sensilla and four cephalic sensilla. This is referred to as the 6 + 6 + 4 pattern. Two chemoreceptor sense organs, the amphids, are primitively located clearly posterior to the three circlets of anterior sensilla, but in more derived forms, such as in Tylenchomorpha, they have migrated forward onto the lip region. Each lip bears an inner and an outer sensillum on its radial axis, this hexaradial pattern being maintained when lip number is secondarily reduced. The four cephalic sensilla are bilaterally arranged (two laterodorsal, two lateroventral) and represent the anteriormost somatic sensilla. In the plant-parasitic Longidoridae and Trichodoridae, the cephalic sensilla have migrated onto the lip region and are close to the outer labial sensilla, thereby forming a single circlet or 6 + (6 + 4) pattern. In Tylenchomorpha, the anterior sensilla are arranged in three circlets but, because of the small size of the cephalic region, the two posterior circlets are located close together.

In general, the six inner labial sensilla protrude from the surrounding cephalic cuticle via a terminal pore on top of a papilla. In a number of plant- and animal-parasitic nematodes, the inner labial sensilla either have pore-like openings around the oral opening or inside the pre-stoma (*Pratylenchus*) (Fig. 1.5F), or pores may be lacking entirely and the receptors end blind in the cuticle of the oral disc (*Hemicyclophora*). Inner labial sensilla in open connection with the environment are chemoreceptive; those covered or embedded in the cuticle are mechanoreceptive. In most tylenches, the inner labial sensilla possess two ciliary receptors and show a combined chemo- and mechanoreceptive function. In *Longidorus*, four such receptors can be found, whilst there are two or three in *Trichodorus*.

The outer labial sensilla may protrude via papillae or setae, but in many plant- and animal-parasitic nematodes they end in simple pores or are embedded in the cephalic cuticle; the cuticle above each termination may show a slight depression. The lateral outer labial sensilla are often reduced, a reduction that may be related to the development of the amphids (*Meloidogyne*).

The cephalic sensilla are sub-median in position and usually protrude from the surrounding cuticle as setae or papillae with a terminal pore. In many parasitic nematodes they are embedded in the cephalic cuticle.

The main constituent parts of an amphid are the aperture, the *fovea*, the *canalis* and the *fusus* or sensillar pouch (Fig. 1.5K). The distal part of the amphid, the fovea, is either an external excavation of the cephalic or body cuticle (as in many free-living

Chromadorida species, Fig. 1.5G and H) or an invagination of the cuticle, thus forming a pocket connected with the exterior through an aperture (as in plant-parasitic nematodes, Fig. 1.5I, J). The amphidial aperture is typically located laterally, but may be shifted dorsad. The amphidial opening varies in form: in Trichodoridae (Fig. 1.5C) it is a post-labial transverse slit, whereas in Longidoridae it is of variable shape and size, being either a post-labial transverse slit or a pore (Fig. 1.5J). In Tylenchomorpha, the amphid openings have migrated onto the lip region and are usually close to the oral opening, their apertures being greatly reduced in size and slit-like to oval in form (Fig. 1.5B). The fovea is the most variable part of the amphid, varying in size and shape according to taxa and even within a taxon between sexes and may be completely or partially filled with a gelatinous substance (*corpus gelatum*) secreted by the amphidial gland and may protrude from the body. In nematodes with an internal fovea there is less variation in form. In Longidoridae, the fovea varies from stirrup- or goblet-shaped to a pouch (Fig. 1.5J), which may be bilobed. In tylenchs, there is no sharp demarcation between fovea and canalis and the sensilla pouch may be located as far posterior as the stylet knobs. The amphids are the largest of the chemoreceptors and possess a much greater number of receptors than other sensilla. In Tylenchomorpha, lip patterns and arrangement of the anterior sensilla and amphids are considered important diagnostic features in identification and for the analysis of relationships.

1.2.5.2. Central nervous system

The nerve ring usually encircles the isthmus of the pharynx, rarely the intestine. The nerve ring is connected to several ganglia, longitudinal nerves running anteriorly towards the anterior sensilla in the cephalic region while posteriorly four large nerves, the largest being the ventral nerve, run through each of the four epidermal chords, with four smaller nerves, two laterodorsal and two lateroventral, running adjacent. The cell bodies of the nerves of the anterior sensilla are located in six separate ganglia anterior to the nerve ring or in a single ganglion (*Caenorhabditis elegans*). The amphidial nerves have an indirect connection with the nerve ring, their cell bodies being located in the paired lateral ganglia. Of the posterior longitudinal nerves, the lateral nerve contains a few ganglia, including the lumbar ganglia in the tail region. The ventral nerve has a chain of ganglia, the anteriormost being the retrovesicular ganglion whereas the dorsal and sub-median nerves lack posterior ganglia. Posterior to the nerve ring, the longitudinal nerves are connected to each other by commissures. The presence and position of some of these commissures are of taxonomic importance. The most important commissure is the lateroventral commissure, which is also known as the hemizonid. This is visible as a refractive body near the S-E pore. At a short distance from the hemizonid lies the hemizonion. Caudalids in the tail region correspond to the paired anal-lumbar commissure, which links the pre-anal ganglion to the lumbar ganglion.

1.2.5.3. Peripheral and inner nervous system

There may be numerous somatic sensilla in addition to the anterior sensilla in the cephalic region, arranged in dorsal, ventral and sub-ventral longitudinal rows. In

free-living marine nematodes a peripheral lattice-like network of nerves connects somatic setae and papillae and coordinates impulses from these somatic sense organs, both with each other and with the central nervous system. In terrestrial nematodes, such as Longidoridae and Trichodoridae, these sensilla are mainly chemosensitive and open to the exterior as pores; they are often associated with glands (e.g. at level of lateral field in Longidoridae). In free-living and plant-parasitic Rhabditia, up to three pairs of laterally located deirids can be found in the neck region. The innervation of deirids (= cervical papillae common in Tylenchomorpha) is from the lateral nerves from the lateral ganglia. Other somatic sense organs common in Tylenchomorpha (although they may be lacking, as in criconematids) are the postdeirids and phasmids, which are usually situated in the tail region. Phasmids have the basic structure of a sensillum, the ciliary receptor being in open connection with the environment via a pore. The pore may be provided with a plug, which is probably secreted by the sheath cell, and which may be small, as in many Tylenchomorpha, or enlarged to a shield-shaped structure, the scutellum (e.g. in *Scutellonema*, Fig. 1.4E). In the posterior body region of males (see Section 1.2.8.2) specialized somatic sensilla or genital supplements may be present.

The pharynx possesses its own visceral nervous system with its own neurons, processes and receptors, but is also connected to the nerve ring. The detailed structure of this system is mainly known from work on *C. elegans*. Internal cephalic receptors have been detected in *Xiphinema*, e.g. in association with the amphidial sheath cell (Wright and Carter, 1979), although their function remains unclear. In *Longidorus* and *Xiphinema*, nerve endings have been observed in association with the cuticular lining of the pharynx at the level of the sinuses of the odontophore, the isthmus and the anterior bulb (Robertson, 1976, 1979). These may be mechanoreceptive, detecting the passage of food or regulating the flow of secretion. Photoreceptors or ocelli have not been observed in plant-parasitic nematodes.

1.2.6. Digestive system

The wide diversity of food sources and methods of ingestion is reflected in the diversity of the structure of the digestive system (Figs 1.6 and 1.7). In general, this system consists of three regions: the stomodeum, mesenteron and proctodeum. Only the mesenteron or mid-gut is of endodermal origin, the stomodeum being of mixed ecto-mesodermal origin and the proctodeum or rectum formed from the ectoderm. The stomodeum region is referred to as the pharyngeal, neck or cervical region.

1.2.6.1. Stomodeum

The stomodeum comprises the mouth opening, stoma *sensu lato* (composed of cheilosome and pharyngostome), pharynx and cardia. The oral aperture is mostly situated terminally. The cheilosome or lip cavity has a hexa- or triradial symmetry and is lined by body cuticle. The cuticle lining may be more or less sclerotized and is supported by simple rods (cheilorhabdia, a synapomorphy of the Chromadorida). Originally, the

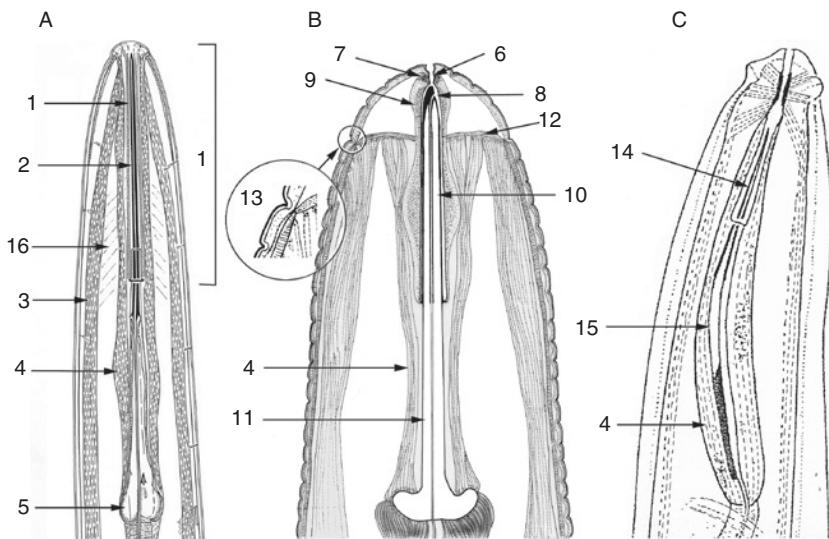


Fig. 1.6. Stoma region and types of feeding apparatus in plant-parasitic nematodes. A: Odontostyle and odontophore (Longidoridae). B: Stomatostylet with detail of body cuticle (inset) at base of cephalic framework (Tylenchomorpha). C: Onchiostyle (Trichodoridae). 1: Cheilostome. 2: Odontostyle. 3: Somatic muscles. 4: Stylet protractor muscles. 5: Odontophore with flanges. 6: Prestoma. 7: Thickening of cuticle around prestoma. 8: Stylet opening. 9: Stoma. 10: Stylet conus. 11: Stylet shaft and knobs. 12: Basal cephalic framework. 13: Body cuticle in detail, showing disappearance of median and striated basal zone in cephalic region. 14–15: Onchiostyle with onchium (14) and onchiophore (15). 16: *Dilatores buccae*. A. from Coomans (1985), B. Adapted from Endo (1980), C. From Maafi and Decraemer (2002).

cheilostome was delimited by the lips but in derived conditions may extend further posteriorly, as in Longidoridae where it represents the stoma *sensu stricto*, extending from the oral opening to the guiding ring (Fig. 1.6A). In Tylenchomorpha, the cheilostome largely lines anteriorly the cavity of the cephalic framework, through which the stylet moves, and extends posteriad until the guide ring. The pharyngostome is triradially symmetrical, as is the pharynx, of which it is a specialized part. Its structure reflects the method of feeding and food source. In plant-parasitic nematodes, some entomophilic and some predatory nematodes, the pharyngostome possesses a protrusible spear or stylet but in Trichodoridae, the spear is a protrusible dorsal tooth. The feeding apparatus in the majority of plant-parasitic nematodes is either a hollow stomatostyle (Tylenchomorpha) or odontostyle (Dorylaimida, Longidoridae). The stomatostyle consists of three parts: a conus with a ventral aperture, a shaft, and a posterior region that may enlarge to form three basal knobs, these acting as attachments for the stylet protractor muscles. According to Baldwin *et al.* (2004) the stylet cone and shaft are formed by arcade syncytia and are homologous with the gymnostom while the stylet knobs are homologous with the prostegostom. Tylenchids have no stylet retractor muscles, the resulting tension in the alimentary tract causing retraction of the stylet when the protractor muscles are relaxed (Fig. 1.6B). The dorylaimid stylet is made up of two parts: an anterior odontostyle with a dorsal aperture

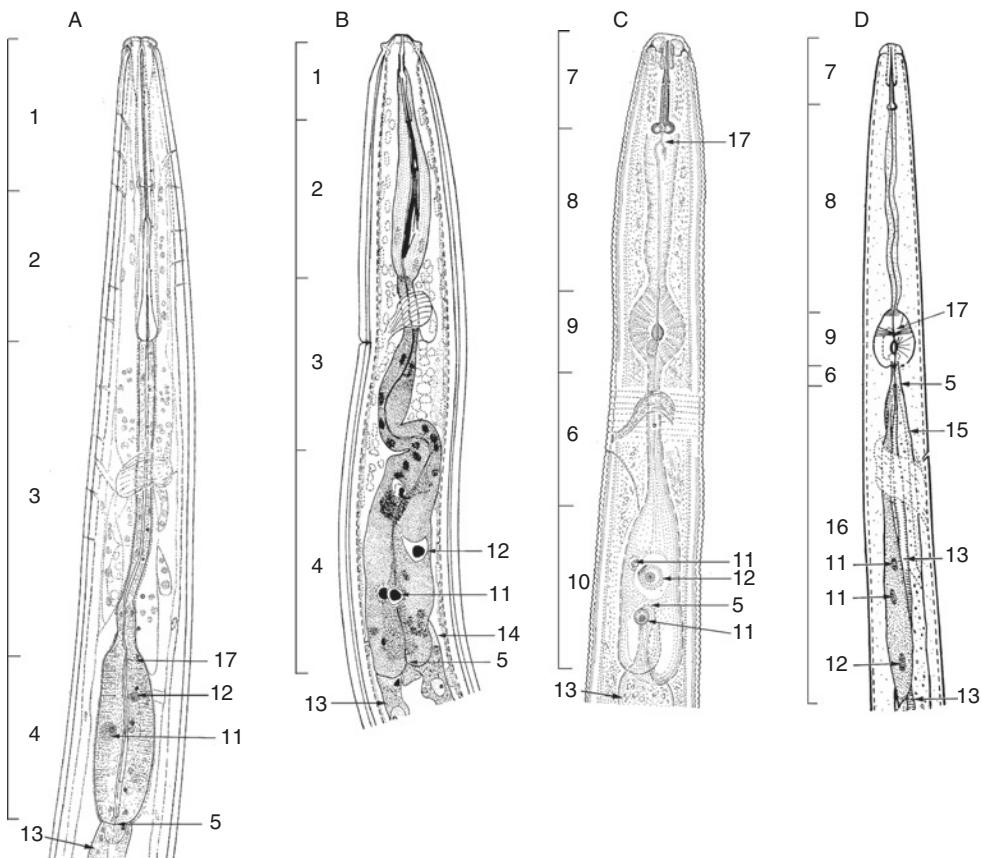


Fig. 1.7. Digestive system of plant-parasitic nematode taxa. A: *Paraphididorus* (Longidoridae). B: *Paratrichodorus* (Trichodoridae). C: *Pratylenchoides* (Pratylenchidae). D: *Aphelenchoides* (Aphelenchoididae). 1: Cheilostome. 2: Pharyngostome. 3: Narrow anterior region of pharynx. 4: Pharyngeal bulb. 5: Pharyngeal-intestinal junction. 6: Isthmus. 7: Stomatostylet. 8: Procorpus. 9: Metacorpus. 10: Post-corpus. 11: Ventrosublateral pharyngeal gland nuclei. 12: Dorsal pharyngeal gland nucleus. 13: Intestine. 14: Intestine dorsally overlapping pharynx. 15: Pharyngeal-intestinal junction valve cell. 16: Pharyngeal lobe. 17: Dorsal pharyngeal gland orifice. A: From Decraemer *et al.* (1998). B: From Decraemer and De Waele (1981). C: From Siddiqi (1986). D: From Shepherd *et al.* (1980).

anteriorly and with its posterior end furcate or simple, and a posterior extension or odontophore, a modification of the anterior pharyngeal region, which acts as a supporting structure for the odontostyle. The base of the odontophore may be enlarged to form three flanges for enhanced insertion of the stylet protractor muscles (Fig. 1.6A). Longidorids possess eight stylet protractor muscles (typical of dorylaims) but true stylet retractor muscles are poorly developed or absent. Pharyngeal retractor muscles at the level of the narrow part of the pharynx assist in retracting the odontostyle. Because of the extreme elongation of the cheilostome, *Xiphinema* species possess a special set of *dilatores buccae* muscles that counteract compression of the cheilostome wall on stylet protraction. In Longidoridae, the odontostyle is long and

needle-like. When it is protruded, the anterior stomatal lining folds along the odontostyle, thereby forming a guiding sheath, the inner cuticular lining being separated from the rest of the wall (except at the level of the guiding ring and at its posterior end) by a fluid-filled cavity that acts as a hydrostatic skeleton. In *Longidorus*, for example, the hydrostatic tissue forms four compensation sacs to regulate the pressure on stylet protraction. The odontostyle is secreted by a cell in the ventrosublateral sector of the pharynx whereas the odontophore, together with the guiding sheath and the pharyngeal lining, is derived from pharyngeal tissue. In Longidoridae, as in all Dorylaimoidea, the replacement odontostyle in the first juvenile stage lies close to the functional one, i.e. its tip is within the lumen of the odontophore. During the first moult, the functional odontostyle is shed together with the body cuticle, cheilstome and inner lining of the anterior stomodaeum. In J2–J4 the replacement odontostyle upon formation moves posteriorly and becomes enclosed in the wall of the pharyngeal isthmus until moulting begins, when the reserve odontostyle moves anteriorly (Radivojeć, 1998). The ventrally curved onchiostyle in Trichodoridae consists of an anterior stylet-like tooth or onchium with a solid tip and a posterior support, the onchiophore, which is formed by the thickened and sclerotized dorsal lumen wall of the pharyngostome (Fig. 1.6C). The protrusion of the onchiostyle occurs upon contraction of protractor muscles of the pharyngostome; there are no retractor muscles. In juveniles, the replacement onchium lies close behind the functional one and is obliquely inserted in the dorsal wall of the odontophore.

The structure of the pharynx itself is related to feeding mode. In Tylenchomorpha, the anterior part of the pharynx (or corpus) is subdivided into an anterior, muscular procorpus and a muscular, larger diameter and more robust metacorpus, which is located posteriorly. The corpus is followed by a non-muscular isthmus (may be very short or absent) and three pharyngeal glands arranged either in a terminal bulb (post-corpus) or in a lobe(s) overlapping the intestine predominantly dorsally, ventrally or laterally (Fig. 1.7C and D). The arrangement of pharyngeal glands is of taxonomic and phylogenetic importance. In the Sphaerularioidea, a group of taxa combining fungus feeding and insect parasitism, the pharynx is simple and lacks differentiation into a procorpus. Species consuming liquid food, such as plant-parasitic tylenchs, have a median bulb or metacorpus with a cuticularized triradiate valvate apparatus attached to well-developed musculature, thereby allowing a stronger pumping action. Within the Tylenchomorpha, the position of the outlet of the dorsal gland is of taxonomic importance and differentiates the Aphelenchoidea (where the outlet is in the metacorpus) from the other taxa of the infraorder (outlet in the procorpus and often close to the stylet base) (Fig. 1.7C and D). In Longidoridae, the pharynx is flask-shaped and posterior to the pharyngostome continues as a narrow flexible tube with a circular lumen followed by an offset, muscular and glandular bulb that is often cylindroid in form (Fig. 1.7A). The terminal bulb in Dorylaimida contains five pharyngeal glands, reduced to three in Longidoridae, and their respective orifi, the cuticular lining of the lumen being reinforced by six platelets. In Trichodoridae, the pharynx consists of a narrow isthmus that gradually expands into a largely glandular basal bulb with five gland nuclei and gland orifi (Fig. 1.7B). The pharyngo-intestinal junction may be directly abutting or characterized by various types of overlap (either a ventral overlap by the pharyngeal glands, or a dorsal overlap by the intestine over the pharynx, or both types of overlap together). In most virus vector trichodorid species, nearly the entire lining of the pharynx anterior to the outlets of the posterior ventrosublateral

glands can act as a retention site for tobraviruses. In longidorid virus vectors, the virus particles adhere to the lumen wall of the odontostyle or inner surface of the guiding sheath (*Longidorus*, *Paralongidorus*) or lumen wall of the odontophore and pharynx (*Xiphinema*). The pharyngo-intestinal junction or cardia exists in many different types. In Tylenchomorpha, the cardia usually consists of two cells, which differ in size and position in relation to the anterior intestine, whereas in Longidoridae and Trichodoridae, the rounded cardia has a triradiate lumen and is small or only weakly developed (Fig. 1.7B and D).

1.2.6.2. Mesenteron

The intestine is entirely of endodermal origin. It is a simple, single layered tube, either cellular or syncytial (criconematids), with or without a clear lumen lined with microvilli. The function of the intestine is mainly absorption (its lumen being bordered with microvilli), storage and secretion of proteins and enzymes. In the evolutionary advanced root parasites, such as *Meloidogyne*, the intestine becomes a storage organ; the thin lumen wall lacks microvilli and loses connection with the anus. Anteriorly, the intestine may be differentiated into a ventricular part that may overlap the pharynx (Trichodoridae) or become modified to house symbiotic bacteria (entomopathogenic *Steinernema* species). Posteriorly, the intestine may be differentiated into a pre-rectum, which is separated from the intestine by a valve-like structure formed by columnar cells (Longidoridae).

1.2.6.3. Proctodeum

The rectum is of ectodermal origin and is lined with body cuticle. It is a very simple short tube and is apparently formed from only a few cells. The junction between the rectum and pre-rectum is guarded by an H-shaped sphincter muscle. Defecation is mediated by the H-shaped *dilator ani*. In criconematids, both rectum and anus are poorly developed and probably non-functional. In *Meloidogyne*, six large rectal glands produce the gelatinous matrix in which the eggs are deposited. In Mononchida and Dorylaimida, males possess rectal glands consisting of three to five pairs of cells located dorsolaterally or laterally from the posterior intestine (pre-rectum). Caudally or dorsocaudally from the spicules the ducts of these rectal glands form loops that run anteriorly between the spicules and open via the dorsal wall of the cloaca (Coomans and Loof, 1986).

1.2.7. Secretory-excretory system

The secretory-excretory (S-E) system is a system of variable complexity (Fig. 1.8). It is often called the ‘excretory system’ on morphological grounds, but physiologically the evidence supports more of a secretory and osmoregulatory function than an excretory one. The S-E system is either: (i) glandular, in general consisting of a renette cell or ventral gland connected to a ventral pore by a duct, the terminal part of which is lined by cuticle; or (ii) tubuloglandular, the most complex system consisting of

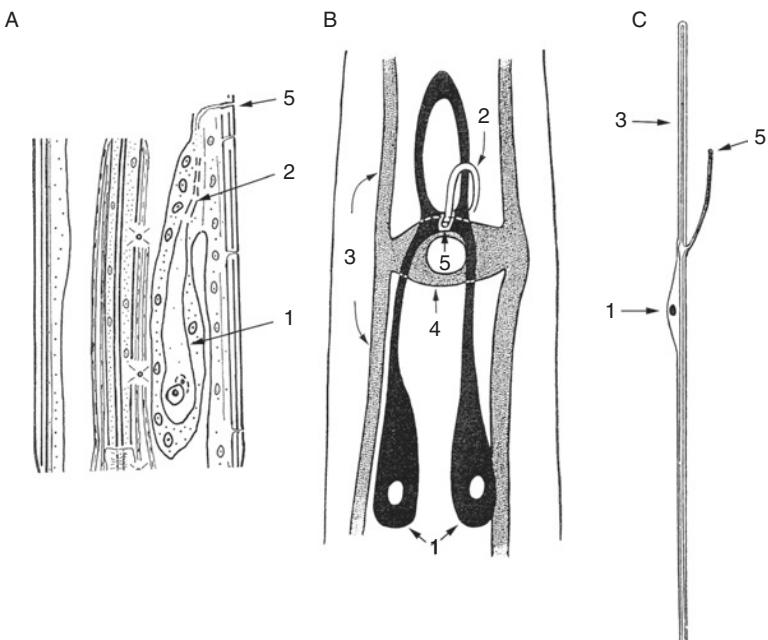


Fig. 1.8. Secretory-excretory system. A: Glandular system in *Longidorus macrosoma* (Longidoridae). B: Complex tubular system in *Caenorhabditis elegans* (Rhabditoidea). C: Simple tubular system in tylenchomorph nematodes. 1: S-E gland cell. 2: S-E duct. 3: Tubular cell. 4: Transverse duct. 5: S-E pore. A: From Aboul-Eid (1969). B: From Nelson *et al.* (1983) in Bird and Bird (1991). C: From de Coninck (1965).

an H-shaped cell with longitudinal canals running in the lateral chords and joined by a transverse duct connected to the S-E pore by a median duct, associated with a S-E sinus and an A-shaped binucleated gland cell (*C. elegans*, Fig. 1.8B). In Tylenchomorpha, the S-E system is of the tubuloglandular type and is asymmetrical with a single renette cell situated laterally or lateroventrally, usually posterior to the pharynx (Fig. 1.8C). The S-E duct leads to the S-E pore, which is usually located posterior to the nerve ring, although exceptionally it may be close to the cephalic region or the vulval region (*Tylenchulus*). In *Tylenchulus*, the renette cell is enlarged and produces a gelatinous matrix into which eggs are deposited. In Trichodoridae, the S-E system is not developed and only a ventral pore and a very short duct are present. In Longidoridae it is not yet clear if a S-E system exists, a glandular structure with two cells being described only from *Longidorus macrosoma* (Fig. 1.8A).

1.2.8. Type of reproduction and reproductive system

Most nematodes are dioecious (having the sexes separate) and gonochoristic (meaning they are either male or female) but bisexual individuals or protandrous hermaphrodites (e.g. *C. elegans*) exist; the latter usually have the appearance of females. Gonochoristic species reproduce by amphimixis or cross-fertilization. Uniparental reproduction or autotoky takes the form of either parthenogenesis, where development occurs through

females producing female offspring (i.e. without fertilization), or automixis or self-fertilization in hermaphrodites (where male and female gametes are produced in the same individual). Parthenogenesis may be either meiotic (following meiosis the diploid chromosome number is restored by fusion with a polar body or by first doubling of the chromosomes), as in *Aphelenchus avenae*, *Pratylenchus scribneri*, *Xiphinema index* or *Meloidogyne hapla* race A, for example, or mitotic (i.e. without meiosis), examples being found in several species of *Meloidogyne* and *Pratylenchus*. In both types of autotoky, males may show up sporadically (i.e. upon environmental stress) and amphimixis may then be possible in some species. Autotoky has arisen independently in several taxa of the phylum. Intersexes are found in some species and should not be confused with hermaphrodites as only one set of reproductive organs is functional, the other being vestigial. Pseudogamy, a way of reproduction intermediate between amphimixis and automixis/parthenogenesis, where development of the egg is activated by a spermatozoon, which then plays no further role, is less common. Sex is mostly determined genotypically, mostly XX in female and hermaphrodites and XO or XX in male, but in some cases the genotype is changed under epigenetic influences.

The reproductive system is quite similar in both sexes and generally comprises one or two (rarely multiple) tubular genital branches (Figs 1.9 and 1.10). Apart from sexual characters, sexual dimorphism is not a common feature among nematodes, but when it occurs it is most evident among parasitic groups, e.g. a swollen saccate female contrasting with the vermiform male (*Verutus*, *Tylenchomorpha*).

1.2.8.1. Female reproductive system (Fig. 1.9)

The basic system is didelphic (i.e. composed of two uteri), amphidelphic (referring to the uteri extending in opposite directions) and connected to a single vagina opening to the outside via a mid-ventral vulva (Fig. 1.9A). A derived system with a single uterus is called monodelphic. A monodelphic system with only the anterior branch developed is described as prodelphic; if it is only the posterior branch that is developed it is called opisthodelphic. The terms monogonic and digonic refer, respectively, to the presence of one or two ovaries. In didelphic systems, the vulva is located at about 50% of the entire body length from the anterior end, although it may be more anterior in monodelphic conditions or virtually subterminal in position as in some monodelphic species or certain obese females. Each genital branch consists of an ovary (= gonad) and a gonoduct. The gonoduct consists of an oviduct and uterus and may have one or two sphincters (valves) and a spermatheca. The spermatheca is either a specialized part of the oviduct or the uterus. Reduction of one of the genital branches is not uncommon, ranging from partial reduction in various degrees (the pseudomonodelphic condition, as in *Xiphinema*) or complete loss, apart from the possible retention of a small post-uterine sac (monodelphic system as in *Criconematidae*). In the didelphic condition, both branches may, in nematodes where the vulva is virtually subterminal (e.g. *Meloidogyne*, *Globodera*), be anteriorly outstretched, i.e. prodelphic.

The ovary is usually a tubular structure, either outstretched (Tylenchomorpha) or reflexed (Longidoridae, Trichodoridae), and consists of three main zones. The blind end usually functions as a germinal zone, the telogonic condition (although primary oocytes may be formed before the last moult), and is followed by the growth zone and ripening zone. In some taxa the oocytes are connected to a central

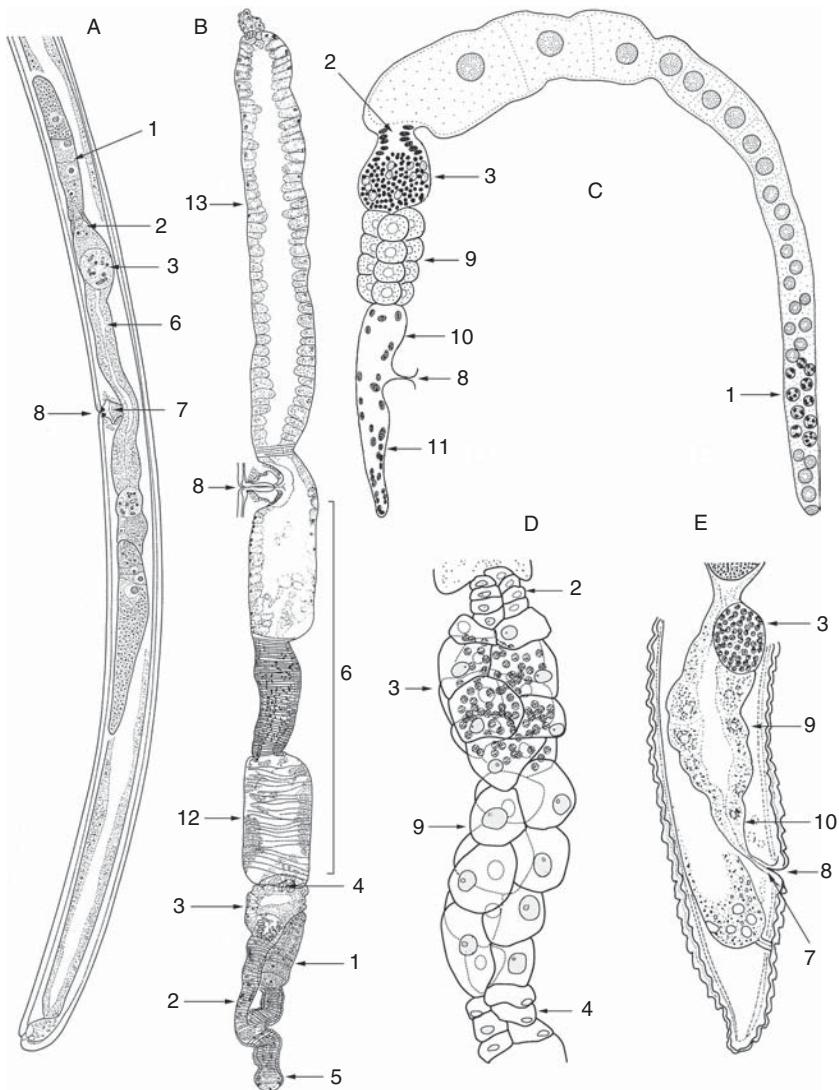


Fig. 1.9. Female reproductive system in plant-parasitic taxa. A: Didelphic-amphidelphic system (*Trichodorus*, Trichodoridae). B: Pseudomonodelphic system (*Xiphinema surinamense*, Longidoridae). C–E: Monodelphic system. C: Total female reproductive system of *Pratylenchus coffeae* (Pratylenchidae). D: Detail of oviduct-uterus region in *Rotylenchus goodeyi* (Tylenchoidea). E: Posterior body region (*Hemicriconemoides minor*, Criconematoidea). 1: Ovary. 2: Oviduct. 3: Spermatheca. 4: Sphincter/value. 5: Ovarial sac. 6: Uterus. 7: Vagina. 8: Vulva. 9: Crustaformeria uterus. 10: Uterine sac. 11: Post-vulval uterine sac. 12: Uterus pars dilata. 13: Reduced anterior branch. 14: Sperm. A: From Decraemer (1991). B: From Decraemer *et al.* (1998). C: From Román and Hirschmann (1969). D: From Bert *et al.* (2003). E: From Decraemer and Geraert (1992).

protoplasmic core or rachis (e.g. *C. elegans*). In species without a rachis, the oocytes are interconnected by protoplasmic bridges.

The oviduct may show great variation in structure and development between taxa, and is considered to be of fundamental importance in nematode systematics

(Geraert, 1983, 2006). In Tylenchomorpha, the oviduct is generally formed by two rows of a few cells (e.g. three or four cells in Tylenchoidea, Criconematoidea and two cells in Aphelenchoididae); the number of cells corresponding to major taxa and being of diagnostic and phylogenetic importance. The tightly packed oviduct cells separate to form a tiny canal when a ripe oocyte is squeezed through. In Trichodoridae, the oviduct consists of two cells, while in Longidoridae the oviduct consists largely of flattened disc-like cells and has a collapsed lumen and a wider part that acts as a spermatheca. The presence or absence of a spermatheca(e) is of diagnostic importance (Trichodoridae). In Tylenchomorpha, the number of cells of the spermatheca, their shape and spatial arrangement is of taxonomic importance (Bert *et al.*, 2003).

The uterus may be a simple tube (Fig. 1.9A) but is usually more complex, being subdivided, for example, into a glandular, a muscular and a non-muscular portion (Fig. 1.9B). In Tylenchomorpha, the uterus *sensu stricto* is restricted to the eggshell-producing region of the gonoduct, the cristaformeria, of which the number and arrangement of cells (tricolumnella, tetracolumnella, quadricolumnella) are of taxonomic importance (Fig. 1.9C and D). In Longidoridae the uterus may vary from very short and simple to very long and complex, with local uterine differentiations such as the Z-organ (*Xiphinema*). The Z-organ probably slows down the descent of the eggs towards the vulva, although its function is not entirely clear. Part of the uterus or uteri opposite the vagina may be differentiated into a muscular ovejector to assist with egg laying. The uterine structure is of taxonomic importance in Longidoridae. The uterus is connected to the vulva by the vagina, which may vary in size and shape (Trichodoridae, Longidoridae) and be of taxonomic importance. The vulva may also vary in shape from pore-like to slit-like (transverse or longitudinal) and may be occluded by a copulation plug (e.g. in *Trichodorus*). Various sets of muscles attach to the vagina, some serving to dilate the lumen while others constrict the vagina or suspend it in the body, thereby preventing it from prolapsing during egg laying. Egg laying is also mediated by the vulval dilators, which connect the vulva to the lateral body wall. Eggs may be laid singly or stuck together in masses in a gelatinous matrix secreted by the female. Such egg masses are associated with species where the females swell and become sedentary, although some obese genera retain all the eggs within the body, the female cuticle tanning on death to form a tough cyst. Egg sacs and cysts serve to protect the vulnerable eggs. Most nematode eggs are morphologically very similar and are ellipsoidal in shape with a transparent shell (except for some animal-parasitic nematodes), and are of similar dimension, irrespective of the size of the adult. Different-sized eggs may occur between free-living and parasitic forms of the same species. The eggshell consists of three main layers: an outer vitelline layer derived from the oolemma and the first layer formed after sperm penetration, a middle chitinous layer, and an inner lipid layer. The lipid layer is largely responsible for the impermeability of the eggshell, which is only permeable to chemicals before the lipid layer is formed during the passage of the egg down the uterus. The permeability of the lipid layer also alters before hatching (see Chapter 7).

1.2.8.2. Male reproductive system (Fig. 1.10)

Nematodes may have a single gonad or testis (monorchic system) or two testes (diorchic system). In Longidoridae, the male reproductive system comprises two testes, the

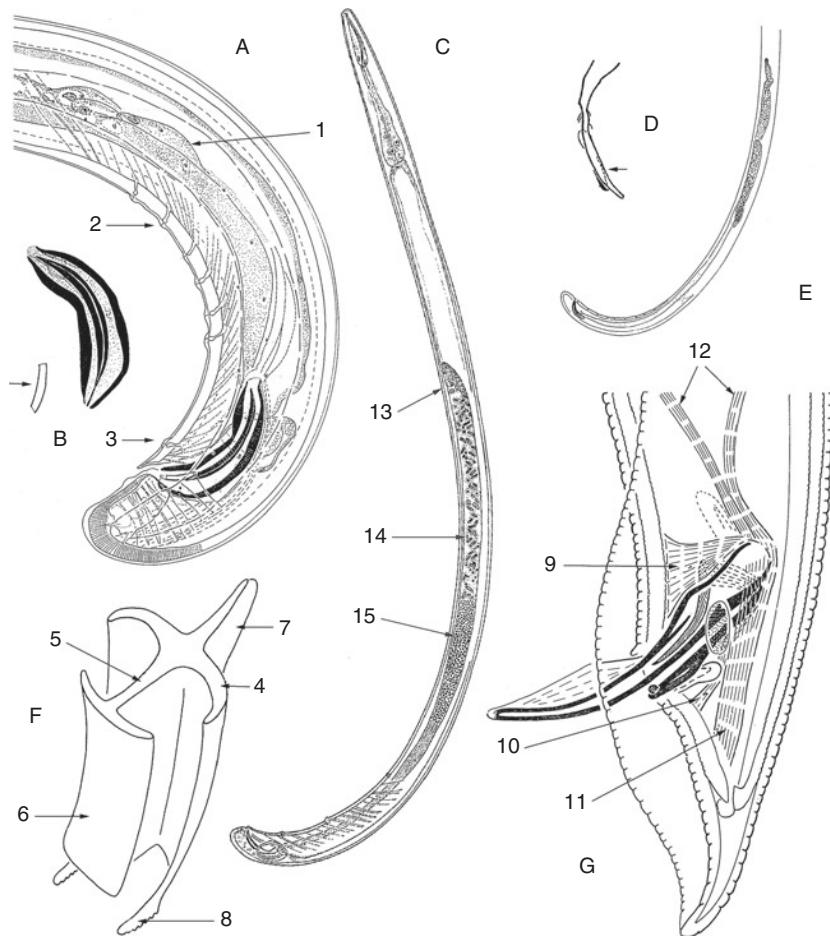


Fig. 1.10. Male reproductive system. A: Posterior body region (*Paraphiphidorus*). B: Spicule and guiding piece or crura (arrow). C: Monorchic system (*Trichodorus*). D: Spicule with velum (arrow). E: Diorchic system (*Xiphinema*). F: Gubernaculum. G: Muscles and copulatory apparatus of *Hoplolaimus*. 1: Ejaculatory gland. 2: Mid-ventral supplement. 3: Adanal supplement. 4: Corpus. 5: Cuneus. 6: Capitulum. 7: Apophysis or apodeme. 8: Crura. 9: Anterior spicule protractor. 10: Gubernaculum protractor. 11: Posterior spicule protractor. 12: Spicule retractor muscles. 13: Germinal zone of testis. 14: *Vesicular seminalis*. 15: *Vas deferens*. A, B: From Decraemer *et al.* (1998). C: From Decraemer (1991). D: From Luc and Comans (1992). F: From Maggenti (1981). G: From Comans (1962).

posterior one being reflexed; the gonoduct consists of a single *vas deferens*, which may be differentiated into a strongly muscular *ductus ejaculatorius* (Enoplia) opening to the cloaca, a common cavity with the outlet of the digestive system. The posterior part of the testis (plural testes) and/or the anterior part of the *vas deferens* may form a *vesicular seminalis* or sperm storage zone. Ejaculatory glands may be associated with the *vas deferens*. Typically, four cells are present along each side of the intestine at pre-rectum level in dorylaims and the ducts merge posteriorly with the *vas deferens*. Such glands are absent in Trichodoridae. Trichodoridae males are monorchic,

as are males of the Tylenchomorpha, except for some *Meloidogyne* males, which occasionally have two testes due to sex reversal during development. Sperm are continuously produced in most plant-parasitic taxa but in genera of the Criconematoidea and some Sphaerularioidea they are produced before the final moult. In such forms the testis appears to be degenerate, although well-developed sperm fill the gonoduct. Sperm size and shape may differ between taxa.

The copulatory apparatus generally consists of two equal, cuticularized, tubular structures called the spicules. Spicules are rarely absent (e.g. the free-living rhabditid *Myolaimus*), may be dimorphic, partly fused (in rhabditids) or reduced to a single spicule (as in some mermithids). Spicules are formed by gradual invagination of the posterior wall of the spicular pouches, which originate from the spicular primordium, specialized cells of the dorsal wall of the cloaca. Each spicule contains sensilla with one or two dendrites and dendritic process or receptor enclosed in a channel leading to a pore or pores near the spicule tip. In several taxa, the spicules are differentiated and show ornamentations such as striae, bristles, a ventral velum or sub-ventral vela (flanges), the presence and form of which are of taxonomic importance. The gubernaculum is a cuticular thickening of the dorsal wall of the spicular pouch and acts as a guide during spicule protrusion. The gubernaculum can be very simple to complex; in Longidoridae only the crura (= lateral guiding pieces) remain (Fig. 1.10B). The copulatory apparatus functions by means of sets of protractor and retractor muscles. There are protractor and retractor muscles for each spicule as well as protractor and retractor muscles attached to the gubernaculum. In Triplonchida (Trichodoridae) the spicule protractor muscles form a capsule of suspensor muscles. Two sets of retractor muscles extend anteriorly and subdorsally from the tip of this capsule and one set runs from tip capsule to the spicule head. Contraction of the retractor muscles pulls the spicules inside the body, whilst contraction of the capsule of the protractor muscles protrudes the spicules. In the caudal region, accessory genital structures such as caudal alae, pre- and post-cloacal supplements, genital papillae or rays on the bursa, setae or suckers may be present. The arrangement of the pre- and post-cloacal supplements (see Longidoridae, Trichodoridae) or genital papillae (Rhabditomorpha) are of taxonomic importance. Paired genital papillae may also be present on the post-cloacal lip (=hypopygium) as in Merliniinae (Tylenchomorpha). Nematode sperm cells are non-flagellate, non-ciliate and show amoeboid motility. They are diverse in size and shape and possess a major sperm protein associated with their unusual motility and correlated with a fibrillar skeleton in mature spermatozoa. Except for Enoplida, mature sperm lack a nuclear envelope.

1.3. Life Cycle Stages

Nematodes typically have an egg stage, four juvenile stages and the adult male and female (Fig. 1.11). The egg is usually ellipsoidal with a chorion/eggshell of varying thickness. Eggs may be deposited singly or in masses. In the latter case they may be held within a gelatinous matrix (root-knot nematodes) or protected within a tough cyst formed from the body of the dead female. Most nematodes moult four times before becoming adult, although there are nematodes, such as certain *Xiphinema* species, that have life cycles shortened to only three moults,

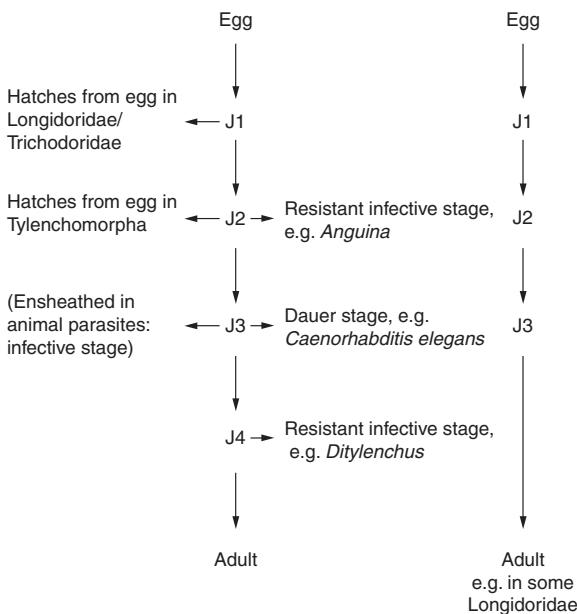


Fig. 1.11. Basic life cycles of plant-parasitic nematodes.

probably due to epogenesis (maturation of gametes before completion of body growth), a phenomenon not uncommon in parthenogenetic organisms. In the Longidoridae and Trichodoridae, the juvenile that hatches from the egg is the first stage or J1, whereas in Tylenchomorpha it is the J2, the first moult occurring within the egg. In some animal-parasitic nematodes, two moults occur within the egg.

Before reaching maturity, the juveniles usually resemble the adult female in morphology, differing in the absence of a mature reproductive system and in certain measurements and proportions. In some groups, one juvenile stage (usually the J3, but may be the J2 or the J4) is more resistant to environmental stress than the other stages (see Chapter 7). This juvenile stage is therefore specialized for dispersal and for surviving inhospitable conditions, and usually represents the infective stage of most animal-parasitic forms. This infective stage is non-feeding and retains the cuticle from the previous stage as a protective sheath around its body. In free-living rhabditid nematodes, this modified stage is often called a dauer larva (from the German for endurance), dauer juvenile or dauer, and may represent an alternative pathway to the normal development process (see Chapter 7). Dauers possess a thicker cuticle, which is often hydrophobic, and are non-feeding, the oral aperture being closed, the non-functional pharynx and intestine reduced in development and the anterior sensilla modified. Many free-living species with dauers are dispersed by insects or other arthropods, the dauers either attaching themselves to the vector or congregating beneath the elytra or within the intersegmental folds. This is known as phoresy, dauers attached to the outside of the insect vectors being ectophoretic whilst those inside the vectors (for example in the Malpighian tubes, intestine, tracheal system or bursa copulatrix) are

endophoretic. When conditions are suitable, the dauer stages can reactivate by absorbing water; the life cycle then continues. The term dauer has also been used for resistant stages of some plant-parasitic nematodes (e.g. J2 of anguinids) although here it does not seem to represent an alternative pathway to the formation of a normal J2.

Nematodes are typically amphimictic and have separate males and females (see Chapter 7). Many species, however, lack males and reproduce either by parthenogenesis (the usual case) or, more rarely, by hermaphroditism. The generation time of nematodes can, depending on the species concerned, vary from a few days to a year or more. Females are usually oviparous, but in some groups juveniles can hatch inside the body of the female (ovoviparity), usually resulting in her death (this is known as endotokia matricida). Ovoviparity can be induced in normally reproducing species by pollutants, e.g. sulphur dioxide (Walker and Tsui, 1968). In some specialized plant parasites, the female body swells and becomes greatly enlarged. The cuticle in some species thickens and tans/darkens on the death of the female to form a tough cyst that surrounds the retained eggs and protects them from deleterious factors, such as drought or extremes of temperature.

1.4. Feeding Groups

Nematodes display a wide range of feeding habits or trophisms. Some species of nematodes are microphagous/microbotrophic, feeding on small microorganisms, while others are saprophagous, feeding on dead and decaying organic matter. Many species of nematodes are phytophagous, obtaining nourishment directly from plants, whilst others are omnivorous or predatory. Parasitism of invertebrates or vertebrates is also common. There are three main types of plant parasitism: ectoparasitic, endoparasitic and semi-endoparasitic.

1. Ectoparasitic – the nematode remains in the soil and does not enter the plant tissues. It feeds by using the stylet to puncture plant cells – the longer the stylet the deeper it can feed within the plant tissues. The majority of ectoparasitic species remain motile whereas others, e.g. *Cacopaurus*, become permanently attached to the root by the deeply embedded stylet.

2. Semi-endoparasitic – only the anterior part of the nematode penetrates the root, the posterior section remaining in the soil phase.

3. Endoparasitic – in this type of parasitism the entire nematode penetrates the root tissue. Migratory endoparasites, such as *Pratylenchus* and *Radopholus*, retain their mobility and have no fixed feeding site within the plant tissue, whereas the more advanced sedentary endoparasites have a fixed feeding site and induce a sophisticated trophic system of nurse cells or syncytia (see Chapter 9). Establishment of a specialized feeding site enhances the flow of nutrients from the host, thereby allowing the females to become sedentary and obese in form and highly fecund. Sedentary endoparasites also have a migratory phase before the feeding site is established. In root-knot and cyst nematodes it is only the J2 and adult male that are migratory but in *Nacobbus*, for example, all juvenile stages, the male and the immature vermiform female are migratory, only the mature female being sedentary.

The above categories are not mutually exclusive as some genera may, depending on the host, be either semi-endoparasitic or migratory ecto-endoparasitic, e.g. *Hoplolaimus* (Tytgat *et al.*, 2000) or *Helicotylenchus*, whilst some sedentary parasites have only the anterior body embedded in the root (= sedentary semi-endoparasites), e.g. *Rotylenchulus*, *Tylenchulus*.

In ectoparasites and most migratory endoparasites, any vermiform stage may feed on, or penetrate, the root, but in those plant-parasitic nematodes where the female becomes obese and sedentary, the infective stage is usually the vermiform J2. This is true for *Heterodera/Globodera* and *Meloidogyne* species, for example, although in *Rotylenchulus* it is the immature female that is the infective stage, the non-feeding juveniles and males remaining in the soil. *Tylenchulus* has a similar life cycle, although here the female juveniles browse on epidermal cells, only the immature female penetrating deeper into the root cortex. In *Nacobbus*, perhaps a less specialized parasite in some ways, all vermiform stages, including the immature female, are infective and may enter and leave roots a number of times. In some Tylenchomorpha the males have a degenerate pharynx and do not feed. Such males are found in *Radopholus*, for example, and occur throughout the Criconematoidea. In *Paratylenchus*, it is the J4 female that lacks a stylet and hence cannot feed on plant cells.

1.5. Classification of Plant-parasitic Nematodes

Nematode classification is currently in a state of flux as molecular phylogenies become increasingly pertinent. For this reason, the higher classification is particularly fluid with proposals for Infraorders, etc., and the bringing together of groups which previously, under classical systematic systems, had been regarded as distantly related (De Ley and Blaxter, 2002). For plant-parasitic nematodes, the most recent classical schemes are those of Siddiqi (1986, 2000), Andrassy (2007) and Manzanilla-López and Hunt (2012) for the Tylenchina, Hunt (1993, 2008), Andrassy (2007, 2009), Kanzaki and Giblin-Davis (2012) and Manzanilla-López and Hunt (2012) for the Aphelenchoidea and/or Longidoridae, and Hunt (1993), Decraemer (1995), Andrassy (2009), Duarte *et al.* (2010) and Manzanilla-López and Hunt (2012) for the Trichodoridae.

In the following scheme, the higher systematic categories are as proposed by De Ley and Blaxter (2002), whilst at subfamily and generic level, a simplification of Siddiqi (2000) and Hunt (1993, 2008) is followed with some genera and many synonyms omitted. The most important plant-parasitic genera are indicated in **bold** type.

It is useful to note that the various systematic ranks have different suffixes attached to the stem:

Class	-ea
Subclass	-ia
Order	-ida
Infraorder	-omorpha
Suborder	-ina
Superfamily	-oidea
Family	-idae
Subfamily	-inae
Tribe	-ini

PHYLUM NEMATODA POTTS, 1932
CLASS CHROMADOREA INGLIS, 1983

Subclass Chromadorea Pearse, 1942

Order Rhabditida Chitwood, 1933

Suborder Tylenchina Thorne, 1949

Infraorder Tylenchomorpha De Ley & Blaxter, 2002

Superfamily Aphelenchoidea Fuchs, 1937¹

Family Aphelenchidae Fuchs, 1937

Subfamily Aphelenchinae Fuchs, 1937

Aphelenchus Bastian, 1865

Subfamily Paraphelenchinae T. Goodey, 1951

Paraphelenchus Micoletzky, 1922

Family Aphelenchoididae Skarbilovich, 1947

Subfamily Aphelenchoidinae Skarbilovich, 1947

Aphelenchoides Fischer, 1894

Schistonchus Cobb, 1927

Subfamily Parasitaphelenchinae Rühm, 1956

Bursaphelenchus Fuchs, 1937

Superfamily Tylenchoidea Örley, 1880

Family Tylenchidae Örley, 1880

Subfamily Tylenchinae Örley, 1880

Aglenchus Andrassy, 1954

Tylenchus Bastian, 1865

Coslenchus Siddiqi, 1978

Filenchus Andrassy, 1954

Subfamily Boleodorinae Khan, 1964

Basiria Siddiqi, 1959

Boleodorus Thorne, 1941

Subfamily Thadinae Siddiqi, 1986

Thada Thorne, 1941

Subfamily Duosulciinae Siddiqi, 1979

Duosulcius Siddiqi, 1979

Malenchus Andrassy, 1968

Miculenchus Andrassy, 1959

Ottolenchus Husain & Khan, 1967

Subfamily Tanzaniinae Siddiqi, 2000

Tanzanius Siddiqi, 1991

Subfamily Ecphyadophorinae Skarbilovich, 1959

Ecphyadophora de Man, 1921

Subfamily Ecphyadophoroidinae Siddiqi, 1986

Ecphyadophoroides Corbett, 1964

Subfamily Atylenchinae Skarbilovich, 1959

Atylenchus Cobb, 1913

Subfamily Eutylenchinae Siddiqi, 1986

Eutylenchus Cobb, 1913

¹ This scheme only covers the plant-parasitic forms. For a complete overview of this group, see Chapter 7.

- Subfamily Tylodorinae Paramonov, 1967
Tylodorus Meagher, 1964
- Subfamily Pleurotylenchinae Andrassy, 1976
Cephalenchus Goodey, 1962
Pleurotylenchus Szczygiel, 1969
- Subfamily Epicharinematiniae Maqbool & Shahina, 1985
Epicharinema Raski, Maggenti, Koshy & Sosamma, 1980
Gracilancea Siddiqi, 1976
- Family Dolichodoridae Chitwood in Chitwood & Chitwood, 1950
 Subfamily Dolichodorinae Chitwood in Chitwood & Chitwood, 1950
Dolichodorus Cobb, 1914
Neodolichodorus Andrassy, 1976
- Subfamily Brachydorinae Siddiqi, 2000
Brachydorus de Guiran & Germani, 1968
- Subfamily Telotylenchinae Siddiqi, 1960
Histotylenchus Siddiqi, 1971
Neodolichorhynchus Jairajpuri & Hunt, 1984
Paratrophurus Arias, 1970
Quinisulcius Siddiqi, 1971
Sauertylenchus Sher, 1974
Telotylenchoides Siddiqi, 1971
Telotylenchus Siddiqi, 1960
Trichotylenchus Whitehead, 1960
Trophurus Loof, 1956
Tylenchorhynchus Cobb, 1913
Uliginotylenchus Siddiqi, 1971
- Subfamily Meiodorinae Siddiqi, 1976
Meidorus Siddiqi, 1976
- Subfamily Macrotrrophurinae Fotedar & Handoo, 1978
Macrotrrophurus Loof, 1958
- Subfamily Merliniinae Siddiqi, 1971
Amplimerlinius Siddiqi, 1976
Geocenamus Thorne & Malek, 1968
Merlinius Siddiqi, 1970
Nagelus Thorne & Malek, 1968
Scutylenchus Jairajpuri, 1971
- Subfamily Belonolaiminae Whitehead, 1960
Belonolaimus Steiner, 1949
Carphodorus Colbran, 1965
Ibipora Monteiro & Lordello, 1977
Morulaimus Sauer, 1966
- Family Hoplolaimidae Filipjev, 1934
 Subfamily Hoplolaiminae Filipjev, 1934
Aorolaimus Sher, 1963
Aphasmatylenchus Sher, 1965
Helicotylenchus Steiner, 1945
Hoplolaimus Daday, 1905

- Scutellonema* Andrassy, 1958
Rotylenchus Filipjev, 1936
Subfamily Rotylenchulinae Husain & Khan, 1967
Acontylus Meagher, 1968
Bilobodera Sharma & Siddiqi, 1992
Rotylenchulus Linford & Oliveira, 1940
Senegalonema Germani, Luc & Baldwin, 1984
Verutus Esser, 1981
Subfamily Heteroderinae Filipjev & Schuurmans Stekhoven, 1941
Betulodera Sturhan, 2002
Cactodera Krall & Krall, 1978
Dolichodera Mulvey & Ebsary, 1980
Globodera Skarbilovich, 1959
Heterodera Schmidt, 1871
Paradolichodera Sturhan, Wouts & Subbotin, 2007
Punctodera Mulvey & Stone, 1976
Vittatidera Bernard, Handoo, Powers, Donald & Heinz, 2010
Subfamily Ataloderinae Wouts, 1973
Atalodera Wouts & Sher, 1971
Bellodera Wouts, 1985
Camelodera Krall, Shagalina & Ivanova, 1988
Cryptodera Colbran, 1966
Ekphymatodera Bernard & Mundo-Ocampo, 1989
Hylonema Luc, Taylor & Cadet, 1978
Rhizonema Cid del Prado, Lownsbery & Maggenti, 1983
Sarisodera Wouts & Sher, 1971
Subfamily Meloidoderinae Golden, 1971
Meloidodera Chitwood, Hannon & Esser, 1956
Subfamily Meloidogninae Skarbilovich, 1959
Meloidogyne Göldi, 1887
Subfamily Nacobboderinae Golden & Jensen, 1974
Bursadera Ivanova & Krall, 1985
Meloinema Choi & Geraert, 1974
Family Pratylenchidae Thorne, 1949
Subfamily Pratylenchinae Thorne, 1949
Apratylenchus Trinh, Waeyenberge, Nguyen, Baldwin, Karssen & Moens, 2009
Pratylenchus Filipjev, 1936
Zygotylenchus Siddiqi, 1963
Subfamily Hirschmanniellinae Fotedar & Handoo, 1978
Hirschmanniella Luc & Goodey, 1964
Subfamily Radopholinae Allen & Sher, 1967
Achlysiella Hunt, Bridge & Machon, 1989
Apratylenchoides Sher, 1973
Hoplotylus s'Jacob, 1960
Pratylenchoides Winslow, 1958
Radopholus Thorne, 1949
Zygradus Siddiqi, 1991

- Subfamily Nacobbinae Chitwood *in* Chitwood & Chitwood, 1950
Nacobbus Thorne & Allen, 1944
- Superfamily Criconematoidea Taylor, 1936
Family Criconematidae Taylor, 1936
Subfamily Criconematinae Taylor, 1936
Bakernema Wu, 1964
Criconema Hofmänner & Menzel, 1914
Lobocriconema De Grisse & Loof, 1965
Neolobocriconema Mehta & Raski, 1971
Ogma Southern, 1914
Subfamily Macroposthoniinae Skarbilovich, 1959
Criconemoides Taylor, 1936²
Discocriconemella De Grisse & Loof, 1965
Xenocriconemella De Grisse & Loof, 1965
Subfamily Hemicriconemoidinae Andrassy, 1979
Hemicriconemoides Chitwood & Birchfield, 1957
- Family Hemicycliophoridae Skarbilovich, 1959
Subfamily Hemicycliophorinae Skarbilovich, 1959
Colbranium Andrassy, 1979
Hemicycliophora de Man, 1921
Subfamily Caloosiinae Siddiqi, 1980
Caloosia Siddiqi & Goodey, 1964
Hemicaloosia Ray & Das, 1978
- Family Tylenchulidae Skarbilovich, 1947
Subfamily Tylenchulinae Skarbilovich, 1947
Trophotylenchulus Raski, 1957
Tylenchulus Cobb, 1913
Subfamily Sphaeronematinae Raski & Sher, 1952
Sphaeronema Raski & Sher, 1952
Subfamily Meloidoderitinae Kirjanova & Poghossian, 1973
Meloidoderita Poghossian, 1966
Subfamily Paratylenchinae Thorne, 1949
Cacopaurus Thorne, 1943
Paratylenchus Micoletzky, 1922
Tylenchocriconema Raski & Siddiqui, 1975
- Superfamily Sphaerularioidea Lubbock, 1861
Family Anguinidae Nicoll, 1935
Subfamily Anguininae Nicoll, 1935
Afrina Brzeski, 1981
Anguina Scopoli, 1777
Diptylenchus Khan, Chawla & Seshadri, 1969
Ditylenchus Filipjev, 1936
Heteroanguina Chizhov, 1980
Indoditylenchus Sinha, Choudhury & Baqri, 1985

² = *Criconemella*, *Macroposthonia* and *Mesocriconema*. Some authors regard *Mesocriconema* as valid based on characters of the labial region and whether the vagina is open or closed.

Litylenchus Zhao, Davies, Alexander & Riley, 2011
Mesoanguina Chizhov & Subbotin, 1985
Nothanguina Whitehead, 1959
Nothotylenchus Thorne, 1941
Orrina Brzeski, 1981
Pseudohalenchus Tarjan, 1958
Pterotylenchus Siddiqi & Lenné, 1984
Safianema Siddiqi, 1980
Subanguina Paramonov, 1967

CLASS ENOPLEA INGLIS, 1983

Subclass Dorylaimia Inglis, 1983

Order Dorylaimida Pearse, 1942

Suborder Dorylaimina Pearse, 1942

Superfamily Dorylaimoidea Thorne, 1935³

Family Longidoridae Thorne, 1935

Subfamily Longidorinae

Australodorus Coomans, Olmos,

Casella & Chaves, 2004

Longidoroides Khan, Chawla & Saha, 1978

Longidorus Micoletzky, 1922

Paralongidorus Siddiqi, Hooper & Khan, 1963

Paraxiphidorus Coomans & Chaves, 1995

Xiphidorus Monteiro, 1976

Subfamily Xiphinematinae

Xiphinema Cobb, 1913

Subclass Enoplia Pearse, 1942

Order Triplonchida Cobb, 1920

Suborder Diphtherophorina Coomans & Loof, 1970

Superfamily Diphtherophoroidea Micoletzky, 1922

Family Trichodoridae Thorne, 1935

Allotrichodorus Rodriguez-M., Sher & Siddiqi, 1978

Ecuadorus Siddiqi, 2002

Monotrichodorus Andrassy, 1976

Nanidorus Siddiqi, 1974

Paratrichodorus Siddiqi, 1974

Trichodorus Cobb, 1913

1.6. Common Morphometric Abbreviations

Nematodes are characterized by a combination of measurements and ratios derived from the various body parts (see Hooper, 1986). Such morphometric characters are usually abbreviated, the most common being listed in Box 1.3. Measurements of, for example, the body, pharynx and tail are taken along the mid-line of the relevant structure (Fig. 1.12). Measurements of the spicule, a

³ There are plant-parasitic forms in other groups (e.g. *Californidorus*, *Longidorella*) although little is known about their importance and they are usually ignored.

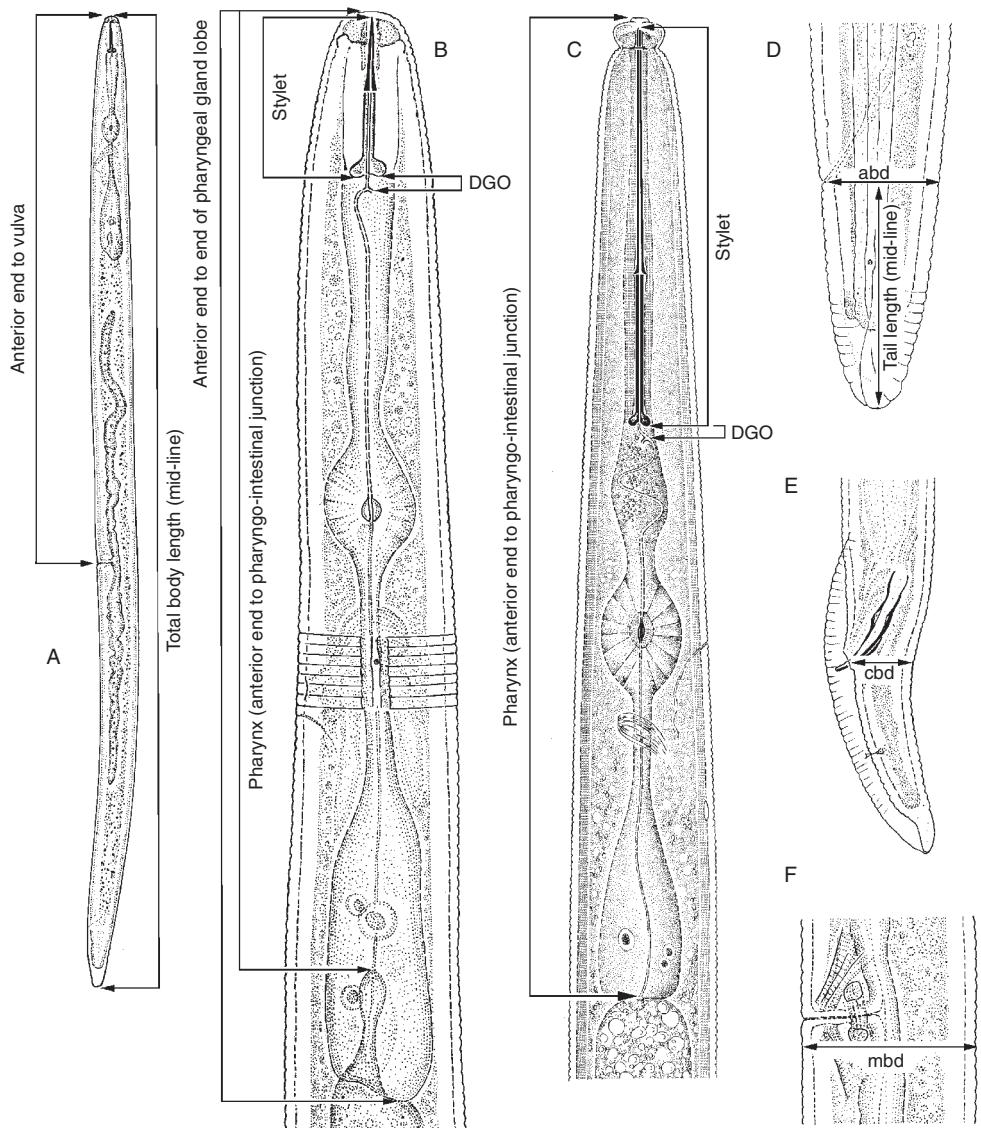


Fig. 1.12. How to take the basic measurements of a nematode. A: Entire nematode showing how to measure total body length along the mid-line from anterior end to tail tip, and distance of vulval aperture from the anterior end, also measured along the mid-line. B, C: Pharyngeal region showing how to measure stylet length, distance of dorsal gland orifice (DOG) from basal knobs, pharynx length (anterior end to pharyngo-intestinal junction) and anterior end to tip of pharyngeal gland lobes (this is not the same as the true pharynx length, but is usually taken in species where the glands overlap the intestine, the pharyngo-intestinal junction itself often being obscure). D: Female tail region showing how to measure tail length, and anal body diameter (abd) by extrapolating a line at 90° to the longitudinal axis. E: Male tail region showing corresponding measurement for body diameter at the cloacal aperture (cbd). Spicule length is measured from tip to tip along the curved median line. F: Mid-body region showing how to take the maximum body diameter (mbd), again at 90° to the longitudinal body axis. Figure digitally compiled from line drawings by Orton Williams (1974) and Siddiqi (1974a), CIH Descriptions of Plant-Parasitic Nematodes, courtesy CABI.

curved structure, are usually taken along the median line (a genuine indicator of actual length), although occasionally (and particularly in aquatic nematodes and in older descriptions) the chord, a straight line joining the two extremities of the spicule, is used. Body diameter should be measured perpendicular to the longitudinal body axis and care should also be taken that the nematode being quantified is not squashed (as a result, for example, of the coverslip not being properly supported with glass rods or beads) as this will produce a higher value than would otherwise be the case. Nematodes that have a long and often fragile tail that may be readily broken, are often measured from the cephalic region to the anal or cloacal aperture instead of to the end of the tail. By removing the error or variable element caused by a long and/or broken tail, ratios resulting from this modified measurement of body length, such as female vulval position, are more consistent and are therefore of greater utility in diagnostics.

Box 1.3. A list of the most commonly used morphometric abbreviations.

L = Total body length (anterior extremity to tail tip).

L' = Body length from anterior end to anal or cloacal aperture (use when the tail is very long and/or frequently in a damaged state).

a = Total body length divided by maximum body diameter.

b = Total body length divided by pharyngeal length (the pharynx is measured from the anterior end to the pharyngo-intestinal junction, i.e. not to the posterior tip of the overlapping gland lobes).

b' = Total body length divided by distance from anterior end of body to posterior end of pharyngeal glands.

c = Total body length divided by tail length.

c' = Tail length divided by body diameter at the anal/cloacal aperture.

V = Position of vulva from anterior end expressed as percentage of body length. Superior figures to the left and right refer to the extent of anterior and/or posterior gonad or uterine sac, respectively, and are also expressed as a percentage of body length.

V' = Position of vulva from anterior end expressed as percentage of distance from head to anal aperture.

T = Distance between cloacal aperture and anteriormost part of testis expressed as percentage of body length.

m = Length of conical part of tylenchid stylet as percentage of total stylet length.

o = Distance of dorsal pharyngeal gland opening posterior to stylet knobs expressed as a percentage of stylet length.

MB = Distance of median bulb from anterior end expressed as a percentage of total pharyngeal length.

Caudal ratio A = Length of hyaline tail divided by its proximal diameter.

Caudal ratio B = Length of hyaline tail divided by its diameter at a point 5 µm from its terminus.

µm = One-thousandth of a millimetre (micron).

2

Molecular Systematics*

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2.1. Phylogenetics and Phylogenomics

The tasks of systematics are: (i) to name, identify and catalogue organisms (taxonomy); (ii) to discover the ancestral relationships among organisms (phylogenetics); and (iii) to organize information about the diversity of organisms into a hierarchical system (classification). Molecular systematics is the application of knowledge of genome information, especially sequence and structure of DNA, RNA molecules and amino acid chains, for addressing questions regarding the phylogeny and taxonomy of organisms.

There are several reasons why molecular data are more suitable for phylogenetic studies than morphological ones. First, DNA and protein sequences are strictly heritable entities, whereas morphological characters can be influenced by various environmental factors. Second, the interpretation of molecular characters, such as the assessment of homologies, is generally easier than that of morphological characters. Third, molecular characteristics generally evolve much more regularly than morphological ones and, therefore, can provide a clearer picture of relationships. Fourth, molecular characters are much more abundant than morphological features, and many can be generated in a relatively short period of time. Various preserved, deformed and partly degraded materials can often be used for molecular studies. Using standard protocols and commercial kits, sequence information of certain genes or DNA fragments can be obtained from a single nematode or even only a part of one. Using specific primers, nematode DNA can be amplified from soil or plant extracts. Moreover, specialized methods enable the extraction of short DNA fragments from long-time preserved, formalin-fixed and glycerine-embedded specimens. Recent achievements in molecular biology and the wide application of molecular techniques have revolutionized our knowledge in taxonomy and phylogeny of nematodes.

The use of such techniques is becoming routine in nematology (Jones *et al.*, 1997; Powers, 2004; Blok, 2005; Perry *et al.*, 2007; Subbotin *et al.*, 2010a,b).

Phylogenetics compares and analyses single or a few genes. However, molecular systematics enters a new era in which many thousands of nucleotides and whole genomes can be obtained inexpensively and in a relatively short period of time. The approach that involves genome data in evolutionary reconstruction is called **phylogenomics**.

2.2. Species Concepts and Delimiting Species in Nematology

There has been considerable debate concerning the definition of a species. Species were at first merely taxonomic units, i.e. the named categories to which Linnaeus and other taxonomists of the 18th century assigned organisms, largely on the basis of appearance. According to the **typological species concept**, the species is considered a community of specimens described by characteristic features of its type specimen. In the early 20th century, taxonomists had accumulated a great deal of evidence leading to the widely used modern concept of species. This species concept was based on two observations: (i) species are composed of populations; and (ii) populations are co-specific if they successfully interbreed with each other. This idea was articulated by E. Mayr (1942) in the **biological species concept**: 'Species are groups of interbreeding natural populations that are reproductively isolated from other such groups'. In the last 50 years several additional species concepts have been proposed. The most popular one in systematics is the so-called **phylogenetic species concept**. This concept does not emphasize the present properties of organisms or their hypothetical future, but rather points at their phylogenetic history. However, the applicability of this concept is debatable, for it proposes operational criteria of how to delimit species only as phylogenetic taxa, rather than describing the role that species play in the living world.

As with any concept, the biological species concept has its limitations. Application of the biological species concept is restricted to sexual, outcrossing populations over a short period of evolutionary time, excluding parthenogenetic organisms. Furthermore, in practice, the diagnosis of biological species is seldom done by testing their propensity to interbreed and produce fertile offspring but is often made by examining the difference in morphology. This should not be a contradiction, because phenotypic characters often, although not always, serve as markers for reproductive isolation. Morphological distinctiveness is a good, but not infallible, criterion for separating species. Sibling species may remain undetected, even after careful morphological examination, unless allozymes or other genetic markers are studied. The genetic difference among related species appears to vary substantially but generally increases with the time elapsed since their reproductive isolation. The degree of the genetic distance among populations, estimated from allozyme frequency, nucleotide divergence, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), microsatellites or other markers, can be evidence to determine whether or not they should be assigned the status of species. These approaches to delimiting species are based on indirect inference of the presence or absence of gene flow.

Another approach for analysis of genetic data deals with tree-based methods. In this approach species are delimited on the properties of a phylogenetic tree, which hypothesizes the relationships of groups bound by monophly, or the shared presence

of apomorphies. These methods can be used to detect asexual species. However, in practice, all of these methods can fail occasionally or be discordant with each other because in nature the process of speciation seems to create diffuse boundaries, or a hybrid zone, between diversifying species. As a result, groups of populations form, sometimes named as subspecies, that are not fully reproductively isolated from each other. Moreover, these methods have different sensitivities and can reflect different properties of speciation. The solution to the problem of delimiting species can be found in the concept of **polyphasic taxonomy and classification** or an integrated approach in systematics.

2.3. Phylogenetics and Classification

Before the 1950s, taxonomists attempted to construct classifications on the dual criteria of common ancestry and their similarities. In the 1950s, the principles of numerical taxonomy were introduced, basing classification not on a few important features but on multiple character data. Numerical methods of analysis were used to create diagrams of overall similarity among species. Such a diagram, called a phenogram, was intended to give an objective basis for classification. This approach was called **phenetics**, giving rise to **phenetic classification**. This approach does not take into account the effects of parallel or convergent evolution in taxonomic interpretations. Another system was based on the argument that classification should rigorously reflect only phylogenetic relationships, not the degree of adaptive divergence or overall similarity. Classifications based on phylogenetic principles are named phylogenetic classifications; only shared, unique character states of similarity provide evidence for phylogenetic relationships. This approach to phylogenetic inference is known as **cladistics**. Branching diagrams constructed by cladistic methods are sometimes called cladograms, and monophyletic groups are called clades. A taxon should be a monophyletic group, originating from a single common ancestor, as opposed to a paraphyletic taxon, which includes only some of the descendants of a common ancestor, or a polyphyletic taxon, whose members share only a distant common ancestor and are usually circumscribed by other characteristics (i.e. **homoplasies**). Several terms are used to describe different character states for taxa under investigation: **plesiomorphy** (ancestral character state), **symplesiomorphy** (shared ancestral character state), **apomorphy** (derived character state), **synapomorphy** (shared derived character state) and **autapomorphy** (derived character state possessed by a single taxon). Within such a framework, the concept of **parsimony** is now widely applied to the reconstruction of phylogenetic relationships. It points out that among the various phylogenetic trees hypothesized for a group of taxa, the best one requires the fewest evolutionary changes, including the fewest homoplasies. Phylogenetic classification must always rely on an inferred phylogenetic tree, which is only an estimated part of a true history of the divergence of a species. In practice, creating a phylogenetic tree to resolve the phylogenetic relation between organisms is not a simple task.

The polyphasic taxonomy, or integrated approach, refers to classifications based on a consensus of all available data and information (phenotypic, genotypic and phylogenetic) used for delimiting taxa at all levels. Such analysis leads to a transition type of taxonomy in which a compromise can be formulated on the basis of results presently at hand.

2.4. Molecular Techniques

Almost all information from the genome and proteome at all levels, including the sequence of fragments of DNA, RNA or amino acids, the structure of molecules, the gene arrangement and presence versus absence of proteins or genes, can be applied to molecular systematics. Various biochemical and molecular techniques have been introduced to nematology for diagnostics, the estimation of genetic diversity of populations and the inference of phylogenetic relationships between taxa. The choice of technique depends on the research question.

2.4.1. Protein-based techniques

These were the first of the molecular techniques to be applied in nematology. Soluble proteins extracted from nematodes are separated on polyacrylamide, starch, and cellulose acetate or agarose gels under an electric field on the basis of different molecular masses. Extracts from nematodes comprise thousands of different proteins but after total staining specific band patterns can be found for each sample. Differences in banding patterns between species or populations may be used as taxonomic markers. Isoelectric focusing (IEF), separating proteins on the basis of their charge in pH gradient, enables more stable profiles to be achieved and resolves proteins into sharp bands. The application of enzyme-staining techniques for characterization of a single protein or small subset of proteins on gels provides another diagnostic method. Extensive characterization of isozymes has been carried out for *Globodera*, *Heterodera*, *Radopholus*, *Meloidogyne*, *Pratylenchus* and other nematode groups. For many groups these studies revealed a wide variation between populations of the same species; however, limited interspecific variation was detected for species of root-knot nematode. The introduction of miniaturized electrophoretic systems, such as the PhastSystem (Pharmacia), has made it possible to study small amounts of soluble protein from a single sedentary female in a fully automatized process. IEF is used as a routine diagnostic technique for *Globodera pallida* and *G. rostochiensis* (Karssen *et al.*, 1995) as well as for the separation of other cyst nematode species (Fig. 2.1). Isozyme phenotypes of adult females, especially of esterase and malate dehydrogenase, are considered to be very useful as reliable markers for identification of root-knot nematodes (see Chapter 3). Because IEF differentiates root-knot nematode species only by specific isozyme patterns of young females, this technique can only be used to separate root-knot nematodes at this life stage.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) provides a better protein separation and fingerprint for any particular sample. In the first dimension, proteins are separated according to their charge; in the second dimension, they are separated on their mass. After staining, the position of individual proteins appears as spots of various size, shape and intensity. This technique has been applied to separate species and populations of *Globodera* and *Meloidogyne*.

2.4.2. DNA-based techniques

Compared with the above approaches, analysis of DNA has several advantages. DNA profiles can be obtained rapidly from a few or even single nematodes and

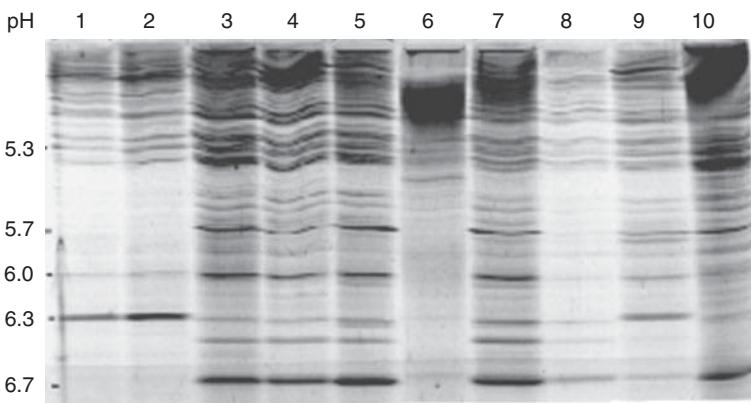


Fig. 2.1. Isoelectric focusing of proteins for species of the *Avenae* group. 1: *Heterodera avenae* (Rinkam, Germany). 2: *H. avenae* (Taaken, Germany). 3: *H. filipjevi* (Chabany, Ukraine). 4: *H. filipjevi* (Chernobyl, Ukraine). 5: *H. filipjevi* (Pushkin, Russia) 6: *H. pratensis* (Putilovo, Russia). 7, 8: *H. filipjevi* (Gorodets, Russia). 9: *H. filipjevi* (Vad, Russia). 10: *H. filipjevi* (Baimak, Russia). (From Subbotin *et al.*, 1996.)

the clarity of the results enables species to be identified very easily without the effects of environmental and developmental variation.

2.4.2.1. DNA extraction

Extraction of DNA is the first step of molecular analysis. Using proteinase K is the most useful, cheap and rapid approach to extracting DNA from nematodes (Waeyenberge *et al.*, 2000). It consists of two steps: (i) mechanical destruction of the nematode body and tissues in a tube using an ultrasonic homogenizer or other tools, or repeatedly freezing samples in liquid nitrogen; and (ii) enzymatic lyses with proteinase K in a buffer for 1 h or several hours with subsequent brief inactivation of this enzyme at a high temperature. Various chemical treatments are also applied to remove cell components and purify the DNA. Phenol or phenol with chloroform extractions is often employed to remove proteins and ethanol is then used to precipitate and concentrate the DNA. Stanton *et al.* (1998) described an efficient method of DNA extraction from nematodes using chemical lyses in alkali solution without prior mechanical breaking of nematode bodies. Effective DNA extraction can also be achieved by using commercial kits developed by various companies.

2.4.2.2. Polymerase chain reaction (PCR)

The polymerase chain reaction (PCR) technique has become one of the most widely used techniques for studying the genetic diversity of nematodes and their identification. PCR is a rapid, inexpensive and simple means of producing relatively large numbers of copies of DNA molecules via an enzyme catalyst. Any DNA fragment can be amplified and detected by PCR. The PCR method requires a DNA template (starting material) containing the region to be amplified, two oligonucleotide primers flanking this target region, DNA polymerase and four deoxynucleotide triphosphates

(dATP, dCTP, dGTP, dTTP) mixed in a buffer containing magnesium ions (Mg^{2+}). A primer is a short, approximately 20-mer, oligonucleotide, which is complementary to the 3' end of each strand of the fragment that should be amplified. Primers anneal to the denatured DNA template and provide an initiation site for the elongation of the new DNA molecule. Universal primers are those complementary to a particular set of DNA for a wide range of organisms; primers matching only to certain species are called species-specific primers. When sequences of the flanking regions of the amplified fragment are unknown, PCR with degenerate primers, containing a number of options at several positions in the sequence that allows annealing and amplification of a variety of related sequences, can be applied.

PCR is performed in a tube in a thermocycler with programmed heating and cooling. The procedure consists of a succession of three steps determined by temperature conditions: (i) template denaturation (95°C for 3–4 min); (ii) primer annealing (55–60°C for 30 s to 2 min), and (iii) extension of the DNA chain (72°C for 30 s to 2 min). PCR is carried out for 30–40 cycles. As the result of PCR, a single target molecule of DNA is amplified into more than a billion copies. The resulting amplified products are electrophoretically separated according to their size on agarose or polyacrylamide gels and visualized using ethidium bromide, which interacts with double-stranded DNA and causes it to fluoresce under UV radiation. Once identified, nematode target DNA generated by PCR amplification can further be characterized by various analyses including restriction fragment length polymorphism (RFLP), dot blotting or sequencing. In some cases, the size of the PCR amplicon may serve as a diagnostic marker for a nematode group or species. It has been shown that primers amplifying the control region of mitochondrial DNA (mtDNA) generate different amplicon sizes for different species of root-knot nematodes; primers amplifying nuclear ribosomal intergenic spacer generated species-specific size polymorphisms for *Meloidogyne chitwoodi*, *M. hapla* and *M. fallax*.

2.4.2.3. PCR-restriction fragment length polymorphism (PCR-RFLP)

Variation in sequences in PCR products can be revealed by restriction endonuclease digestion. The PCR product obtained from different species or populations can be digested by a restriction enzyme, after which the resulting fragments are separated by electrophoresis. If differences in fragment length occur within restriction sites, the digestion of the PCR products will yield restriction fragment length polymorphism (RFLP), i.e. different RFLP profiles. PCR-RFLP of the internal transcribed spacer (ITS) regions of the ribosomal DNA is a very reliable method of identifying many plant-parasitic nematode groups including cyst (Fig. 2.2), root-knot, lesion and gall-forming nematodes, as well as nematodes from the genera *Bursaphelenchus* and *Aphelenchoides*. Using six to nine restriction enzymes enables most of the economically important species of cyst nematodes to be distinguished from each other as well as from their sibling species. RFLP of the ITS-rDNA obtained after restriction with several enzymes and their combination identifies important root-knot nematode species; however, it fails to separate species from the tropical group, including *M. javanica*, *M. incognita* and *M. arenaria*. PCR-RFLP of the mtDNA fragment between cytochrome oxidase subunit II gene and large subunit (LSU) has been applied successfully for diagnostics of these nematodes (Powers and Harris, 1993).

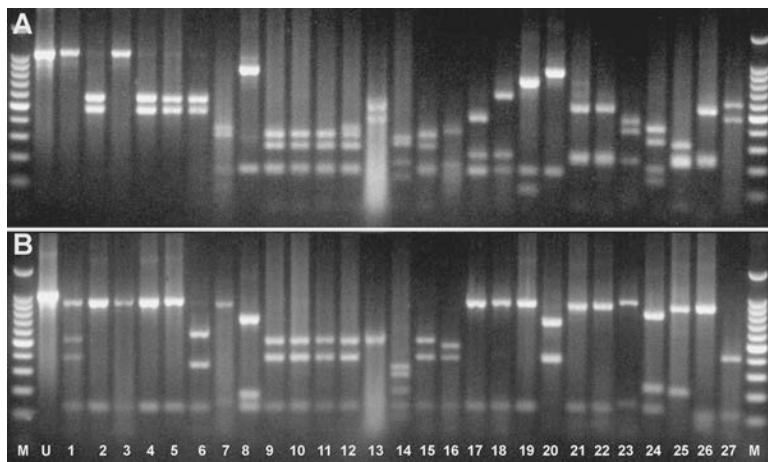


Fig. 2.2. RFLP profile of PCR-ITS rDNA generated by *Alul* (A) and *Bsh1236I* (B) for cyst-forming nematodes. M: 100 bp DNA ladder. U: unrestricted PCR product. 1 and 2: *Heterodera avenae*. 3: *H. arenaria*. 4: *H. filipjevi*. 5: *H. aucklandica*. 6: *H. ustinovi*. 7: *H. latipons*. 8: *H. hordecalis*. 9: *H. schachtii*. 10: *H. trifolii*. 11: *H. medicaginis*. 12: *H. ciceri*. 13: *H. saliphila*. 14: *H. oryzicola*. 15: *H. glycines*. 16: *H. cajani*. 17: *H. humuli*. 18: *H. ripae*. 19: *H. fici*. 20: *H. litoralis*. 21: *H. carotae*. 22: *H. cruciferae*. 23: *H. cardiolata*. 24: *H. cyperi*. 25: *H. goettingiana*. 26: *H. urticae*. 27: *Meloidodera alni*. (From Subbotin *et al.*, 2000.)

2.4.2.4. Multiplex PCR

This type of PCR constitutes a major development in DNA diagnostics and enables the detection of one or several species in a nematode mixture by a single PCR test, decreasing diagnostic time and costs. In multiplex PCR, two or more unique targets of DNA sequences in the same sample are amplified by different primer pairs in the same amplification reaction. Multiplex PCR for detection of a single nematode species uses two sets of primers. One set is to amplify an internal control (e.g. universal primers for D2–D3 expansion regions of the 28S rRNA gene) confirming the presence of DNA in the sample and the success of PCR; the second set, including at least one species-specific primer, is targeted to nematode DNA sequences of interest (Fig. 2.3). Diagnostics using multiplex PCR with species-specific primers have been developed for a wide range of plant-parasitic nematodes.

2.4.2.5. Random amplified polymorphic DNA (RAPD)

This method uses a single random primer of about ten nucleotides long for creating genomic fingerprints. This technique is often used for estimating genetic diversity between individuals, populations or closely related species. In this PCR approach, the short primer anneals to numerous similar sequences within the genome during the annealing step of the PCR cycle, which occurs at a lower temperature than does ‘classical’ PCR. If two complementary sequences are present on

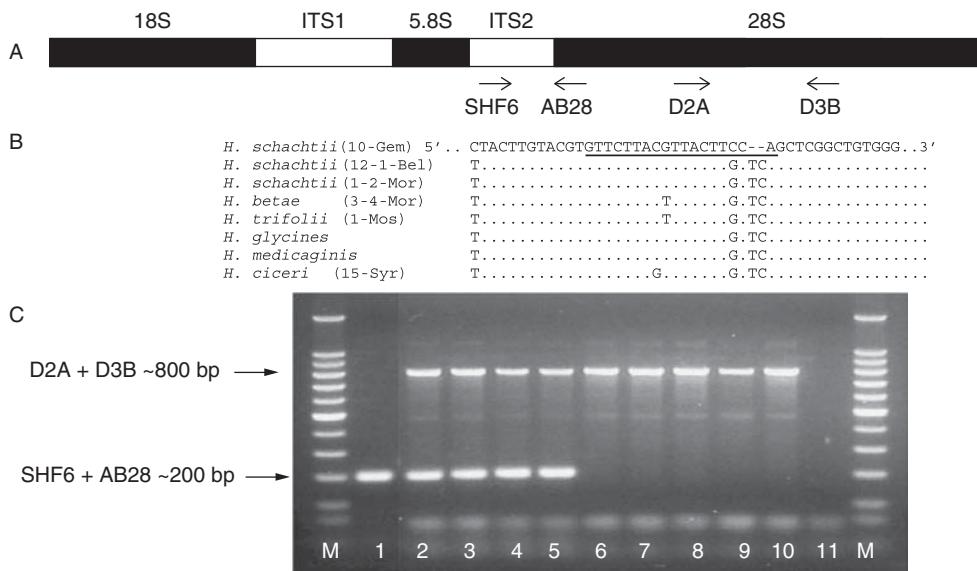


Fig. 2.3. PCR with a species-specific primer for the sugar beet cyst nematode *Heterodera schachtii*. A: Positions of specific (SHF6) and universal primers in the rRNA gene. B: Sequence alignment for *H. schachtii* and closely related species with the sequence of the specific primer underlined. C: Agarose gel with PCR products generated with specific and universal primers (SHF6 + AB28) and universal (D2A + D3B) primers, or control band. 1–5: samples with *H. schachtii*. 6–10: nematode samples without *H. schachtii*. 11: a sample without nematode DNA ladder. M: 100 bp DNA ladder. 1: resultant amplicon obtained with SHF6 + AB28 primer sets; resultant amplicons obtained with SHF6 + AB28 and D2A + D3B primer sets. (Modified from Amiri *et al.*, 2002.)

opposite strands of a genomic region in the correct orientation and close enough to one another, the DNA fragment between them can be amplified by PCR. Amplified DNA fragments obtained using different random primers from different samples are separated on gels and compared. RAPD polymorphisms result from the fact that if a primer hybridization site in a genome differs by even a single nucleotide, the change can lead to elimination of a specific amplification product. The resulting individual bands are considered as equivalent independent characters (Fig. 2.4). The band polymorphism can be binary scored and the data matrix is used for calculating the genetic distance between the samples under study and then presented as a dendrogram. Reproducibility of results is the most critical point for application of this technique.

The RAPD technique has been widely applied for separation of closely related species and studies of intraspecific variability of *G. pallida*, *Heterodera glycines*, *Radopholus similis*, *Ditylenchus dipsaci* and many other species (Powers, 2004; Blok, 2005). Specific sequences for certain species or races, called sequence-characterized amplified regions (SCAR) can be derived from RAPD fragments. Specific pairs of SCAR primers have been designed for identification of *M. chitwoodi*, *M. fallax*, *M. hapla* and other root-knot nematode species (Zijlstra *et al.*, 2000), as well as identification of stem nematodes *D. dipsaci* and *D. gigas*.

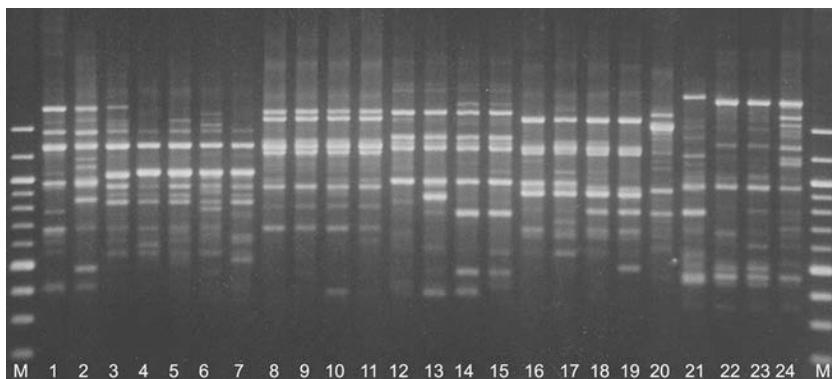


Fig. 2.4. Random amplified polymorphic DNA patterns for 26 populations of the *Heterodera avenae* complex. Primer G-10: 5'-AGGGCCGTCT-3'. 1: *H. avenae* (Taaken, Germany). 2: *H. avenae* (Santa Olalla, Spain). 3: *H. avenae* (Çukurova plain, Turkey). 4: *H. avenae* (Saudi Arabia). 5: *H. avenae* (Ha-hoola, Israel). 6: *H. avenae* (Israel). 7: *H. avenae* (near Delhi). 8: *H. australis* (South Australia, sample 3). 9: *H. australis* (Beulah, Australia). 10: *H. australis* (Victoria, Australia). 11: *H. australis* (Yorke Peninsula, Australia). 12: *H. mani* (Bavaria, Germany). 13: *H. mani* (Heinsberg, Germany). 14: *H. mani* (Andernach, Germany). 15: *H. mani* (Germany). 16: *H. pratensis* (Missunde, Germany). 17: *H. pratensis* (Östergaard, Germany). 18: *H. pratensis* (Lindhöft, Germany). 19: *H. pratensis* (Lenggries, Germany). 20: *H. aucklandica* (Auckland, New Zealand). 21: *H. filipjevi* (Saratov, Russia). 22: *H. filipjevi* (Akenham, UK). 23: *H. filipjevi* (Torralba de Calatrava, Spain). 24: *H. filipjevi* (Selçuklu, Turkey). M: 100 bp DNA ladder (Biolab). (After Subbotin *et al.*, 2003.)

2.4.2.6. Amplified fragment length polymorphism (AFLP)

One of the most popular fingerprinting techniques, AFLP is also a random amplification technique, which does not require prior sequence information. AFLP produces a higher number of bands than is obtained by RAPD. It is a much more reliable and robust technique, unaffected by small variations in amplification parameters; however, it is more expensive. The AFLP technique represents a conceptual and practical advance in DNA fingerprinting. It comprises the following steps: (i) restriction of the total DNA with two restriction enzymes; (ii) ligation of double-stranded adapters to the ends of the restriction fragments; (iii) amplification of a subset of the restriction fragments using two 17–21 nucleotide primers complementary to the adapter and one that is 1–3 nucleotides adjacent to the restriction sites; (iv) separation and visualization of the AFLP-PCR fragments with a variety of techniques, usually on denaturing polyacrylamide gels with further staining. A comparative study of *Globodera* species and populations using AFLP revealed greater inter- and intraspecific variability than obtained by RAPD, and enabled subspecies of *G. tabacum* to be distinguished. AFLP analysis also showed a clear distinction between species of the *D. dipsaci* complex (Fig. 2.5) (Esquibet *et al.*, 2003).

2.4.2.7. Real-time PCR

DNA technology also provides several methods for quantification of nematodes in samples. Real-time PCR requires an instrumentation platform that consists of a thermal

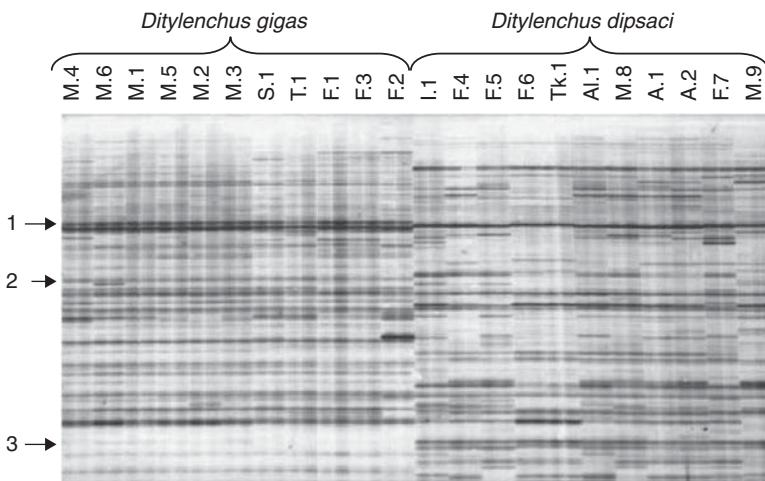


Fig. 2.5. Silver-stained 6% polyacrylamide gel showing AFLP amplification products generated using E-AA and M-CTG primers from 22 populations of *Ditylenchus dipsaci* and *D. gigas*. Two replicates were done for each population. Some polymorphic bands among races or populations are indicated by arrows. 1: *D. gigas*. 2: Population-specific band. 3: *D. dipsaci*. (From Esquibet *et al.*, 2003.)

cycler, optics for fluorescence excitation and emission collection, and computerized data acquisition and analysis software. The PCR quantification technique measures the number of nematodes indirectly by assuming that the number of target DNA copies in the sample is proportional to the number of targeted nematodes. Most of the difficulties with the PCR technique arise because only a very small number of the cycles (4–5 out of 40) contain useful information. The early cycles have an undetectable amount of DNA product; the final cycles, or the so-called plateau phase, are almost as uninformative. Quantitative information in a PCR comes from those few cycles where the amount of DNA grows exponentially from just above background to the plateau. The real-time technique allows continuous monitoring of the sample during PCR using hybridization probes (TaqMan, Molecular Beacons and Scorpions), allowing simultaneous quantification of several nematode species in one sample, or double-stranded dyes, such as SYBR Green, providing the simplest and most economical format for detection and quantification of PCR products in real-time reactions. Compared with traditional PCR methods, real-time PCR has advantages. It allows for faster, simultaneous detection and quantification of target DNA. The automated system overcomes the laborious process of estimating the quantity of the PCR product after gel electrophoresis. Real-time PCR has been used for detection and quantification of *Paratrichodorus pachydermus*, *H. schachtii*, *G. pallida* and *D. dipsaci*, as well as for estimating the number of virus vectoring trichodorid nematodes.

2.4.2.8. Loop-mediated isothermal amplification (LAMP)

LAMP is a novel approach to nucleic acid amplification. The LAMP reaction requires a DNA polymerase with strand displacement activity (*Bst* polymerase) and a set of 4–6

specially designed primers based on distinct regions of the target DNA. Due to the specific nature of the action of these primers, the amount of DNA produced in LAMP is considerably higher than PCR-based amplification. The reaction occurs under isothermal conditions (60–65°C) and yields large amounts of product in a short time (30–60 min). LAMP products can be visualized either by gel electrophoresis or the naked eye by adding DNA intercalating dyes such as ethidium bromide or SYBR Green I in a tube. DNA concentration can also be detected by real-time detection methods. Because LAMP does not require an expensive thermal cycler and optical detection equipment and all LAMP steps are conducted within one reaction tube, this method clearly holds potential for testing in the field or in under-equipped laboratories. LAMP assays have been developed for detection of the pinewood nematode (Fig. 2.6) and common root-knot nematode species.

2.4.2.9. DNA hybridization arrays

DNA arrays provide a powerful method for the next generation of diagnostics. The distinct advantage of this approach is that it combines DNA amplification with subsequent hybridization to oligonucleotide probes specific for multiple target sequences. DNA arrays can be used to detect many nematode species based on differences in the rRNA gene. In general, arrays are described as macroarrays or micro-arrays, the difference being the size and density of the sample spots, the substrate of hybridization and the type of production. Although the potential of DNA array methods for nematological diagnostics has been recognized, little progress had been made in their use,

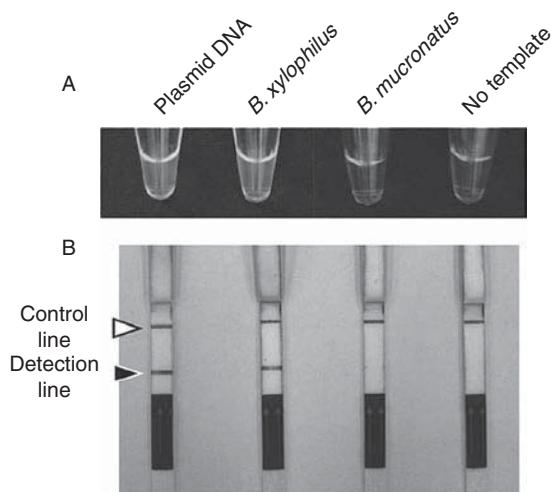


Fig. 2.6. Visual inspection and lateral-flow strips used for the detection of loop-mediated isothermal amplification (LAMP) products. A: LAMP products visualized by fluorescent detection reagent and UV light. B: FAM-labelled probe detected by lateral-flow strips. LAMP reaction was carried out using internal transcribed spacer primers in the presence of plasmid DNA containing the target sequence (positive control) and genomic DNA of *Bursaphelenchus xylophilus* and *B. mucronatus*, and in the absence of template DNA (negative control). (After Kikuchi *et al.*, 2009.)

and only few research papers have been published on this technique (Blok, 2005). A reverse dot-blot assay has been developed for identification of several *Pratylenchus* species using oligonucleotides designed from the sequences of the ITS region of rRNA (Uehara *et al.*, 1999).

2.4.2.10. Sequencing of DNA

The process of determining the order of the nucleotide bases along a DNA strand is called sequencing. Several different procedures have been developed for DNA sequencing, i.e. the chemical degradation (Maxam–Gilbert) method and the chain termination (Sanger dideoxy) method, the latter being more commonly used. The chain termination sequencing method is similar to PCR in that it involves the synthesis of new strands of DNA complementary to a single-stranded template. The sequencing reaction components are template DNA, DNA polymerase with reaction buffer, one primer and the mixture of all four deoxynucleotides (dNTP) and four dideoxynucleotides (ddNTP) labels, each with a different colour fluorescent dye. As all four deoxynucleotides are present, chain elongation proceeds until, by chance, DNA polymerase inserts a dideoxynucleotide. As the dideoxy sugar lacks a 3'-hydroxyl group, continued lengthening of the nucleotide chain cannot occur. Thus, the dideoxynucleotide acts analogously to a specific chain-terminator reagent. Therefore, the result is a set of new chains with different lengths. These fluorescently labelled fragments are then separated by size using capillary electrophoresis. As each label fragment migrates through the gel pass, a laser excites the fluorescent molecule, which sends out light of a distinct colour. The detection system records the chromatogram output on a computer. A computer program (Chromas) then presents the sequencing result as chromatogram sequence files (Box 2.1).

The high demand for low-cost sequencing has driven the development of several high-throughput sequencing technologies that parallelize the sequencing process, producing thousands or millions of sequences at once. One of these is the 454 pyrosequencing technology developed by 454 Life Sciences. Pyrosequencing is based on the ‘sequencing by synthesis’ principle. The method amplifies DNA inside water droplets in an oil solution. A single DNA template attached to a single primer-coated bead then forms a clonal colony. The sequencing machine contains many picolitre-sized wells each containing a single bead and sequencing enzymes. Pyrosequencing differs from Sanger sequencing in that it relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides.

2.5. Genes used for Molecular Systematics

A gene is usually defined as a DNA segment that codes for a polypeptide or specifies a functional RNA molecule. Eukaryotic protein-coding genes consist of transcribed and untranscribed parts, called flanking regions. Flanking regions are necessary for controlling transcription and processing pre-messenger RNA. A pre-mRNA consists of coding regions (exons), which encode amino acids, and non-coding regions containing information necessary for regulation of polypeptide production. Some segments of the non-coding regions (introns) are spliced out in the process of production of a mature mRNA.

Box 2.1. Computer programs

A large amount of software is available for performing phylogenetic analysis. The most comprehensive list of software is given at: <http://evolution.genetics.washington.edu/phylip/software.html>

Packages for manipulation and align of sequences

Chromas (<http://www.technelysium.com.au/chromas.html>) is a program for displaying, editing and exporting chromatogram sequence files.

Clustal (<http://www.clustal.org>) is a package of multiple sequence alignment programs for DNA and proteins. It provides an integrated environment for performing multiple sequence and profile alignments and analysing the results.

BioEdit (<http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) is a biological sequence alignment editor. An intuitive, multiple document interface with convenient features makes alignment and manipulation of sequences relatively easy. Several sequence manipulation and analysis options and links to external analysis programs facilitate a working environment that allows one to view and manipulate sequences with simple point-and-click operations.

General phylogenetic packages

PAUP* (<http://paup.csit.fsu.edu>) is the most sophisticated and user-friendly program for phylogenetic analysis, with many options. It includes parsimony, distance matrix and maximum likelihood (ML) methods.

PHYLIP (<http://evolution.genetics.washington.edu/phylip/>) includes programs to carry out parsimony, distance matrix methods and ML, including bootstrapping and consensus trees. There are programs for data types including DNA and RNA, protein sequences, gene frequencies, restriction sites and restriction fragments, and discrete and continuous characters.

MrBayes (<http://mrbayes.sourceforge.net/>) is a program for the Bayesian estimation of phylogeny. The program uses a Markov chain Monte Carlo (MCMC) technique to approximate the posterior probabilities of trees. One of the program features is the ability to analyse nucleotide, amino acid and morphological data under different models in a single analysis.

MacClade (<http://macclade.org/>) is a program for phylogenetic analysis with analytical strength in studies of character evolution. It also provides many tools for entering and editing data and many descriptive statistics as well as for producing tree diagrams and charts.

Package for selecting models of evolution

ModelTest (<https://code.google.com/p/jmodeltest2/>) is a program for selecting the model of nucleotide substitution that best fits the data.

Packages for tree visualization and tree analysis

TreeView (<http://taxonomy.zoology.gla.ac.uk/rod/treeview.html>) is a simple program for displaying and manipulating phylogenetic trees.

TreeMap (<http://taxonomy.zoology.gla.ac.uk/rod/treemap.html>) is an experimental program for comparing host and parasite trees.

Component (<http://taxonomy.zoology.gla.ac.uk/rod/cpw.html>) is a program for analysing evolutionary trees and is intended for use in studies of phylogeny, tree shape distribution, gene trees/species trees, host-parasite co-speciation and biogeography.

The eukaryotic cell contains two different genomes: that of the nucleus and that of mitochondria. Molecular systematics use data from both genomes. Mitochondria are inherited maternally, whereas the nucleus is biparental. The genome of *Caenorhabditis elegans* is organized as five pairs of autosomal chromosomes (coded I, II, III, IV and V) and a pair of sex chromosomes (X). The nuclear genome of this species is about 100 million base pairs in length, and encodes approximately 20,000 protein-coding genes. The mitochondrial genome of nematodes varies and in *C. elegans* consists of 13,794 base pairs.

To determine true evolutionary relationships between organisms, it is essential that the correct gene fragment or molecule is chosen for sequence studies. This is important for several reasons: (i) the molecule should be universally distributed across the group chosen for study; (ii) it must be functionally homologous in each organism, i.e. the phylogenetic comparisons must start with molecules of identical function; and (iii) it is critical in sequence comparisons to be able to align the molecules properly in order to identify regions of sequence homology and sequence heterogeneity.

2.5.1. Nuclear ribosomal RNA genes

Historically, the only nuclear genes with a high enough copy number for easy study were ribosomal genes. These genes code rRNAs, which are nearly two-thirds of the mass of the ribosome. The genes encoding rRNA are arranged in tandem, in several hundred copies, and are organized in a cluster that includes a small subunit (SSU or 18S) and a large subunit (LSU or 26–28S) gene, which are themselves separated by a small 5.8S gene. The whole set of genes is transcribed as a single unit. Another ribosomal gene, a ubiquitous component of large ribosomal subunits in the eukaryotic cell, is 5S rRNA. The gene is found at different localizations in different organisms. The 5S rRNA gene linked to the intergenic spacers (IGS) regions of rRNA repeated units has been described for several root-knot nematode species.

There are 55 copies of the rRNA genes on chromosome I and 110 5S rRNA genes on chromosome V in *C. elegans*. In addition to these coding sequences, the rDNA array also contains spacer sequences, which contain the signals needed to process the rRNA transcript: an external transcribed spacer (ETS) and two internal transcribed spacers, ITS1 and ITS2. A group of genes and spacer sequences together make up an rRNA transcript unit. These units are separated from each other by an IGS region, also known as a non-transcribed spacer (NTS).

The rRNA (18S and 28S) genes evolve slowly and can be used to compare distant taxa that diverged a long time ago, whereas external and intergenic spacers have higher evolution rates and so have been used for reconstructing relatively recent evolutionary events and for the comparison of closely related species and subspecies. The IGS region contains many repeats and is more variable than the ITS region.

Although rRNA genes are present in many copies, their sequences are almost identical, because the highly repetitive sequences undergo homogenization processes known as concerted evolution. If a mutation occurs in one copy of a sequence, it is generally corrected to match the other copies, but sometimes the non-mutated copies are corrected to match the mutated one, so that nucleotide

changes propagate throughout the arrays. However, this process may be disrupted, so that several different copies of this gene may be present in the genome. The risk of incorporating ITS paralogues into phylogenetic studies should be considered with caution. Inspection of some basic features of the sequence, including the integrity of the conserved motifs and the thermodynamic stability of the secondary structures of the RNA transcripts, enables rRNA pseudogenes to be excluded from the dataset.

2.5.2. Nuclear protein-coding genes

Protein-coding genes have some advantages over rRNA genes and their spacers in that the alignment of sequences is less problematic. Protein sequences also lend themselves to different phylogenetic weighting of bases by codon position. The intron position patterns may also serve as decisive markers for phylogenetic analysis. Heat shock proteins, RNA polymerase II, actin, major sperm protein and other genes have been used for phylogenetic studies of cyst, root-lesion and other nematodes.

2.5.3. Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been used to examine population structure and evolutionary relationships between different nematode groups. All nematode mtDNAs are circular, double-stranded DNA molecules. The mitochondrial genome of the majority of nematodes includes: (i) 12 protein-coding genes, all components of the oxidative phosphorylation system including subunits of cytochrome *c* oxidase (COI-COIII); (ii) 22 transfer RNA genes; and (iii) rRNA genes encoding SSU and LSU rRNAs (Fig. 2.7). In addition, there is usually a non-coding AT-rich region, or a region with high levels of the nucleotides adenine and thymine in the mitochondrial genome containing an initiation site for replication and transcription. The remainder of the approximately 1000 mitochondrial proteins is nuclear-encoded and is imported in the organelle. The arrangement of genes in the mitochondrial genome is not consistent within Nematoda. Nematodes are characterized by a surprising variation in gene order. A unique feature of the mitochondrial genome organization of nematodes is that some of them, e.g. *G. pallida*, contain at least six small circular mtDNA molecules varying in size from 6.3 to 9.5 kb.

Nematode mtDNA sequences accumulate substitution changes much more quickly than the ITS sequences and tend to be extremely A+T rich, with typical values as high as 75 and 80%. The T content seems to be greater at the third codon position, compared with the first and second positions. Although the high rate of substitution makes mtDNA very useful for low-level phylogenetic applications, failure to correct for this severe substitution bias can potentially lead to phylogenetic error.

The relatively rapid rate of evolution and rearrangements that occur in mtDNA has limited the design of universal primers and, thus, mtDNA has not been as widely used as other markers for nematode phylogenetic or diagnostic purposes, except for

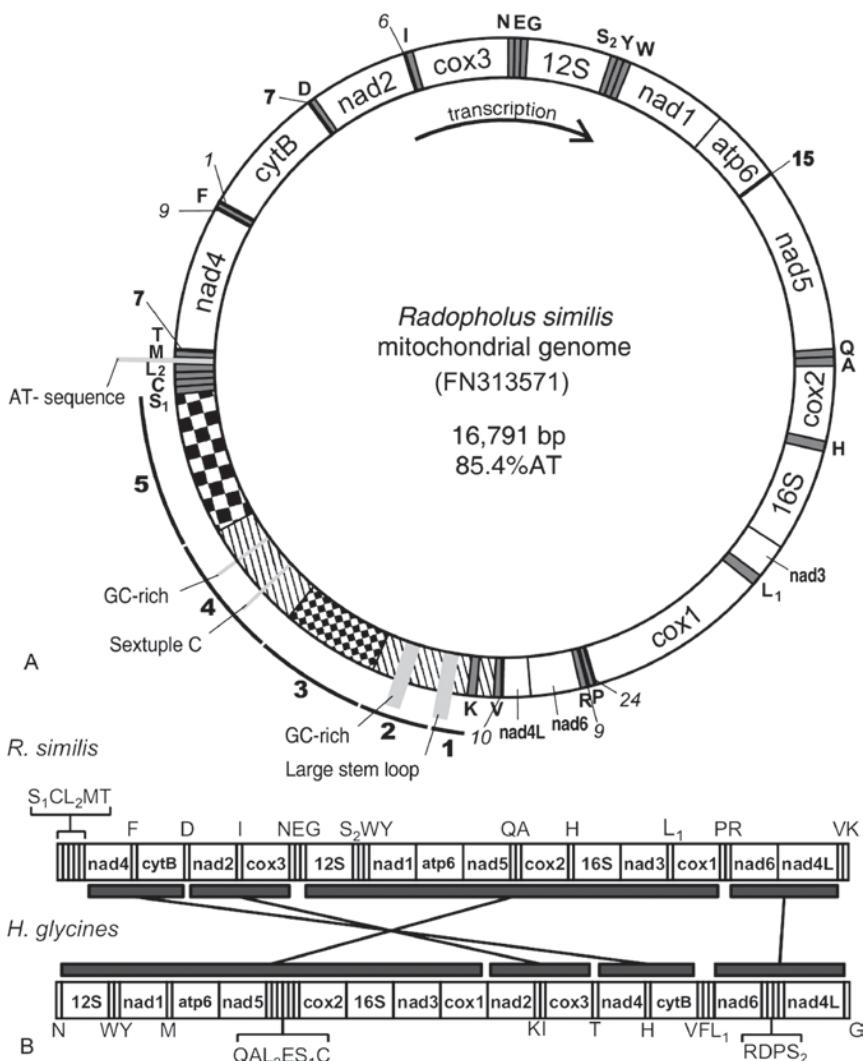


Fig. 2.7. A. Overview of the organization of the circular mitochondrial DNA of *Radopholus similis*. Genes and non-coding regions are indicated: in white, the protein-coding and rRNA genes, in grey, the tRNA genes called by their amino acid symbol (S₁: Ser-AGN, S₂: Ser-UCN, L₁: Leu-CUN, L₂: Leu-UUR). Bold and italic numbers indicate non-coding and overlapping nucleotides between neighbouring genes, respectively. The pattern-filled part represents the large non-coding region. The repeat region of 302 bp is filled with large checkers and the 26 bp repeat region is filled with small checkers. (After Jacob *et al.*, 2009.) B. Partial mitochondrial genome organization of *R. similis* and *Heterodera glycines*. Bars joined by lines indicate regions of conserved genome organization. (After Gibson *et al.*, 2011.)

root-knot nematodes. The region between the COII and the LSU RNA gene containing an intergenic region with unique size and nucleotide polymorphism might be utilized for distinguishing different species and host races of *Meloidogyne* (Powers and Harris, 1993).

2.6. Microsatellites

Microsatellites or simple sequence repeats (SSRs) are short, 1–6 base nucleotide sequences (e.g. AAG) that are repeated many times in tandem (...AAGAAGAAG...). Microsatellites are found in all eukaryotic genomes. Overall, $(AT)_n$, $(AG)_n$, $(CT)_n$, $(AAT)_n$ and $(ATT)_n$ are the most frequent microsatellite motifs presently known in nematode genomes. They are present in both coding and non-coding regions, and found covering from 0.09 to 1.20% of the nematode genomes (Castagnone-Sereno *et al.*, 2010). A very high mutation rate, from 10^{-4} to 10^{-3} mutations per microsatellite and per generation is usually associated with microsatellite loci, resulting in high heterozygosity and the presence of multiple alleles at a given locus. They are present in both coding and non-coding regions and are usually characterized by a high level of length polymorphism. Despite the fact that the mechanism of microsatellite evolution is still unclear, SSRs have been widely used as powerful markers for studies in population genetics. Microsatellites mutate over time, their alleles diverging in the number of sequence repeats. The flanking regions surrounding the microsatellites can be conserved across genera or even higher taxonomic levels and, therefore, are used as designs for PCR primers to amplify microsatellite loci. Based on analysis of the microsatellite variation in populations, inferences can be made about population structures and differences, genetic drift and the date of a last common ancestor (Jarne and Lagoda, 1996).

2.7. DNA Bar Coding

DNA bar coding is a taxonomic method that uses a fragment containing the first half of the COI gene of mtDNA to identify it as belonging to a particular species. It is based on a relatively simple concept: most eukaryote cells contain mitochondria and mtDNA has a relatively fast mutation rate and more differences than the ribosomal genes, which results in significant variance in mtDNA sequences between species and, in principle, a comparatively small variance within species. Molecular bar coding involves isolation of the nematodes (as individuals or in bulk), amplification of the target gene, cloning, sequencing and phylogenetic analysis leading to the assessments of species content, abundance and diversity. However, DNA bar coding is only as good as the reference database: it cannot be used to identify species not already catalogued. Bar coding will also be most reliable for identification of potential putative new species, but only for species groups whose genetic diversity has been well surveyed. DNA bar coding cannot replace the traditional methods of species description. Currently, there is insufficient information in databases for extensive nematode species identification based on the COI gene. However, the increasing deposition of DNA sequences in GenBank and NemATOL databases will be beneficial for diagnostics.

2.8. Phylogenetic Inference

Phylogenetic analysis is a complex field of study that embraces a variety of techniques that can be applied to a wide range of evolutionary questions. However, a

complete understanding of all assumptions involved in analysis is essential for a correct interpretation of the results. A possible work flow of a molecular phylogenetic project could be presented as a flow diagram: (i) selection and sampling of a group of organisms; (ii) choice of molecular markers; (iii) sequencing and assembling of sequence data; (iv) alignment, or establishment of homology between molecules; (v) construction of phylogenetic tree using distance or discrete methods and making an assessment of the reliability of its branches; and (vi) testing of different alternative hypotheses.

2.8.1. Alignment

The first step of any phylogenetic study is the construction of alignment or establishment of positional homology between nucleotides or amino acid bases that have descended from a common ancestral base. Errors incurred in this step can lead to an incorrect phylogeny. The best way to compare the homologous residues is to align sequences one on top of another in a visual display, so that, ideally, each homologous base from different sequences line up in the same column. Three types of aligned pairs are distinguished: (i) **matches** (same nucleotide appears for all sequences); (ii) **mismatches** (different nucleotides were found in the same position); and (iii) **gaps** (no base in a particular position for at least one of the sequences). A gap indicates that a deletion has occurred in one sequence or an insertion has occurred in another sequence. However, the alignment itself does not enable these mutational events to be distinguished. Therefore, insertions and deletions are sometimes collectively referred to as **indels**. For closely related species the optimal alignment of sequences having the same length can easily be done manually; for distantly related organisms, where many deletion or insertion mutations have occurred, alignments are usually constructed using computer programs with particular algorithms.

The optimal automatic alignment is considered to be that in which the numbers of mismatches and gaps are minimized according to the desired criteria. The program Clustal (Box 2.1) is one of the most commonly used computerized alignment programs using a progressive alignment approach. Sequences are aligned in pairs to generate a distance matrix, which then is used for calculating a neighbour-joining guide tree. This tree gives the order in which progressive alignment should be carried out. Progressive alignment is a mathematical process that is completely independent of biological reality. The use of structural components of the given molecule can significantly improve estimations of homology, thus generating a better alignment.

2.8.2. Methods for inferring phylogenetic trees

The methods for constructing phylogenetic trees from molecular data can be categorized into two major groups: (i) distance methods; and (ii) discrete methods. In distance methods such as analysis by minimum evolution, sequences are converted into a distance matrix that represents an estimate of the evolutionary distances between sequences, from which a phylogenetic tree is constructed, by considering the relationships among these distance values, which are supposed to represent distances between

taxa. In discrete methods, maximum parsimony, maximum likelihood (ML), Bayesian inference methods map the history of characters onto a tree. Each method requires some assumptions about evolution.

2.8.2.1. Minimum evolution method

The minimum evolution method is very useful for analysing sequences. In this method, the sum of all branch lengths is computed for all plausible trees and the tree that has the smallest sum value is chosen as the best tree. The neighbour joining (NJ) method applies the minimum evolution principle and estimates the tree based on data transformed into a pairwise distance matrix. This method does not examine all possible topologies but at each stage of taxon clustering a minimum evolution principle is used. The NJ algorithm is extremely popular because it is relatively fast and performs well when the divergence between sequences is low.

2.8.2.2. Maximum parsimony

Maximum parsimony is an important method of phylogenetic inference. The goal of parsimony is to find the tree with the minimum total tree length, or the minimum amount of evolutionary changes, i.e. the transformation of one character state to another. The better a tree fits the data, the fewer homoplasies will be required and the fewer number of character state changes will be required. Several different parsimony methods have been developed for treating datasets. The problems of finding optimal trees under the maximum parsimony criterion are twofold: (i) determining the tree length; and (ii) searching over all possible trees with the minimum length. When the number of taxa is small, it is possible to evaluate each of the possible trees, or to conduct an exhaustive search. An exhaustive search is carried out by finding each of the possible trees by a branch-additional algorithm. However, if the number of trees is large, the application of this approach is near impossible, and a heuristic strategy is used.

As with any method, maximum parsimony has its pitfalls. If some sequences have evolved much faster than others, homoplasies have probably occurred more often among the branches leading to these sequences than in others, so that parsimony tends to cluster these highly divergent branches together. This effect, called long-branch attraction, can be reduced by sampling additional taxa related to those terminating the long branches, so that the branches may be broken up into smaller ones.

2.8.2.3. Maximum likelihood

Maximum likelihood (ML) is the method that is generally considered to make the most efficient use of the data to provide the most accurate estimates of phylogeny. The likelihood is not the probability that the tree is the true tree; rather it is the probability that the tree has given rise to the data that were collected. The basic idea of the likelihood approach is to compute the probability of the observed data assuming it has evolved under a particular evolutionary tree and a given probabilistic model of

substitution. The likelihood is often expressed as a natural logarithm and referred to as the log-likelihood. The tree with the highest likelihood is the best estimate of the true phylogeny. The main obstacle to the widespread use of ML methods is computing time, because algorithms that find the ML score must search through a multidimensional space of parameters to find a tree. ML requires three elements: a model of sequence evolution, a tree and the observed data.

2.8.2.4. Bayesian inference

Bayesian inference of phylogeny is based on a quantity called the posterior probability of a tree. The posterior probability of a tree can be interpreted as the probability that the tree is correct. The posterior probability involves a summation over all trees and, for each tree, integration over all possible combinations of branch length and substitution model parameter values. This method is almost impossible to complete by exhaustive analysis, and so the Markov chain Monte Carlo (MCMC) is a search method used to approximate the posterior probabilities of trees. ML and Bayesian analysis are both based upon the likelihood function, although there are fundamental differences in how the two methods treat parameters.

2.8.2.5. Evolutionary models

In order to reconstruct an evolutionary tree some assumptions about the evolutionary process for the studied molecules should be made. Evolutionary substitution models for DNA are implemented in a different way in distance, ML and Bayesian analysis. The substitution model is a description of the way sequences evolved in time by nucleotide replacements. The nucleotide substitution process of a DNA sequence can be described by a so-called homogeneous Markov process that uses the Q matrix, which specifies the relative rates of change of each nucleotide along the sequences. The Jukes–Cantor model (JC69) was one of the first proposed and is perhaps the simplest model of sequence evolution. It assumes that the four bases have equal frequencies, and that all substitutions are equally likely. The general time-reversible model (GTR) is the most general model, where all eight free parameters of reversible nucleotide rate Q matrix are specified. The best-fit model of evolution for a dataset can be selected through statistical testing. The fit to the data of different models can be compared through likelihood ratio tests (LRTs) or information criteria to select the best-fit model within a set of possible ones.

2.8.3. Phylogenetic tree and tree terminology

The result of a molecular phylogenetic analysis is expressed in a phylogenetic tree. A tree consists of nodes, which are connected by branches. The branch length usually represents the evolutionary distance between two consecutive nodes. Terminal nodes (leaves) are often called operational taxonomic units (OTUs). Internal nodes represent hypothetical ancestors and may be called hypothetical taxonomic units (HTUs). The ancestor of all the taxa that comprise the tree is the root of the tree. An outgroup

is a terminal taxon whose most recent common ancestor with any taxon within a given clade occurs at a node outside that clade. The OTUs within a given clade are called ingroup taxa. A group of taxa that belong to the same branch is called a cluster. Sister groups or sister taxa refer to two groups on a tree with the same immediate common ancestor, or are more closely related to each other than either is to any other taxon. The branching patterns, or the order and arrangement of nodes, are collectively called the topology of the tree. If three branches connect to an internal node, then the node represents a bifurcation, or dichotomy. If more than three branches connect to an internal node, then the node represents polytomy. A tree implicitly assumes that once two lineages appear, they subsequently never interact with each other. However, in reality such interactions might have occurred, such as through hybridization (rare in animals) or reticulate evolution, and such relationships can be presented as a network.

Parsimony analyses often arrive at multiple trees with the same length but with different branch order. Rather than choosing among these trees, systematists may simply want to determine what groups can be found in all the shortest trees. There are approaches to summarizing information which are common to two or more trees in a single tree. The resulting tree is called a **consensus tree**. A **strict consensus tree** shows only those relationships that were hypothesized in all the equally parsimonious trees, whereas a **majority consensus rule tree** shows those relationships hypothesized in more than half the trees being considered.

2.8.4. Evaluation of the reliability of inferred trees

The estimation of phylogeny should be accompanied by an indication of its confidence limit. Phylogenetic trees should always be evaluated for reliability, which could be measured as the probability that the taxa of a given clade are always members of that clade. Bootstrap and jack-knife analyses are the techniques used most often for this purpose. Bootstrapping and jack-knifing are so-called re-sampling techniques, because they estimate the sampling distribution by repeatedly re-sampling data from the original dataset. These methods differ in their methods of re-sampling. Bootstrapping is the more commonly used approach for phylogenetic reconstruction. To estimate the confidence level by bootstrapping, or the bootstrap value of a clade, a series of pseudo samples or pseudo alignments is first generated by randomly re-sampling the sites in the original alignment with replacement. In such pseudo alignments some characters are not included at all, while others may be included twice or more. Secondly, for each pseudo alignment, a tree is constructed, and the proportion of each clade among all the bootstrap replicates is computed in a majority-rule consensus tree. If the value of support of the clade obtained as a result of these analyses is greater than 95%, the branch is considered to be statistically significant. Branch support less than 70% should be treated with caution.

Confidence in maximum parsimonious trees can also be evaluated by calculating the decay index, or Bremer support, which expresses the number of extra steps required for each node not to appear in the tree, i.e. the length difference between the shortest trees including the group and the shortest trees that exclude this group. The higher the decay index, the better the support for the group.

2.8.5. Testing of hypotheses

Once phylogenetic trees are obtained from a molecular dataset using different methods, they should be compared with each other or with trees generated from other, non-molecular datasets. There are several tests that allow the evaluation of alternative hypotheses and determine whether one tree is statistically worse than another: the Templeton test, Shimodaira–Hasegawa test and parametric bootstrapping.

2.9. Reconstruction of Historical Associations

Historical associations, when a lineage tracks another lineage, can be divided into three basic categories: (i) genes and organisms; (ii) organisms and organisms; and (iii) organisms and areas (Page and Charleston, 1998). In each association, one entity tracks the other with a degree of fidelity that depends on the relative frequency of four events: co-divergence, duplication, horizontal transfer and sorting event. The testing of hypotheses for co-divergence could be made using tree topology or data-based methods. Both methods are not without problems, and should rely on the estimation of inferred phylogenies or adequacy of models of sequence evolution.

Genes and organisms. Each gene has a phylogenetic history that is intimately connected with, but not necessarily identical to, the history of the organisms in which the gene resides. Processes such as gene duplication, horizontal gene transfer, gene loss and lineage sorting can produce complex gene trees that differ from organismal trees.

Organisms and organisms. Several associations between nematodes and other organisms are known, e.g. nematodes of the Heteroderidae or Anguinidae and their host plants; nematodes from the *Xiphinema americanum* group and symbiotic bacteria of the genus *Xiphinematabacter*; seed gall forming nematodes of the genus *Anguina* and host plants or bacteria from the genus *Rathayibacter*; and soil-inhabiting nematodes and parasitic bacteria of the genus *Pasteuria*. The phylogenetic tests for co-divergence have been made for associations: anguinid nematodes and their host plants and soil-inhabiting nematodes and parasitic *Pasteuria*. Molecular data suggest that anguinid groups are generally associated with host plants from the same or related systematic groups. Although the strict co-speciation hypothesis for seed-gall nematodes and grasses was rejected, the analysis showed a high level of co-speciation events, which cannot be explained as a result of random establishments of host–parasitic association. Analysis of phylogenies of *Pasteuria* and their nematode hosts suggest that horizontal host switching is the most common event in this association (Sturhan *et al.*, 2005).

Organisms and areas. Organisms can track geological history such that sequences of geological events are directly reflected in the phylogenies of those organisms. Phylogeography is the study of the historical processes (vicariance, sympatry, dispersal and extinction) that take place in this association (Page and Charleston, 1998). Studies of the geographical distributions of genealogical lineages within and between species of some genera give interesting views on origin and dispersal of some nematode groups (Fig. 2.8).

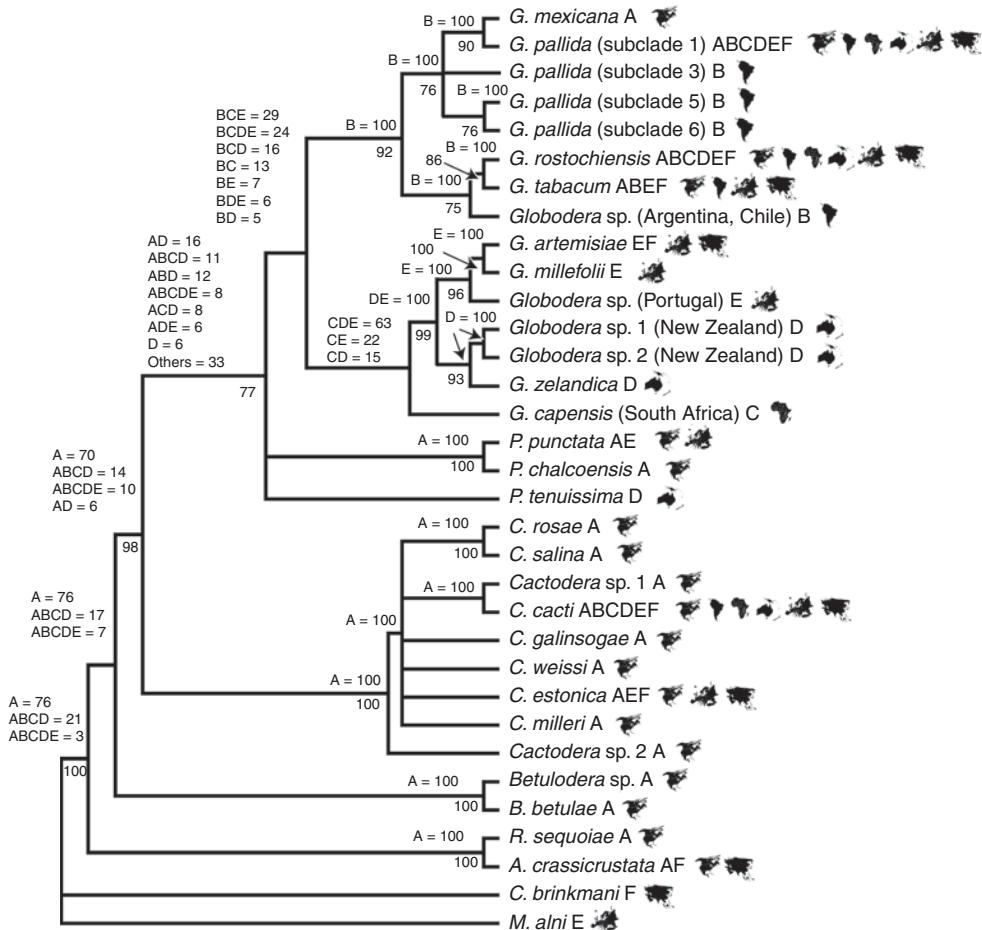


Fig. 2.8. Phylogeny of *Globodera* and *Punctoderinae* *sensu* Krall & Krall, 1978. Strict consensus of 28 maximum parsimony trees obtained from analysis of the ITS rRNA gene sequences. Letters at nodes indicate putative ancestral areas with frequency of occurrence of the node: A: North America, B: South America, C: Africa, D: Oceania (New Zealand), E: Europe. Letters to the right of taxon name indicate present distribution and thus coding for the ancestral area analysis. Phylogeographic analysis suggested a North American origin of *Punctoderinae* with possible further long-distance dispersal to South America, Africa and other regions. (From Subbotin *et al.*, 2011.)

2.10. Databases

Phylogenetic analyses are often based on data accumulated by many investigators in different databases. All novel sequences have to be submitted in a public database. Databases are essential sources for modern bioinformatics, as they serve as information storage equipped with powerful query tools and a well-developed system of cross-references (Box 2.2).

Box 2.2. Databases.

Numerous genetic databases are spread out all over the world. The biggest public databases containing nucleotide sequence information are as follows: **GenBank** (National Center for Biotechnology Information, USA) (<http://www.ncbi.nlm.nih.gov>), **EMBL** (European Molecular Biology Laboratory) (<http://www.ebi.ac.uk/embl>) and **DDBJ** (DNA Data Bank of Japan) (<http://www.ddbj.nig.ac.jp>). Exchange of data between these international collaborating databases occurs on a daily basis.

TreeBASE (<http://www.treebase.org>) is a relational database designed to manage and explore information on phylogenetic relationships. Its main function is to store published phylogenetic trees and data matrices. It also includes bibliographic information on phylogenetic studies, and some details on taxa, characters, algorithms used and analyses performed.

There are several specialized nematode databases:

WormBase (<http://www.wormbase.org>) is the central data repository for information about *Caenorhabditis elegans* and related nematodes. As a model organism database, WormBase extends beyond the genomic sequence, integrating experimental results with an extensively annotated view of the genome. WormBase also provides a large array of research and analysis tools.

NemaGene (<http://www.nematode.net>) is a web-accessible resource for investigating gene sequences from nematode genomes. The database is an outgrowth of the parasitic nematode expressed sequence tags (EST) project. ESTs are usually shorter than the full-length mRNA from which they are derived and are prone to sequencing errors. The database provides EST cluster consensus sequence, enhanced online BLAST search tools and functional classification of cluster sequences.

NEMBASE4 (<http://www.nematodes.org/nembase4/>) is a resource for nematode transcriptome analysis, and a research tool for nematode biology, drug discovery and vaccine design. Users may query the database on the basis of BLAST annotation, sequence similarity or expression profiles.

NemAToL (<http://nematol.unh.edu/>) is an open database dedicated to collecting, archiving and organizing video images of other morphological information, DNA sequences, alignments, and other reference materials for study of the phylogeny and diversity, and taxonomy, systematics and ecology of nematodes.

2.11. Examples of Molecular Phylogenies

2.11.1. Position of Nematoda within metazoans

The relative position of nematodes in animal phylogeny remains uncertain. In the traditional, morphologically based view, bilateral organisms are subdivided according to their internal organization and emerged in a universal phylogenetic tree with the following order: (i) the Acoelomata, lacking a body cavity (mainly the platyhelminths and nemertines); (ii) the Pseudocoelomata (nematodes and some other minor phyla), with an internal cavity outside the mesoderm; and (iii) the Coelomata,

which have true coelomic cavities splitting the mesoderm. Another vision of the phylogenetic relationships between metazoan phyla was obtained after analysis of molecular data. The concept is known as the Ecdysozoa hypothesis, grouping moulting organisms, including arthropods and nematodes, in a single clade based on analysis of 18S rRNA gene sequences and recently also supported by studies of the 28S rRNA. By contrast, the phylogenetic analyses using many protein-coding genes showed the strongest and most consistent support for the coelomate topology.

2.11.2. The phylum Nematoda

Since the first publications of general phylogenies of nematodes based on 18S rRNA in 1998, the massive nematode rRNA database has been accumulated. The analysis of the phylum highlights a number of paraphyletic taxa and indicates new relationships between previously unconnected taxa. The Nematoda seems to have arisen from adenophorean ancestry; the classic split into Adenophorea and Secernentea is not supported. Holterman *et al.* (2006) presented a subdivision of the phylum Nematoda into 12 clades. It was suggested that animal parasitism arose independently at least five times, and plant parasitism three times: Tylenchomorpha, Dorylaimida and Diphtherophorina (Fig. 2.9) (Blaxter *et al.*, 1998; De Ley and Blaxter, 2004; van Megen *et al.*, 2009). However, the correctness of the phylogenetic reconstruction for nematodes using SSU might be influenced by two main factors: (i) grouping of long branches occurring as a result of abnormally high evolution rate and (ii) a total deficit of informative characters. Because the SSU tree is reconstructed based on a single gene, efforts are continuously made to sequence other genes. Multigene phylogeny will become available when sequences of genes from nematode genome projects are obtained.

2.11.3. The infraorder Tylenchomorpha

The evolutionary relationships of tylenchid and aphelenchid nematodes have been evaluated using sequence data of the 18S and the 28S rRNA genes. The order Tylenchida *sensu* Siddiqi containing plant-parasitic nematodes appears to be clearly monophyletic. The order Aphelenchida *sensu* Siddiqi comprising fungal-feeding Aphelenchidae and Aphelenchoididae is polyphyletic in all molecular analyses. Several studies have confirmed the sister relationship of tylenchids with the bacteriovorous Cephalobidae (Blaxter *et al.* 1998). The molecular datasets showed that the order Tylenchida *sensu* Siddiqi comprises lineages that largely correspond to two suborders, Hoplolaimina and Criconematina, and other taxonomic divisions by Siddiqi (2000). Molecular analysis supported the classical hypothesis of the gradual evolution of feeding types from simple forms of plant parasitism, such as root hair and epidermal feeding and ectoparasitism towards more complex forms of endoparasitism. Sedentary endoparasitism has also evolved several times

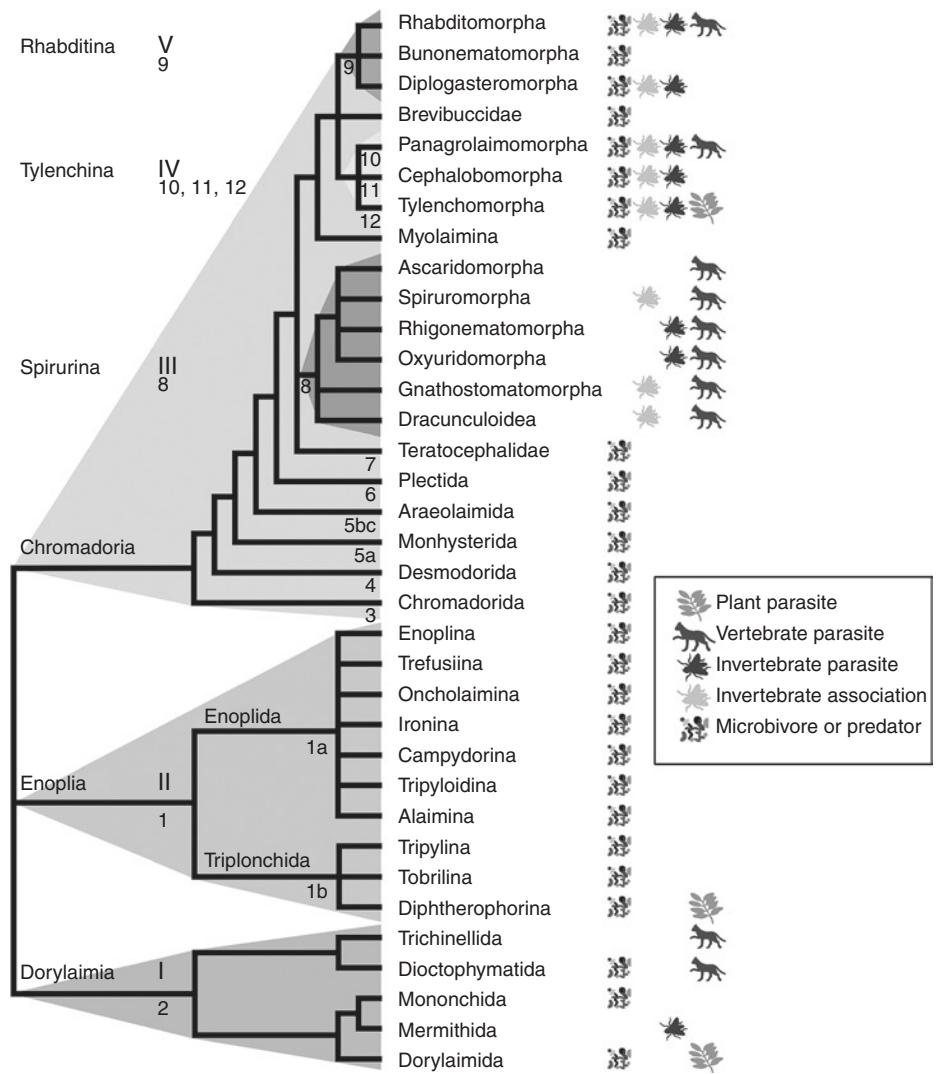


Fig. 2.9. An overview of phylogeny of the phylum Nematoda based on the small subunit ribosomal RNA gene. The systematic names given by De Ley and Blaxter (2004) are given, as is the ‘clade’ naming convention introduced by Blaxter *et al.* (1998). More recently, Helder and colleagues (Holterman *et al.*, 2006; van Meegen *et al.*, 2009) have introduced a numerical clade name scheme; this is given in outlined letters below the relevant branches. Feeding mode, and animal- and plant-parasitic and vector associations, are indicated by small icons (Blaxter, 2011).

independently: (i) cyst and non-cyst nematodes of Heteroderidae probably evolved from migratory ectoparasitic nematodes; (ii) root-knot nematodes appear to be related to the false root-knot nematode *Nacobbus* and have evolved from migratory endoparasitic nematodes; and (iii) sedentary nematodes from Tylenchulidae and Sphaeronomematidae (Criconematidae).

2.11.4. Root-knot nematodes of the family Meloidogynidae

The genus *Meloidogyne* contains more than 90 nominal species. The evolutionary relationships of root-knot nematodes have been inferred from several types of data: isozymes, DNA hybridization, DNA amplification fingerprinting, RAPD-PCR, sequencing of SSU rRNA, D2-D3 expansion segments of LSU rRNA, ITS rRNA and mtDNA. The SSU rRNA gene sequence data resolve deep relationships within *Meloidogyne*. There are several supported clades on trees generated from the SSU sequence datasets: (i) mitotic parthenogenetic species from the tropical group (*M. incognita*, *M. arenaria* and *M. javanica*) and the meiotic parthenogenetic *M. floridensis*; (ii) obligatory amphimictic *M. microtyla*, meiotic parthenogenetic *M. hapla* and two species with unknown reproductive strategy (*M. duytsi* and *M. maritima*); and (iii) meiotic parthenogenetic *M. exigua*, *M. graminophila*, *M. chitwoodi*, *M. fallax*, *M. minor* and mitotic parthenogenetic *M. oryzae*. The basal *Meloidogyne* species consist of the polyphagous *M. artiellia* and the oligophagous *M. mali*, *M. ulmi* and *M. ichinohei* (De Ley *et al.*, 2002; Holterman *et al.*, 2009) (Fig. 2.10).

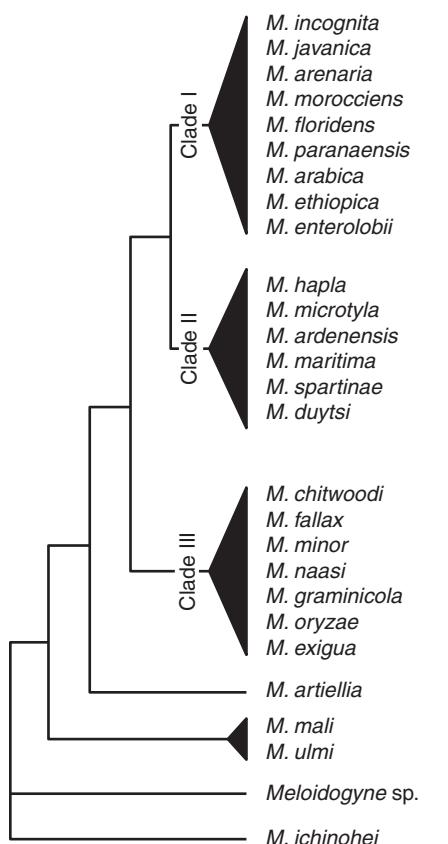


Fig. 2.10. Schematic overview of the phylogeny of the Meloidogynidae derived from SSU rDNA sequence data (Holterman *et al.*, 2009; Bert *et al.*, 2011).

2.11.5. Cyst nematodes of the family Heteroderidae

Cyst nematodes are highly evolved sedentary plant parasites. Phylogenetic analysis of the ITS rRNA and D2–D3 expansion segment of the 28S rRNA gene sequences confirmed the monophyly of the subfamilies Punctoderinae and Heteroderinae with the genus *Heterodera*. The combination of molecular data with morphology of the vulval structure and the number of incisures in the lateral field of second-stage juveniles (J2) enabled six main groups within *Heterodera* to be recognized: *Avenae*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari* and *Schachtii* groups. Close relationships were revealed between the *Avenae* and *Sacchari* groups and between the *Humuli* group and the species *H. turcomanica* and *H. salicophila*. Some inconsistencies between molecular phylogeny and earlier proposed morphological groupings may be attributed to homoplastic evolution, e.g. a bifenestrated vulval cone developed independently at least three times during the evolution of cyst nematodes. Likewise, the presence of three incisures in the lateral field of J2 seems to have arisen twice independently (Subbotin *et al.*, 2001). Molecular data suggested an early divergence of tropical and temperate heteroderid species and often revealed association of nematodes with their host plants from related families (Fig. 2.11).

2.11.6. Stem and gall-forming nematodes of the family Anguinidae

The stem nematode, *D. dipsaci*, occurs as more than 20 biological races. Molecular approaches using RAPD-PCR, AFLP, PCR-RFLP and sequences of the ITS rRNA confirmed that *D. dipsaci* constitutes a complex sibling species. The phylogenetic analysis of the ITS sequences of plant-parasitic species of *Ditylenchus* revealed two main clades: (i) *D. dipsaci sensu stricto* with diploid chromosome numbers and comprising most isolates from agricultural, ornamental and several wild plants; and (ii) a complex of *Ditylenchus* species with polyploid chromosome numbers, including *D. gigas* from *Vicia faba*, *D. weischeri* and several species parasitizing various Asteraceae and a species from *Plantago maritima*. Molecular methods failed to distinguish biological races within *D. dipsaci sensu stricto*.

Over 40 nominal species of gall-forming nematodes have been described. Testing of recognized anguinid classifications using the ITS sequences strongly supported monophyly of the genus *Anguina* and paraphyly of the genera *Mesoanguina*, *Heteroanguina sensu* Chizhov & Subbotin and *Subanguina sensu* Brzeski. Molecular data demonstrate that the main anguinid groups are generally associated with host plants belonging to the same or related systematic groups. The molecular analysis supports the concept of narrow host-plant specialization of seed-gall nematodes, shows that *Anguina agrostis* causing elongate galls occurs only in one host, *Agrostis capillaries*, and reveals several undescribed species infecting other species of grass (Fig. 2.12) (Subbotin *et al.*, 2004).

2.11.7. Needle nematodes of the family Longidoridae

Longidorids are a group of ectoparasitic nematodes with hundreds of species. The analysis of 18S and partial 28S rRNA gene sequences revealed several major

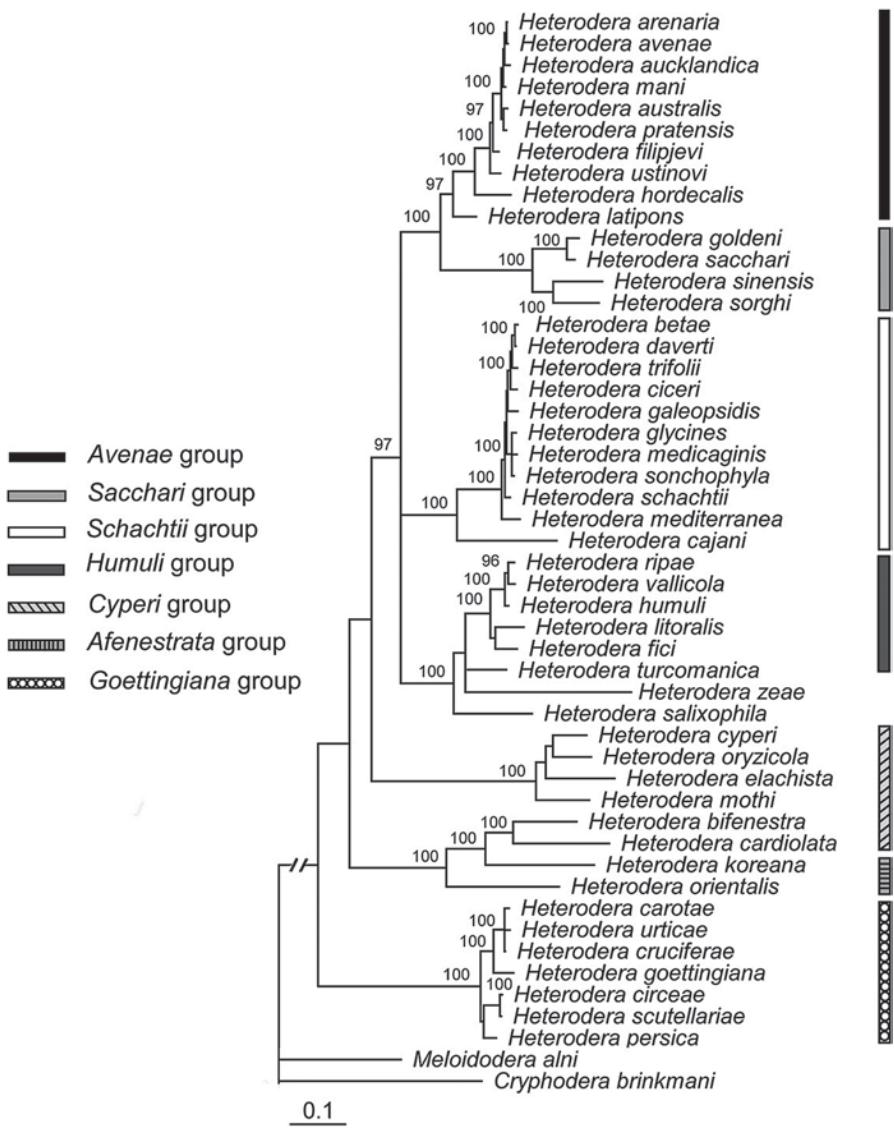


Fig. 2.11. Phylogenetic relationships among *Heterodera*: Bayesian 50% majority rule consensus tree from two runs as inferred from ITS1-5.8S-ITS2 sequences of rRNA gene alignment. Posterior probabilities more than 70% are given for appropriate clades. (Modified from Subbotin *et al.*, 2010b.)

groups within Longidoridae: *Longidorus*, *Paralongidorus*, the *X. americanum* group, other *Xiphinema* species and *Xiphidorus*. The grouping of *Longidorus* species on the tree is well correlated with amphidial shapes. Although the result of the alternative phylogenetic hypotheses tests did not refute the monophyly of the genus *Xiphinema*, the species of this genus were split into two distinct clades in all trees.

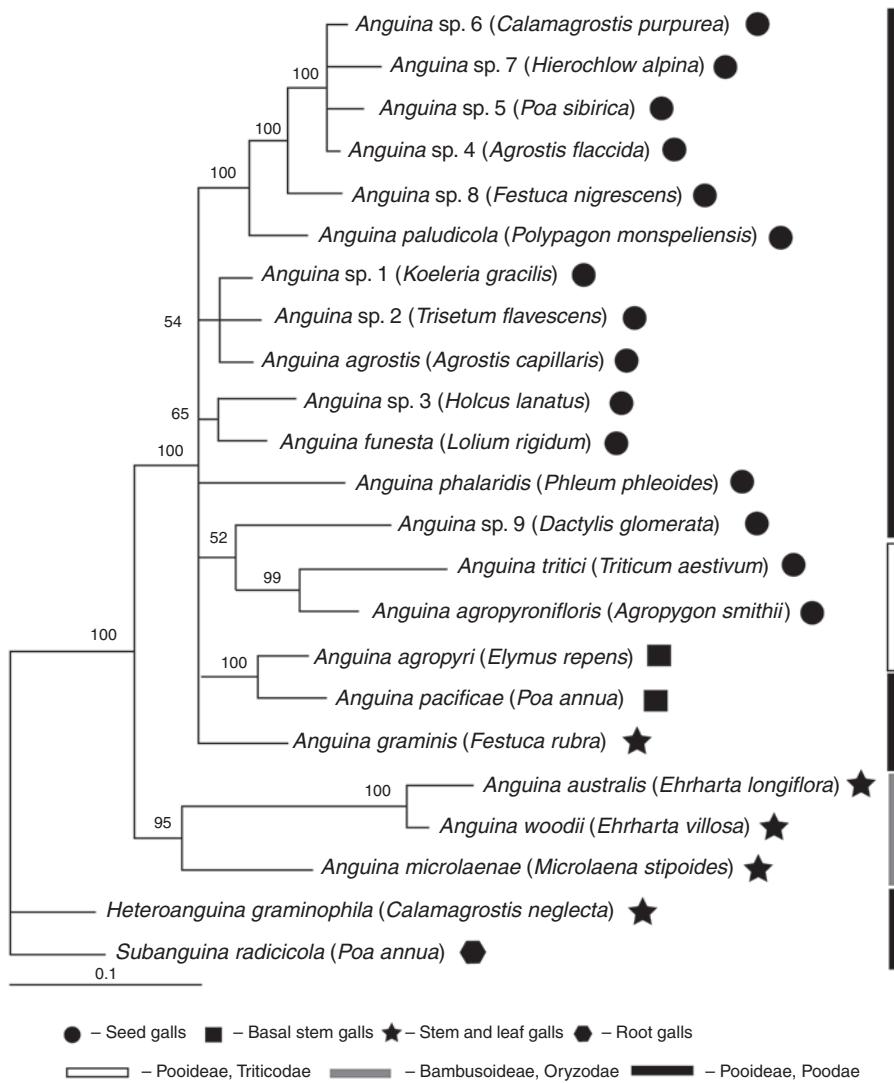


Fig. 2.12. Phylogenetic relationships within the genus *Anguina* as inferred from the Bayesian analysis of the ITS1 rRNA gene sequences with mapping gall types and systematic position of host plants (subfamily, tribe). Posterior probabilities are given on appropriate clades. (From Subbotin and Riley, 2012.)

2.11.8. Root-lesion nematodes of the family Pratylenchidae

Phylogenetic analyses of the D2–D3 of 28S and 18S rRNA gene sequences of large numbers of geographically diverse isolates of genus *Pratylenchus* species confirmed that most classical morphospecies are monophyletic and revealed several species complexes. Analyses revealed at least six distinct major clades of examined *Pratylenchus* species and these clades are generally congruent with those defined

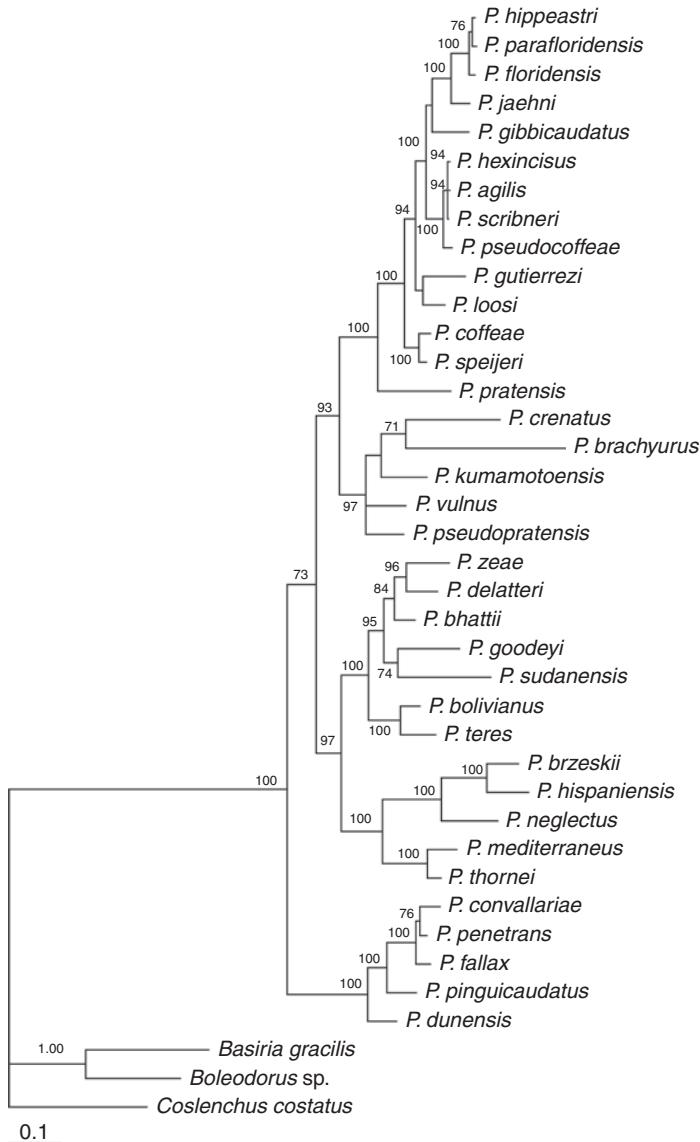


Fig. 2.13. Phylogeny of the genus *Pratylenchus*. Bayesian majority rule consensus tree as inferred from analysis of the D2–D3 expansion segments of 28S rRNA gene sequence alignment. Posterior probabilities are given on appropriate clades.

by characters derived from lip patterns, numbers of lip annules and shape of the spermatheca (Fig. 2.13).

2.11.9. Pinewood nematode and other *Bursaphelenchus* species

A phylogeny of *Bursaphelenchus* species from Europe, North America, Central America and Asia representing much of the known biological diversity in this genus

has been reconstructed using sequences of the 18S, 28S and ITS of rRNA and COI genes. Phylogenetic analyses using several methods of inference were congruent, with the greatest resolution obtained with combined datasets. Phylogenetic analysis revealed *B. abruptus* as the basal taxon among all investigated *Bursaphelengus* species and a large number of significantly supported monophyletic groups that are largely consistent with morphological and life history variation in the genus. The genus is divided into seven groups with four incisures, four groups with three incisures and two groups with two incisures. MtDNA data were limited by non-stationary base composition and apparent saturation above the species level (Ye *et al.*, 2005).

3

Root-knot Nematodes*

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* A revision of Karssen, G. and Moens, M. (2006) Root-knot nematodes. In: Perry, R.N. and Moens, M. (eds) *Plant Nematology*, 1st edn. CAB International, Wallingford, UK.

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3.1. Introduction to Root-knot Nematodes

Root-knot nematodes are included within the genus *Meloidogyne* Göldi, 1887 (*Meloidogyne* = apple-shaped female) and belong to a relatively small but important polyphagous group of highly adapted obligate plant pathogens. They are distributed worldwide and parasitize nearly every species of higher plant. Typically, they reproduce and feed within plant roots and induce small to large galls or root-knots (Fig. 3.1). Root-knot is one of the oldest known nematode diseases of plants. Other plant-parasitic nematodes, like the false root-knot nematode *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 and *Subanguina radicicola* (Greef, 1872) Paramonov, 1967, also cause root galls and can be confused with *Meloidogyne*. Due to their endoparasitic way of living and feeding, root-knot nematodes disrupt the physiology of the plant and may reduce crop yield and product quality and, therefore, are of great economic importance. For reliable identification of root-knot nematodes, the best approach is to integrate morphological, isozyme and DNA data, together with information on mode of reproduction, chromosome number, host plants and distribution. Field samples often include mixtures of *Meloidogyne* species.

The systematic position of the root-knot nematodes at family level has been the subject of discussion for many years. Box 3.1 shows the classification of *Meloidogyne* after De Ley and Blaxter (2002). At genus level, root-knot nematodes have been confused with cyst nematodes (*Heterodera* Schmidt, 1871) for a long time. Between 1884 and 1932 root-knot nematodes were generally named *Heterodera radicicola*, whilst between 1932 and 1949 the name *Heterodera marionii* was commonly used. Highlights of the taxonomical history of the genus are given in Box 3.2. A more comprehensive description of the taxonomical history of root-knot nematodes can be found in KarsSEN (2002) and aspects covered below are dealt with in greater detail in the book *Root-knot Nematodes* (Perry *et al.*, 2009).

3.2. Life Cycle and Behaviour

Root-knot nematode eggs are enclosed in gelatinous egg sacs that are usually deposited on the surface of galled roots (Fig. 3.2). Sometimes they occur within the galls. Following embryogenesis, the first moult occurs within the egg giving rise to the

second-stage juvenile (J2). Hatching of *Meloidogyne* J2 from the eggs is temperature driven and occurs without requiring stimulus from plant roots; however, root diffusates sometimes stimulate hatching. Occasionally, the requirement for a hatching stimulus from the host plant is dependent on plant age. Hatching of J2 of *M. chitwoodi* produced

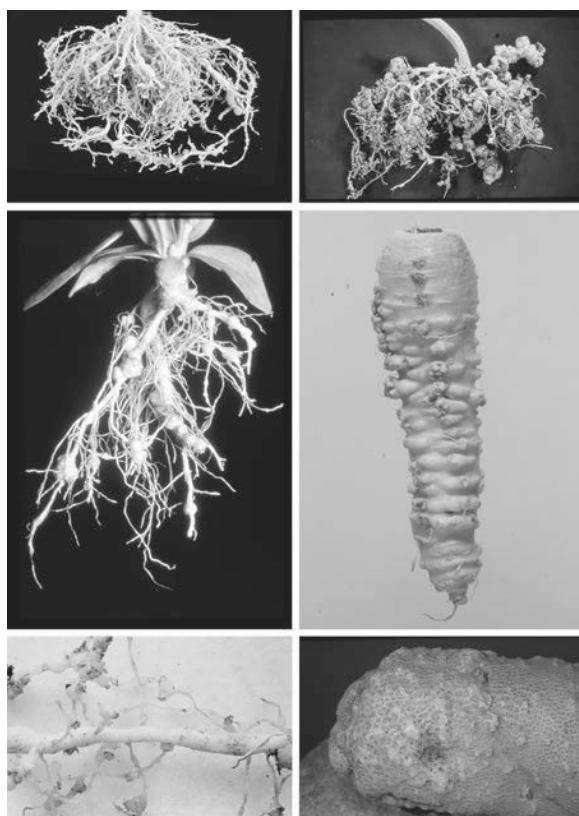


Fig. 3.1. Root-knot nematode galls of, respectively (starting top left, clockwise): *Meloidogyne arenaria* on tomato, *M. incognita* on cucumber, *M. fallax* on carrot, *M. chitwoodi* on potato, *M. hapla* on lettuce and *M. javanica* on *Eustoma* spp.

Box 3.1. Classification of the genus *Meloidogyne* Göldi, 1887 within the phylum Nematoda, after De Ley and Blaxter (2002).

Phylum Nematoda Potts, 1932
Class Chromadorea Inglis, 1983
Order Rhabditida Chitwood, 1933
Suborder Tylenchina Thorne, 1949
Infraorder Tylenchomorpha De Ley & Blaxter, 2002
Superfamily Tylenchoidea Örley, 1880
Family Meloidogynidae Skarbilovich, 1959
Genus *Meloidogyne* Göldi, 1887

Box 3.2. Highlights of the taxonomical history of the genus *Meloidogyne*.

- 1855 First illustrated report of root-knot disease by Berkeley (UK).
- 1879 First root-knot nematode description (*Anguillula marioni*) by Cornu (France).
- 1884 Müller (Germany) described and illustrated a perineal pattern for the first time.
- 1885 Treub (Indonesia) described *Heterodera javanica* (= *M. javanica*).
- 1889 Neal (USA) described *Anguillula arenaria* (= *M. arenaria*).
- 1887 Göldi (Brazil) described *Meloidogyne exigua*.
- 1919 Kofoid and White (USA) described *Oxyuris incognita* (= *M. incognita*).
- 1949 Revision of the genus (five species) and the name *Meloidogyne* revived by Chitwood (1949).
- 1968 *Meloidogyne* monograph (23 species) by Whitehead (1968).
- 1987 *Meloidogyne* monograph (51 species) by Jepson (1987).
- 2002 Revision of the European *Meloidogyne* species by Karssen (2002).

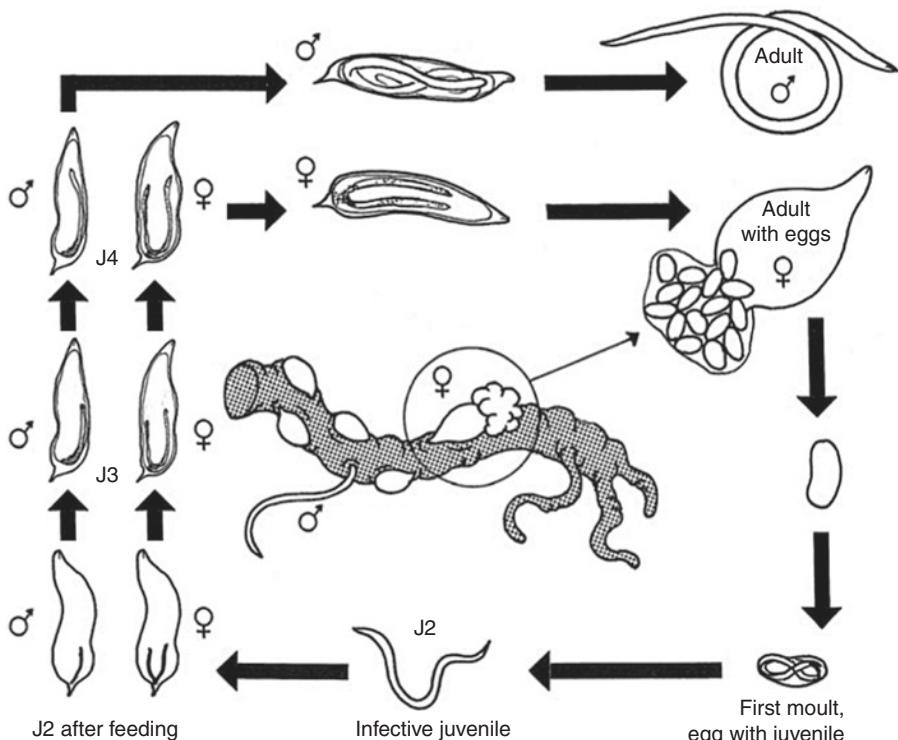


Fig. 3.2. Diagram of the life cycle of the root-knot nematode *Meloidogyne*. J2: second-stage juvenile; J3: third-stage juvenile; J4: fourth-stage juvenile.

on young plants does not require host root diffusate stimulus, whereas at the end of the plant growing season, egg masses contain a percentage of unhatched J2 that require host root exudate to cause hatch. The closely related species, *M. fallax*, does not show this difference. The eggshell becomes flexible immediately before hatching and enzymes are thought to be involved in altering eggshell structure (see Chapter 7).

When J2 leave the egg masses, they infect nearby galled roots or enter new roots. J2, i.e. the infective stage, and males are the stages of *Meloidogyne* that can be found freely in the soil. J2 can survive in the soil in a quiescent state for an extended period. However, during that period they consume the food reserves stored in their intestine. As infectivity is related to food reserves (see Chapter 7), their infectivity will be reduced after long periods spent out of the roots. A comprehensive review of hatch and location of *Meloidogyne* is given by Curtis *et al.* (2009).

J2 are attracted to plant roots, and root location depends on perception of gradients of attractants emanating from the plant root. The infective J2 accumulate at the region of cell elongation just behind the root cap, even of plants resistant to root-knot nematodes. They are also attracted to apical meristems, points where lateral roots emerge, and penetration sites of other J2. There is little difference in the attractiveness of isolated susceptible and resistant plant roots; however, when both are present, the susceptible ones are much more attractive. The nature of the stimuli produced by the roots and perceived by J2 is not clear. Many organic and inorganic compounds excreted by roots form gradients from the root surface into the soil and may influence the nematodes. Carbon dioxide is frequently considered as being the most important factor for attracting plant-parasitic nematodes to the root area and specific attractants may be responsible for ensuring the J2 reaches the preferred invasion site (see Chapter 8).

When root-knot nematodes come into contact with plant roots, they often penetrate immediately. Penetration usually occurs directly behind the root cap but it can occur at any site. J2 penetrate the rigid root cell walls by a combination of physical damage via thrusting of the stylet and breakdown by cellulolytic and pectolytic enzymes. Following penetration, especially with multiple infections on the same root, the root tip may enlarge and root growth often stops for a short period.

After penetration into the root, J2 migrate intercellularly in the cortex to the region of cell differentiation. This migration causes cells to separate along the middle lamella. Cells along the path become distended but only rarely show signs of nematode feeding. Increases in levels of oxidoreductive enzyme activity have also been found; this is an indication of increased metabolic activity. To circumvent the barrier formed by the endodermis, the J2 migrate towards the root tip and turn around when they arrive in the apical meristematic region. Subsequently, they move back up in the vascular cylinder towards the zone of differentiation. After migrating over a short distance, J2 become sessile in the cortical tissue in the zone of differentiation. The head of the J2 is embedded in the periphery of the vascular tissue where they feed on protoxylem and protophloem; the rest of the body is in the cortex parallel with the longitudinal axis of the root.

3.3. Host Response to Parasitism

Susceptible plants react to feeding by juveniles and undergo pronounced morphological and physiological changes. Giant cells (2 to 12, usually about 6), feeding sites for the root-knot nematode, are established in the phloem or adjacent parenchyma (Fig. 3.3). These cells are highly specialized cellular adaptations. The induction and maintenance of these giant cells have been the subject of several hypotheses. It is suggested that both induction and maintenance are controlled by stylet secretions originating from the sub-ventral (early stages of giant cell formation) and dorsal pharyngeal (later stages

of giant cell formation and maintenance) glands of the feeding juveniles (see Chapter 9). Removal of solutes from giant cells may be the stimulus necessary for maintaining the active metabolism of giant cells. If the host does not respond by the formation of giant cells, J2 fail to develop. The J2 will either starve or, if they have sufficient food reserves, may migrate out of the root and locate another root.

Giant cells induced by root-knot nematodes divide without forming new cell walls; they are most likely formed after repeated endomitosis without cytokinesis. Cell wall dissolution does not occur in the first 72 h of giant cell initiation. In normal divisions, cell plate vesicles coalesce during telophase to form a cell wall separating daughter nuclei. In giant cells around *Meloidogyne* these vesicles are not consolidated; they disperse and are resorbed in the cytoplasm to yield binucleate cells. At later periods, cell wall stubs (plates, flanges) can be formed. The appearance of these flanges is different from that of wall fragments of syncytia of cyst nematodes in that they are not perforated (Fig. 3.4).

At the beginning of the giant cell formation the cells are occupied predominantly by the cell vacuole; the nuclei are located in the peripheral cytoplasm. Within a cell, mitosis of the different nuclei occurs synchronically (Fig. 3.5). In a more advanced giant cell, synchrony of the nuclear divisions may not be constant. As cytoplasmic contents (Golgi apparatus, endoplasmic reticulum, mitochondria, plastids and ribosomes) increase, the cells expand laterally. Walls that separate giant cells are apparently

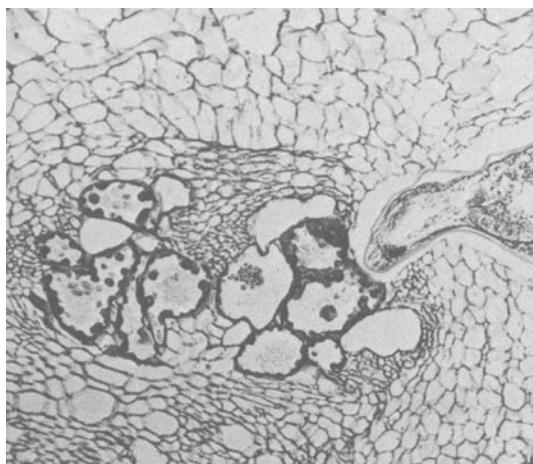


Fig. 3.3. Light micrograph of a cross-section of a root gall infected with a *Meloidogyne* female showing several giant cells. (After Eisenback and Hirschmann Triantaphyllou, 1991.)

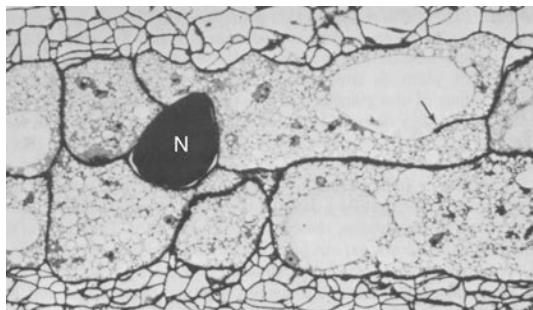


Fig. 3.4. Longitudinal section of giant cells within 6-day-old gall induced by *Meloidogyne incognita* in balsam root, and associated nematode (N). The cell vacuole is greatly reduced and the cells are filled with cytoplasm and secondary vacuoles. A single cell wall stub (arrow) is present. (From Jones and Payne, 1978.)

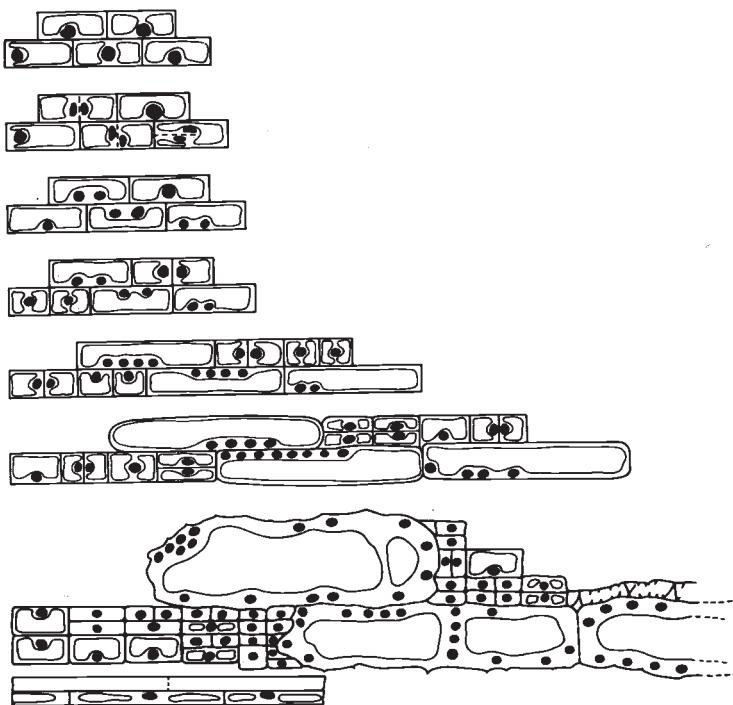


Fig. 3.5. Diagrammatic representation of the events that occur up to 72 h after the induction of three giant cells by *Meloidogyne* spp. Multinucleate cells develop after the failure of cytokinesis. Mitosis within one cell is synchronous, but not necessarily at the same rate as in other cells. Vascular elements start to differentiate outside the giant cells, and rapid division of neighbouring cells allows for their expansion. (From Jones and Payne, 1978.)

continuous, without ‘gaps’ or ‘perforations’. However, there are alternating thick and thin areas on the same wall. Plasmodesmata are found only in the thin portions of the wall. As the food demand of the nematode increases with increasing egg laying, the giant cell cytoplasm shows signs of intense metabolic activity. This is demonstrated by the presence of aneuploid nuclei with 14–16 times more DNA than nuclei of non-infected root tip cells. Further details of giant cell induction and maintenance are given in Chapter 9.

At the same time as the establishment of giant cells, root tissues around the nematode undergo hyperplasia and hypertrophy, causing the characteristic root gall usually associated with *Meloidogyne* infections. Galls usually develop 1 or 2 days after J2 have penetrated the root. Their size is related to the host plant, the number of J2, and the nematode species. Gall formation is not essential for nematode development.

Plant growth regulators have been implicated in the development of giant cells and galls. Auxins (promoters of cell growth) have been identified in higher concentrations in root-knot infected tissue than in non-galled tissue. Cytokinins (promoters of cell division) may also increase in *Meloidogyne*-infected plants. Application of these plant growth regulators to resistant plants reverses the resistance response and makes plants susceptible. These regulators have also been identified in different stages of

Meloidogyne. Another plant growth regulator, ethylene, has been associated with gall formation. It may be involved in the hypertrophy of cortical parenchyma tissue during gall formation.

3.4. Post-infection Biology

Following penetration of host tissue and establishment of the feeding site, J2 undergo several morphological changes (Fig. 3.6). The nematode grows slightly in length. Simultaneously, enlargement of the pharyngeal glands and metacorpus occur. The cells of the genital primordial divide and six rectal glands, which secrete the ovisac, develop in the posterior end of the female juvenile stages. The genital primordium is V-shaped in the female, consisting of two limbs joined posteriorly to the vaginal rudiment that lies close to the posterior body wall. The male gonad develops as a single limb, the *vas deferens* differentiating at the hind end and eventually joining with the rectum. During their further development, the juveniles gradually assume a flask shape and undergo three moults. The last moult is a true metamorphosis for the male, which appears as a long filiform nematode folded inside the moulted cuticle of the fourth-stage juvenile, which is retained as a sheath. The adult female at first retains the same shape as the last juvenile stage but enlarges as it matures and eventually becomes pyriform. In the amphimictic species, the males mate after escaping from the last juvenile sheath. Females secrete a gelatinous matrix into which they extrude a large number of eggs.

The energy necessary for completion of the third and fourth moults must be obtained by the animal before the second moult because it is unable to feed on its host from the start of the second moult to the completion of the fourth moult. This happens because during this period the nematode has no stylet and remains within the sheath of the second cuticle. This cuticle retains a characteristic spike-like tail although the enclosed juvenile is bluntly rounded posteriorly. By contrast, the developing juveniles of *Heterodera* and *Globodera* have no tail spike. After the fourth moult the stylet is re-formed and digestive and reproductive organs are developed. When the male is mature it uses its stylet to break through the cuticle and root tissue.

It is well known that the proportion of males in a population, at least in parthenogenetic species, varies according to the host plant and the environmental conditions. Food supply may be an important factor as males are more abundant under adverse conditions for development. It is known that under such conditions, female juveniles can become adult males. Sometimes true intersexes (males with vulva) appear.

The number of generations per year varies according to species and food availability. Usually there are many, but in some species there is only one (e.g. *M. naasi*). In a favourable host several hundred eggs are produced by each female. Each female may lay 30–80 eggs per day, the number depending on the host plants and the environmental conditions. Eggs of *Meloidogyne* are deposited in a gelatinous matrix, which is not retained in the body (as with most cyst-forming nematodes). If the female is deeply embedded in the plant tissue, the egg sac may also be embedded and the gelatinous matrix remains soft. If the egg sac is exposed on the root surface the outer matrix layers may dry and become tough; sometimes they become orange-brown.

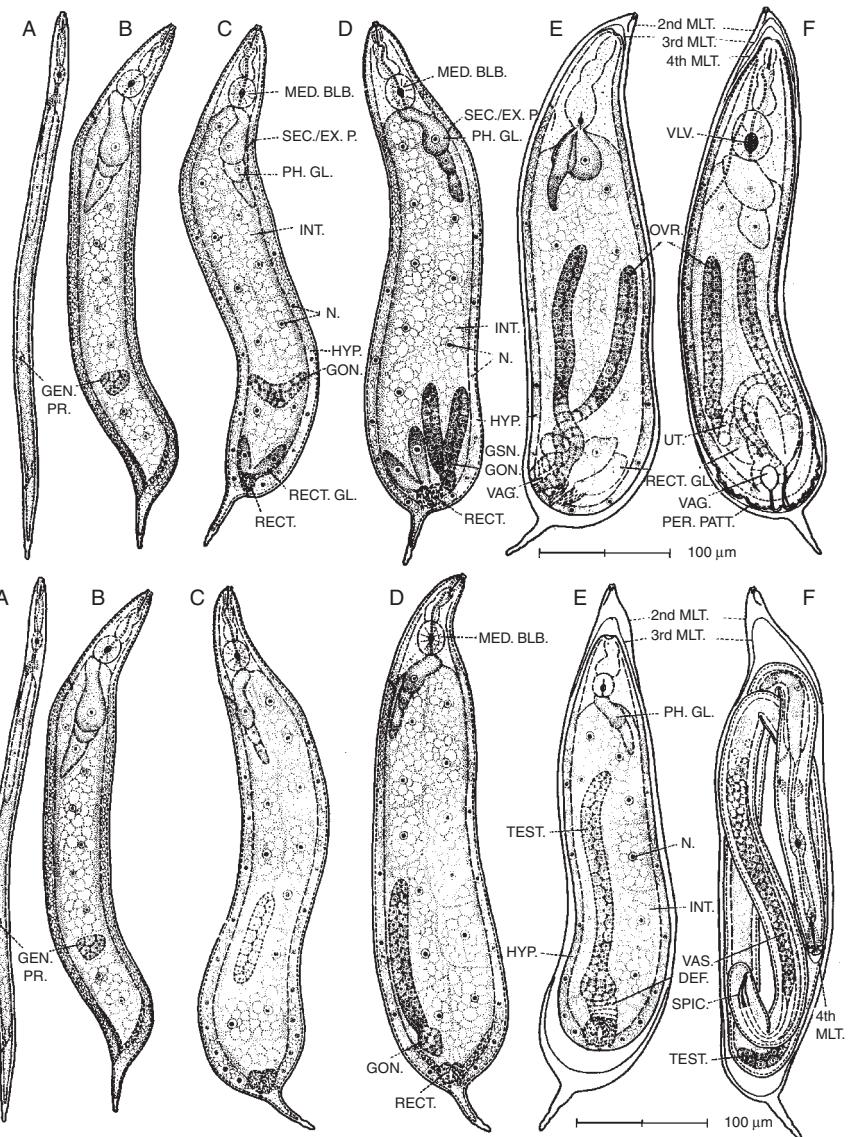


Fig. 3.6. Development of a female (top) and male (bottom) of *Meloidogyne incognita* from second-stage juvenile to adult. A: Second-stage infective juvenile. B: Swollen, sexually undifferentiated second-stage juvenile. C: Early second-stage juvenile differentiating into a female/male. D: Second-stage female/male juvenile shortly before second moult. E: Fourth-stage female/male juvenile. F: Adult female/male shortly after fourth moult. SEC./EX. P.: secretory-excretory pore. GEN. PR.: genital primordium. GON.: gonad. HYP.: hypodermis. INT.: intestine. MED. BLB.: median bulb. 2nd MLT.: second moult. 3rd MLT.: third moult. 4th MLT.: fourth moult. N.: nucleus. PH. GL.: pharyngeal glands. OVR.: ovary. PER. PATT.: perineal pattern. RECT. GL.: rectal glands. RECT.: rectum. SPIC.: spicule. TEST.: testis. UT.: uterus. VAG.: vagina. VAS. DEF.: vas deferens. VLV.: valve. (Modified from Eisenback and Hirschmann Triantaphyllou, 1991.)

In either case, the eggs are partially protected against drying and can survive in soil for a period that depends on temperature and moisture and on the nematode species.

Root-knot nematodes are known to have unbalanced sex ratios. Cross-fertilizing species (*M. carolinensis*, *M. spartinae*) usually have a 1:1 male-to-female ratio. Species that reproduce by facultative or obligatory parthenogenesis (*M. hapla*, *M. incognita*) have variable sex ratios. Depending on the environmental conditions, males may be absent or rare or abundant. Depending on the developmental stage at which sex reversal occurs, sex-reversed males may have one to two gonads of variable length.

3.5. Effect on Plant Growth and Yield

As described above, the impact of root-knot nematodes on roots of host plants is very characteristic. The severity of the symptoms can be scaled with a root gall index (Greco and Di Vito, 2009). Crops from which tubers or taproots are harvested can be worthless due to quality loss despite the normal total yield in tons (see Chapter 10). Above-ground symptoms observed on infected plants are similar to those produced on any plants having a damaged and malfunctioning root system. Symptoms include: (i) suppressed shoot growth and accompanying decreased shoot-root ratio; (ii) nutritional deficiencies showing in the foliage, particularly chlorosis; (iii) temporary wilting during periods of mild water stress or around midday, even when adequate soil moisture is available; and (iv) suppressed plant yields. The importance of these symptoms is often related to the number of juveniles penetrating and becoming established within the root tissue of young plants. The common explanation for these above-ground symptoms is that *Meloidogyne* infection affects water and nutrient uptake and upward translocation by the root system.

Most of the studies focusing on the relationship between root-knot infection and plant nutrition demonstrated that *Meloidogyne* infection increased N, P and K concentrations in above-ground plant parts. However, this was not always the situation; sometimes nutrients appeared to accumulate in the roots. Supplying nutrients to infected plants often increases tolerance to *Meloidogyne*. The few studies on the water relationships of *Meloidogyne*-infected plants suggest that water consumption is not affected by nematode infection if soil moisture is not limiting, but under periodic stress, consumption is reduced. Plant development is suppressed by infections that inhibit root growth during moisture stress and prevent roots from extending into moist soil.

Meloidogyne infection of roots decreases the rate of photosynthesis in leaves. The nematodes interfere with the production of root-derived factors regulating photosynthesis. *Meloidogyne* functions as a metabolic sink in diseased plants. The increased metabolic activity of giant cells stimulates mobilization of photosynthetic products from shoots to roots and, in particular, to the giant cells where they are removed and utilized by the feeding nematode. Mobilization and accumulation of substances reaches a maximum when the adult females start egg laying and declines thereafter. Root branching and the degree of root extension are frequently affected by *Meloidogyne* infection. Abnormal root growth results in reduced root surface area and limits the capacity of a root system to explore the soil.

As a consequence, the primary cause of poor nutrient uptake and suppressed growth of infected plants could be related to the reduced root system.

3.6. Survival

Meloidogyne eggs and juveniles do not survive as long as those of the closely related genera *Heterodera* and *Globodera*, where eggs in cysts remain viable for several years. Eggs of *Meloidogyne* are deposited in a gelatinous matrix, which may be white or brown. Egg masses formed early in the host growing cycle contain eggs, which hatch immediately, making several generations per growing cycle possible. Egg masses formed later or in adverse conditions by old or poorly nourished females are brown and contain eggs that are dormant and do not hatch immediately. This form of dormancy is termed diapause; more information is provided in Chapter 7. These latter egg masses ensure some carry-over from one season to another.

Temperature is an important factor in several stages of the development of nematodes. As with all poikilothermic animals, temperature influences distribution, survival, growth and reproduction. Within the genus *Meloidogyne*, two distinct groups can be distinguished: thermophils and cryophils, which can be separated by their ability to survive lipid-phase transitions that occur at 10°C (see Chapter 7). *Meloidogyne hapla*, *M. chitwoodi*, *M. fallax* and probably *M. naasi* are cryophils and are able to survive soil temperatures below 10°C. *Meloidogyne chitwoodi* and *M. fallax* were found to stay infective for more than 300 days in soil at 5°C. *Meloidogyne incognita*, *M. javanica* and *M. exigua* are thermophils and do not have extended survival at temperatures below 10°C. Like survival, hatching is primarily controlled by temperature. Thermotypes exist within a species. Such populations can be distinguished from each other by small differences in the minimum temperatures needed for hatching, probably due to adaptation of geographical populations to local temperature regimes.

Soil texture, moisture, aeration and osmotic potential are interacting factors and it is difficult to determine the effect of each one separately. *Meloidogyne* species are active in soils with moisture levels at 40–60% of field capacity. As the soils either dry or increase in moisture, nematode activity decreases. In drying environments, the gelatinous matrix of the egg sac appears to maintain a high moisture level and provides a barrier to water loss from eggs. Survival of eggs in egg masses at temperature extremes was found to be higher in dry soil compared with moist soil. Embryos and first-stage juveniles are more resistant to water loss than unhatched J2 because of changes in the egg membrane after the first moult. In drying soils, nematodes may be subjected to increased osmotic pressures, especially after fertilizer applications. Even small changes in osmotic potential may influence nematode behaviour. However, as discussed in Chapter 8, osmotic pressure within the soil is unlikely to influence the water balance of plant-parasitic nematodes as the soil dries because most water will be extracted from nematodes by other forces before osmotic pressure is physiologically important. Increased crop damage is often associated with alkaline soils. This seems to be associated with stress on the host plant. As *Meloidogyne* prefer sandy soils, their agricultural importance is most frequently associated with sandy soils. *Meloidogyne artiellia* is an exception and causes severe damage in both sandy soils and in soils containing 30–35% clay.

3.7. Cytogenetics

The cytogenetic status of the genus *Meloidogyne* is complex but important in understanding the overall biology and evolution of these nematodes. The major root-knot nematodes have been well studied cytogenetically (Triantaphyllou, 1985). Within the genus *Meloidogyne* there are three modes of reproduction: amphimixis, automixis and apomixis (Table 3.1). Only a small number of species reproduce by amphimixis, i.e. with the obligatory fusion of a male and female gamete. The majority of species of *Meloidogyne* reproduce by parthenogenesis (automixis or apomixis). During maturation of the oocytes, the apomictic species undergo only a single mitotic division. Meiosis is completely absent in these obligatory mitotic parthenogens. In automictic species, oocytes undergo a meiotic division. When males are present, the sperm and egg nuclei fuse together. However, without the presence of a sperm nucleus, the nucleus of the second polar body fuses with the egg pronucleus and restores the diploid state (meiotic parthenogenesis). Therefore, automictic root-knot nematode species are facultatively parthenogenetic.

Most of the amphimictic and automictic species are diploid with a haploid chromosome number of 18. The majority of the apomictic species are polyploid or aneuploid and usually show a wide variation in chromosome number. *Meloidogyne incognita* reproduces exclusively by apomixis. The majority of those populations of this species that have been studied have $2n = 42\text{--}44$ chromosomes and are considered to be triploid or hypotriploid (i.e. derived from triploid forms through the loss of chromosomes); however, some populations have 32 or 38 chromosomes and represent a diploid form ($n = 16\text{--}18$). *Meloidogyne hapla* is cytogenetically the most complex species of the genus. Most populations reproduce by automixis (termed race A) with a haploid chromosome number of 17; some populations with lower numbers (13–15) are known. Also polyploid *M. hapla* females ($n = 28\text{--}34$) have been observed within some diploid populations reproducing by automixis; even conversion from polyploid to diploid was noted. Other *M. hapla* populations reproduce by apomixis (race B), with usually 43–48 chromosomes and a few populations have 30–32 chromosomes. Direct polyploidization within the apomictic *M. microcephala* has also been recorded, i.e. through doubling of its somatic chromosome number from a diploid ($2n = 37$) to a tetraploid ($2n = 74$) state. Usually polyploidization mainly affects nematode morphometrics, resulting in an increased size of various structures, but rarely affects morphology.

3.8. General Morphology

Female. Sedentary adult root-knot nematode females are pearly white with a rounded to pear-shaped body and a protruding, sometimes bending, neck (Fig. 3.7). A cyst stage is not present. Females range in length from 350 µm to 3 mm and in maximum width from 300 to 700 µm. In full-grown females the cuticle annulation is only visible in the head region and the posterior part where a characteristic unique cuticular pattern or perineal pattern can be observed around the perineum (vulva–anus region). The shape of this pattern is often variable and influenced by several developmental factors. The perineum is terminal with the phasmids just above the anus.

Table 3.1. Chromosome number and mode of reproduction of some root-knot nematodes.

Meloidogyne species	Chromosome number		Mode of reproduction
	n	2n	
<i>M. carolinensis</i>	18		
<i>M. kikuyensis</i>	7		
<i>M. megatyla</i>	18		
<i>M. microtyla</i>	18–19		Amphimixis
<i>M. pini</i>	18		
<i>M. spartinae</i>	7		
<i>M. subartica</i>	18		
<i>M. chitwoodi</i>	14–18		
<i>M. exigua</i>	18		
<i>M. fallax</i>	18		
<i>M. floridensis</i>	18		Facultative
<i>M. graminicola</i>	18		meiotic
<i>M. graminis</i>	18		parthenogenesis
<i>M. hapla</i> (race A)	13–17		(automixis)
<i>M. minor</i>	17		
<i>M. naasi</i>	18		
<i>M. ottersoni</i>	18		
<i>M. trifoliophila</i>	18		
<i>M. arenaria</i>	30–38		
	40–48		
	51–56		
<i>M. cruciani</i>	42–44		
<i>M. enterolobii</i>	44–46		
<i>M. ethiopica</i>	36–44		
<i>M. hapla</i> (race B)	30–32		
	43–48		
<i>M. hispanica</i>	33–36		
<i>M. incognita</i>	32–38		Obligatory
	41–46		mitotic
<i>M. inornata</i>	54–58		parthenogenesis
<i>M. izalcoensis</i>	44–48		(apomixis)
<i>M. javanica</i>	42–48		
<i>M. konaensis</i>	44		
<i>M. microcephala</i>	36–40		
<i>M. morocciensis</i>	47–49		
<i>M. oryzae</i>	51–55		
<i>M. paranaensis</i>	50–52		
<i>M. partityla</i>	40–42		
<i>M. petuniae</i>	41		
<i>M. platani</i>	42–44		
<i>M. querciana</i>	30–32		
<i>M. salasi</i>	36		

After Triantaphyllou (1985) and updated by the authors.

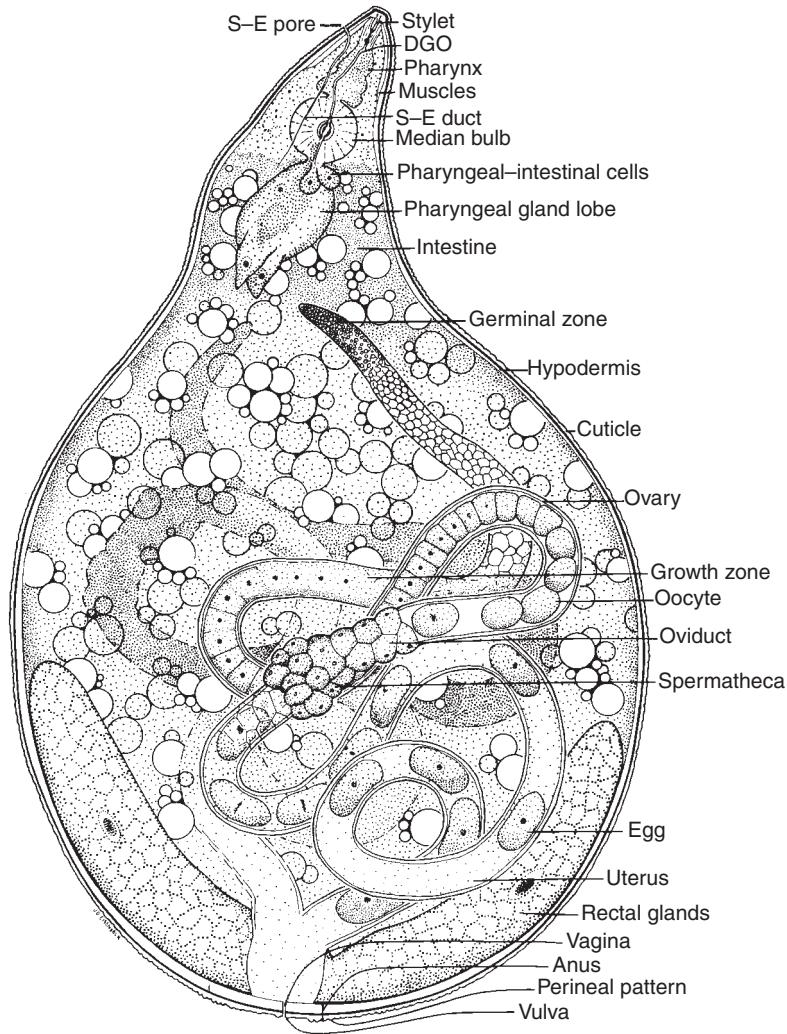


Fig. 3.7. Drawing of a female root-knot nematode. (From Eisenback and Hirschmann Triantaphyllou, 1991.)

Usually the anus is covered with a small cuticular fold and the perineum can be slightly elevated. Some species have lateral lines or sub-cuticular punctuations above the perineum (Fig. 3.8).

The head is usually not or only slightly set off with a distinct but delicate cephalic framework present. The labial disc is not to slightly raised and fused with the medial and lateral lips. Two slit-like amphidial and ten small sensilla openings are present around the stylet stoma (Fig. 3.9). The delicate stylet ranges in length from 10 to 25 μm ; in most species the cone is slightly curved dorsally, and the shaft is straight and includes three basal knobs. Stylet knob shape varies from rounded to transversely elongate and can be set off or is posteriorly sloping. The dorsal pharyngeal gland orifice (DGO) is located between 2.5 and 9.0 μm behind the stylet knobs.

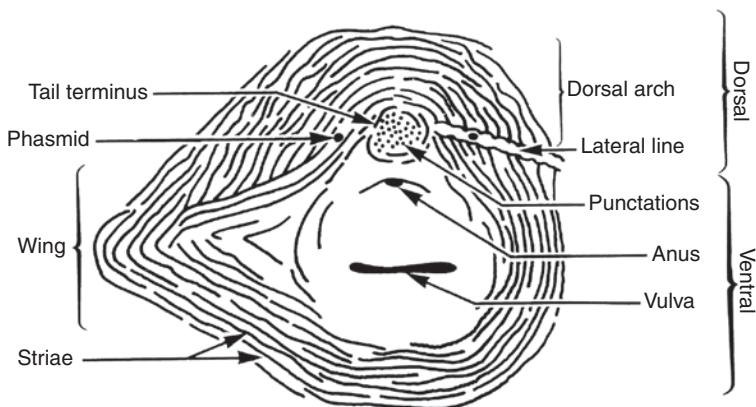


Fig. 3.8. Diagram of a root-knot nematode perineal pattern. (From Eisenback and Hirschmann Triantaphyllou, 1991.)

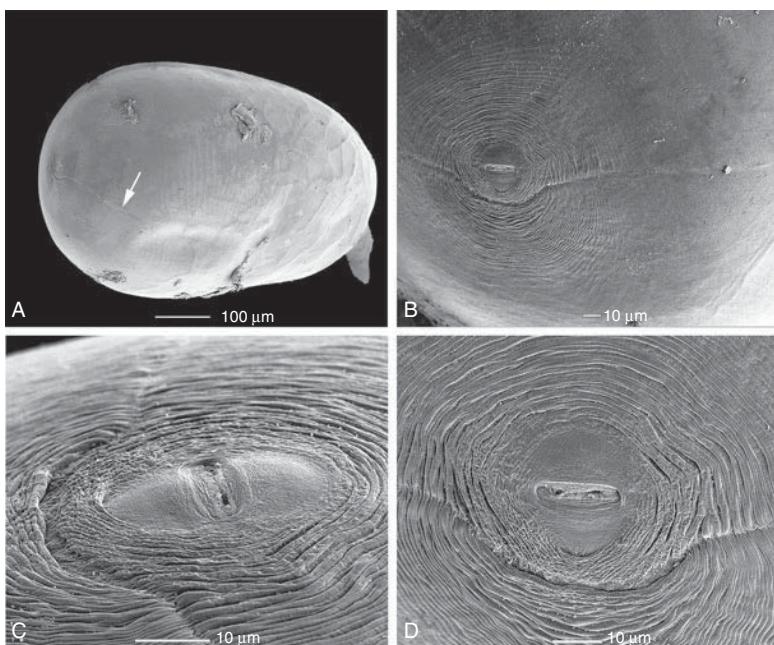


Fig. 3.9. Female root-knot nematode scanning electron microscope photographs. A: *Meloidogyne hapla* body (arrow = lateral line). B–D: *M. hapla* perineal patterns.

The secretory-excretory (S-E) pore is located usually between the stylet knobs and the metacorpial level. The metacorpus itself is relatively large and connected posteriorly with the pharyngeal glands. These glands are highly variable in size and shape and overlap the intestine ventrally. Two long didelphic, partly convoluted gonads are present. Each gonad is composed of an ovary with a germinal and growth zone,

an oviduct, a large lobed spherical spermatheca and a very long uterus. Most of the unembryonated eggs are deposited in an egg sac, produced by six large unicellular rectal glands and secreted through the anus.

Male. The non-sedentary male is vermiform, clearly annulated and ranges in length from 600 to 2500 µm (Fig. 3.10). The head is composed of a head cap and head region (= post-labial annule). The head region can be offset and/or partly subdivided by transverse incisures or annulations. The head cap has a relatively large rounded labial disc and is usually fused with four medial lips. Six inner labial sensilla are centred around the oral opening and one cephalic sensillum is present on each medial lip. The two large slit-like amphidial openings are located between the labial disc and the lateral lip. In some species, these lateral lips are reduced or absent (Fig. 3.11). The cephalic framework and straight stylet are well developed, the latter ranges in length from 13 to 33 µm. The DGO is located 2 to 13 µm behind the three stylet knobs; stylet knob shape is as in females.

The metacorpus is much smaller compared to females. The S-E pore and the hemizonid are located between the metacorpial level and the ventral overlapping of the pharyngeal glands. The hemizonid is positioned anterior or sometimes posterior

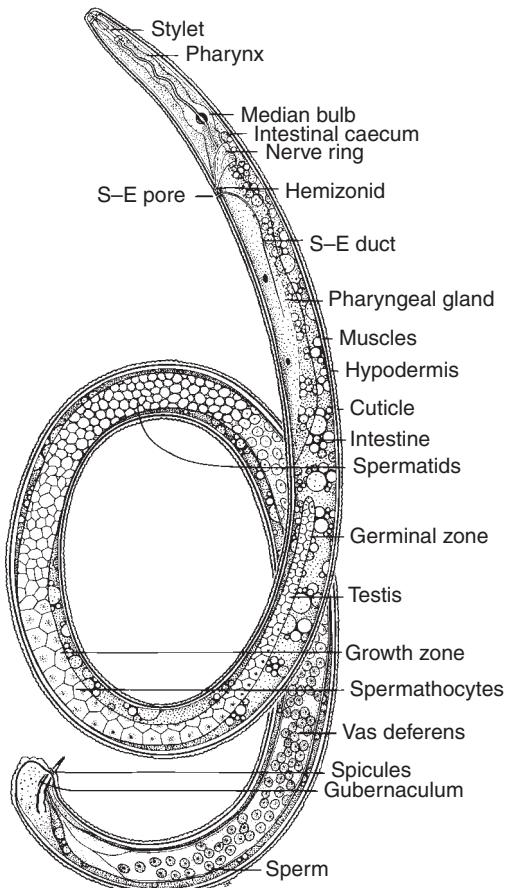


Fig. 3.10. Drawing of a male root-knot nematode. (After Eisenback and Hirschmann Triantaphyllou, 1991.)

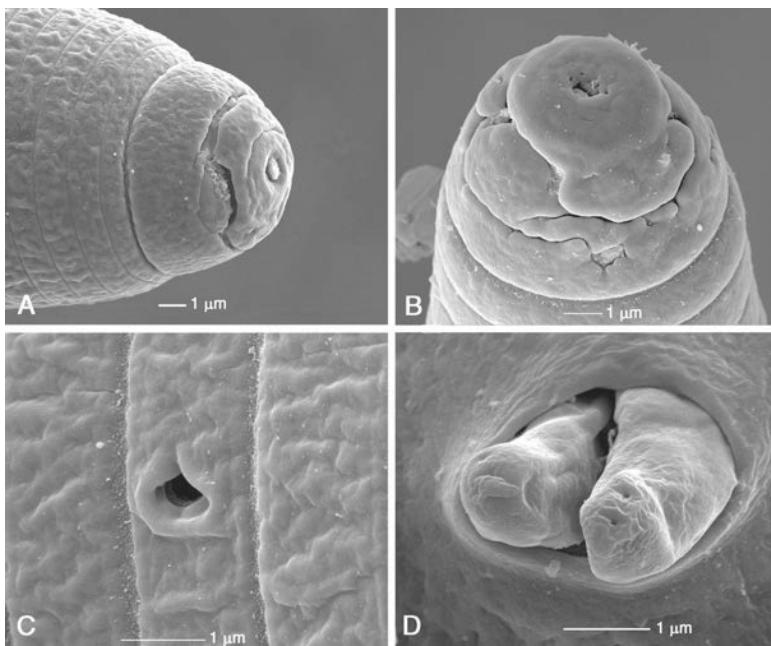


Fig. 3.11. Male root-knot nematode scanning electron microscope photographs.
A: *Meloidogyne duytsi* head region (lateral view). B: *M. minor* anterior end.
C: *M. minor* S-E pore. D: *M. duytsi* spicule.

to the S-E pore. The pharyngeal gland nuclei are usually reduced to two. Usually one long testis is present; sometimes, however, two reduced ones can be observed in sex-reversed males. In most species, the lateral field has four incisures and the outer bands are often areolated. The tail is very short, bluntly rounded and without bursa. The small phasmids are positioned near the cloaca. The spicules are slender and 20–40 µm long, while the crescentic gubernaculum is about 10 µm long.

Second-stage juvenile. The infective J2 is vermiform, annulated and ranges in length from 250 to 600 µm (Fig. 3.12). The head structure is as in males but much smaller and with a weakly sclerotized cephalic framework. The delicate straight stylet is about 9–16 µm long, and the position of the DGO ranges from 2 to 12 µm behind the stylet knobs.

The metacorpus is relatively small with well-developed valve plates. The hemizonid is usually positioned anterior or posterior to the S-E pore. Three pharyngeal glands are present and ventrally overlap the intestine. Usually the rectum is clearly inflated. The tail is 15–100 µm long and tapers towards the tail tip and ends in a hyaline tail part (tail terminus). Phasmids are very small and located about one-third of the tail length below anus level. The lateral field has four incisures with the outer bands usually areolated.

The third- and fourth-stage juvenile stages are sedentary inside the root and swollen; they have no stylet and develop within the J2 cuticle. A detailed description of the ultrastructure of *Meloidogyne* is given by Bird and Bird (1991). Box 3.3 summarizes the criteria used for morphological identification of *Meloidogyne* species.

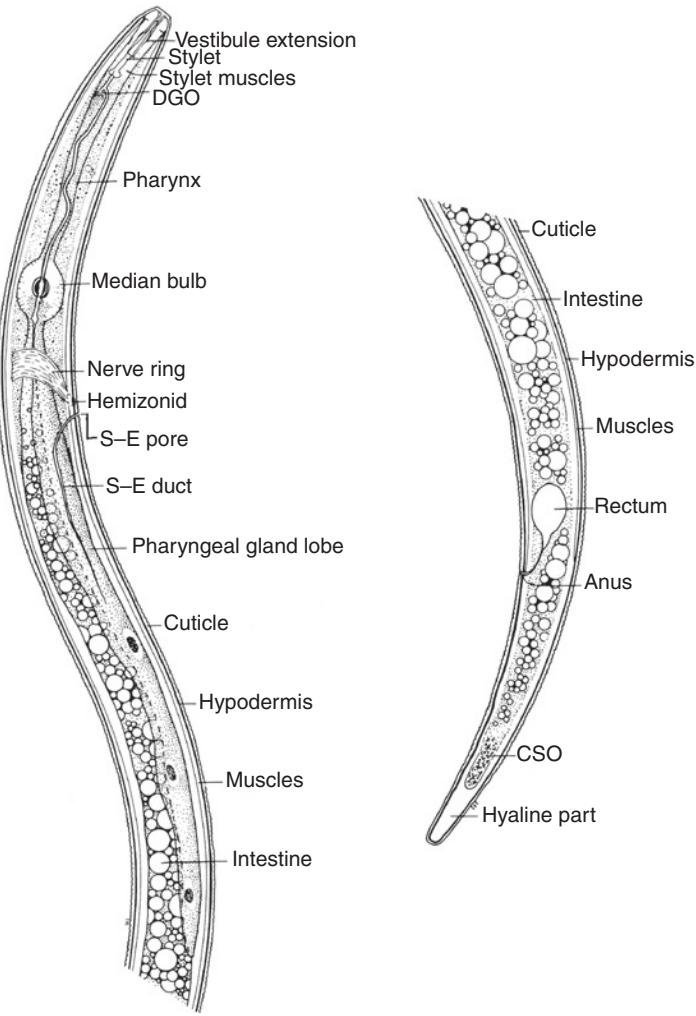


Fig. 3.12. Drawing of a second-stage juvenile root-knot nematode. (From Eisenback and Hirschmann Triantaphyllou, 1991.)

Box 3.3. Morphological identification.

Characters used for identification of root-knot nematodes using light microscopy are:

Female: Body shape, stylet length, knob shape and perineal pattern shape.

Male: Head shape, stylet length, knob shape and DGO-stylet knob length.

Second-stage juvenile: Body length, tail and hyaline tail length, DGO-stylet knob length, hemizonid position, tail and hyaline tail shape.

For a reliable morphological observation of *Meloidogyne* species, it is very important to use correct methods for extraction, isolation, microscopic preparation and observation, as described in detail by Jepson (1987).

3.9. Principal Species

A list of nominal root-knot nematodes and synonyms is given in Table 3.2. At the end of 2012, nearly 100 nominal species of root-knot nematodes had been described. The number of descriptions of new species continues to increase but, unfortunately, they are not always well described and compared with other species from the genus. It is not surprising that, at present, a satisfactory tool for the identification of all root-knot nematodes has not been developed. The following species of root-knot nematodes are economically important agricultural pests and described briefly (Figs 3.13 and 3.14); Jepson (1987) and Eisenback and Hirschmann Triantaphyllou (1991) provide extensive information on these and other species.

3.9.1. *Meloidogyne arenaria*

Female. Body pear-shaped and without posterior protuberance. Stylet 13–17 (16) µm long, knobs rounded and backwardly sloping. Perineal pattern rounded with low dorsal arch and distinct striae, lateral lines sometimes weakly visible.

Male. Head not offset from the body. Head cap rounded, labial disc not elevated, lateral lips absent. Stylet 20–27 (23) µm long, knobs rounded and backwardly sloping. DGO to stylet knobs: 4–8 (6) µm.

Second-stage juvenile. Body 400–600 µm long. Hemizonid anterior adjacent to the S–E pore. Tail long (45–70 µm) and slender, hyaline tail part 6–15 µm long, anterior region usually not clearly delimited, tip finely rounded to pointed.

Isozymes. Esterase A1, A2 and A3 type and malate dehydrogenase N1 and N3 type (Fig. 3.15). A few atypical esterase phenotypes have been described.

Hosts. Able to reproduce on many monocotyledonous and dicotyledonous plants.

Distribution. *Meloidogyne arenaria*, *M. incognita* and *M. javanica* are the three most common species of root-knot nematodes, although *M. arenaria* is not as common as the other two species. Known worldwide; in temperate regions restricted to greenhouses.

Remarks. Induces large galls, which sometimes appear as a string of pearls along the root.

3.9.2. *Meloidogyne artiellia*

Female. Body pear-shaped with short neck region and without posterior protuberance. Stylet 13–15 (14) µm long; knobs relatively small, ovoid and backwardly sloping. Perineal pattern striking, rounded with fine striae, lateral area with coarse ridges, dorsal arch angular and obscure lateral lines present.

Male. Head offset from the body. Head cap distinct, labial disc not elevated, lateral lips present. Stylet 15–19 (17) µm long, knobs backwardly sloping and ovoid shaped. DGO to stylet knobs: 4–6 µm.

Second-stage juvenile. Body 320–370 µm long. Hemizonid anterior adjacent to the S–E pore. Tail conical and short (19–25 µm), hyaline tail part 4–8 µm in length with a rounded tail tip.

Table 3.2. Nominal root-knot nematode species (*Meloidogyne* Göldi, 1887). For a species list including full synonymy, see Hunt and Handoo (2009).

Type species

M. exigua Göldi, 1887

syn. *Heterodera exigua* (Göldi, 1887) Marcinowski, 1909

Species

M. acronea Coetzee, 1956

syn. *Hypsoperine acronea* (Coetzee, 1956) Sledge & Golden, 1964

Hypsoperine (Hypsoperine) acronea Coetzee, 1956 (Siddiqi, 1986)

M. actinidiae Li & Yu, 1991

M. africana Whitehead, 1960

M. aquatilis Ebsary & Eveleigh, 1983

M. arabicida López & Salazar, 1989

M. ardenensis Santos, 1968

syn. *M. deconincki* Elmiligy, 1968

M. litoralis Elmiligy, 1968

M. arenaria (Neal, 1889) Chitwood, 1949

syn. *Anguillula arenaria* Neal, 1889

Heterodera arenaria (Neal, 1889) Marcinowski, 1909

M. arenaria arenaria (Neal, 1889) Chitwood, 1949

M. arenaria thamesi Chitwood, 1952

M. thamesi (Chitwood, 1952) Goodey, 1963

M. thamesi gyulai Amin, 1993

M. gyulai Amin, 1993

M. artiellia Franklin, 1961

M. baetica Castillo, Vovlas, Subbotin & Troccoli, 2003

M. brasiliensis Charchar & Eisenback, 2002

M. brevicauda Loos, 1953

M. californiensis Abdel-Rahman & Maggenti, 1987

M. camelliae Golden, 1979

M. caraganae Shagalina, Ivanova & Krall, 1985

M. carolinensis Eisenback, 1982

M. chitwoodi Golden, O'Bannon, Santo & Finley, 1980

M. chosenia Eroshenko & Lebedeva, 1992

M. christiei Golden & Kaplan, 1986

M. cirriformis Zhang, 1991

M. citri Zhang, Gao & Weng, 1990

M. coffeicola Lordello & Zamith, 1960

syn. *Meloidodera coffeicola* (Lordello & Zamith, 1960) Kirjanova, 1963

M. cruciani García-Martínez, Taylor & Smart, 1982

M. cynariensis Pham, 1990

M. decalineata Whitehead, 1968

M. donghaiensis Zheng, Lin & Zheng, 1990

M. dunensis Palomares Rius, Vovlas, Troccoli, Liébanas, Landa & Castillo, 2007

M. duyseni Karssen, van Aelst & van der Putten, 1998

M. enterolobii Yang & Eisenback, 1983

syn. *M. mayaguensis* Rammah & Hirschmann, 1988

M. ethiopica Whitehead, 1968

M. fallax Karssen, 1996

Continued

Table 3.2. Continued.

- M. fanzhiensis* Chen, Peng & Zheng, 1990
M. floridensis Handoo, Nyczepir, Esmenjaud, van der Beek, Castagnone-Sereno, Carta, Skantar & Higgins, 2004
M. fujianensis Pan, 1985
M. graminicola Golden & Birchfield, 1965
M. graminis (Sledge & Golden, 1964) Whitehead, 1968
syn. *Hypsoperine graminis* Sledge & Golden, 1964
Hypsoperine (Hypsoperine) graminis Sledge & Golden, 1964 (Siddiqi, 1986)
M. hainanensis Liao & Feng, 1995
M. hapla Chitwood, 1949
M. haplanaria Eisenback, Bernard, Starr, Lee & Tomaszewski, 2003
M. hispanica Hirschmann, 1986
M. ichinohei Araki, 1992
M. incognita (Kofoid & White, 1919) Chitwood, 1949
syn. *Oxyuris incognita* Kofoid & White, 1919
Heterodera incognita (Kofoid & White, 1919) Sandground, 1923
M. incognita incognita (Kofoid & White, 1919) Chitwood, 1949
M. incognita acrita Chitwood, 1949
M. acrita (Chitwood, 1949) Esser, Perry & Taylor, 1976
M. kirjanovae Terenteva, 1965
M. elegans da Ponte, 1977
M. grahami Golden & Slana, 1978
M. incognita wartellei Golden & Birchfield, 1978
M. indica Whitehead, 1968
M. inornata Lordello, 1956
syn. *M. incognita inornata* Lordello, 1956
M. izalcoensis Carneiro, Almeida, Gomes & Hernández, 2005
M. javanica (Treub, 1885) Chitwood, 1949
syn. *Heterodera javanica* Treub, 1885
Anguillula javanica (Treub, 1885) Lavergne, 1901
M. javanica javanica (Treub, 1885) Chitwood, 1949
M. javanica bauruensis Lordello, 1956
M. bauruensis (Lordello, 1956) Esser, Perry & Taylor, 1976
M. lordelloi da Ponte, 1969
M. lucknowica Singh, 1969
M. jianyangensis Yang, Hu, Chen & Zhu, 1990
M. jinanensis Zhang & Su, 1986
M. kikuyensis De Grisse, 1960
M. konaensis Eisenback, Bernard & Schmitt, 1994
M. kongi Yang, Wang & Feng, 1988
M. kralli Jepson, 1984
M. lini Yang, Hu & Xu, 1988
M. lusitanica Abrantes & Santos, 1991
M. mali Itoh, Oshima & Ichinohe, 1969
M. maritima Jepson, 1987
M. marylandi Jepson & Golden, in Jepson, 1987
M. megadora Whitehead, 1968
M. megatyla Baldwin & Sasser, 1979
M. mersa Siddiqi & Booth, 1992

Continued

Table 3.2. Continued.

- M. microcephalus* Cliff & Hirschmann, 1984
M. microtyla Mulvey, Townshend & Potter, 1975
M. mingnanica Zhang, 1993
M. minor Karssen, Bolk, v. Aelst, v.d. Beld, Kox, Korthals, Molendijk, Zijlstra, v. Hoof & Cook, 2004
M. morocciensis Rammah & Hirschmann, 1990
M. naasi Franklin, 1965
M. nataliei Golden, Rose & Bird, 1981
M. oryzae Maas, Sanders & Dede, 1978
M. oteifae Elmiligy, 1968
M. ottersoni (Thorne, 1969) Franklin, 1971
syn. *Hypsoperine ottersoni* Thorne, 1969 (Siddiqi, 1986)
M. ovalis Riffle, 1963
M. panyuensis Liao, Yang, Feng & Karssen, 2005
M. paranaensis Carneiro, Carneiro, Abrantes, Santos & Almeida, 1996
M. partityla Kleynhans, 1986
M. petuniae Charchar, Eisenback & Hirschmann, 1999
M. phaseoli Charchar, Eisenback, Charchar & Boiteau, 2008
M. pini Eisenback, Yang & Hartman, 1985
M. piperi Sahoo, Ganguly & Eapen, 2000
M. pisi Charchar, Eisenback, Charchar & Boiteau, 2008
M. platani Hirschmann, 1982
M. polycephannulata Charchar, Eisenback, Vieira, Fonseca-Boiteux & Boiteux, 2009
M. propora Spaull, 1977
syn. *Hypsoperine (Hypsoperine) propora* Spaull, 1977 (Siddiqi, 1986)
M. querciana Golden, 1979
M. salasi López, 1984
M. sasseri Handoo, Huettel & Golden, 1993
M. sewelli Mulvey & Anderson, 1980
M. silvestris Castillo, Vovlas, Troccoli, Liébanas, Palomares Rius & Landa, 2009
M. sinensis Zhang, 1983
M. spartinae (Rau & Fassuliotis, 1965) Whitehead, 1968
syn. *Hypsoperine spartinae* Rau & Fassuliotis, 1965
Hypsoperine (Hypsoperine) spartinae Rau & Fassuliotis, 1965 (Siddiqi, 1986)
M. subarctica Bernard, 1981
M. suginamiensis Toida & Yaegashi, 1984
M. tadzhikistanica Kirjanova & Ivanova, 1965
M. thailandica Handoo, Skantar, Carta & Erbe, 2005
M. trifoliophila Bernard & Eisenback, 1997
M. triticoryzae Gaur, Saha & Khan, 1993
M. turkestanica Shagalina, Ivanova & Krall, 1985
M. ulmi Palmisano & Ambrogioni, 2001
M. vandervegtiae Kleynhans, 1988

Species inquirendae

- M. marioni* (Cornu, 1879) Chitwood & Oteifa, 1952
syn. *Anguillula marioni* Cornu, 1879
Heterodera marioni (Cornu, 1879) Marcinowski, 1909
M. megriensis (Poghossian, 1971) Esser, Perry & Taylor, 1976
syn. *Hypsoperine megriensis* Poghossian, 1971

Continued

Table 3.2. Continued.

- Hypsoperine (Hypsoperine) megriensis* Poghossian, 1971 (Siddiqi, 1986)
M. poghossianae Kirjanova, 1963
syn. *M. acronea apud* Poghossian, 1961
M. vialae (Lavergne, 1901) Chitwood & Oteifa, 1952
syn. *Anquillula vialae* Lavergne, 1901
Heterodera vialae (Lavergne, 1901) Marcinowski, 1909

Nomina nuda

- M. californiensis* Abdel-Rahman, 1981
M. carolinensis Fox, 1967
M. goeldii Santos, 1997
M. panyuensis Liao, 2001
M. zhanjiangensis Liao, 2001

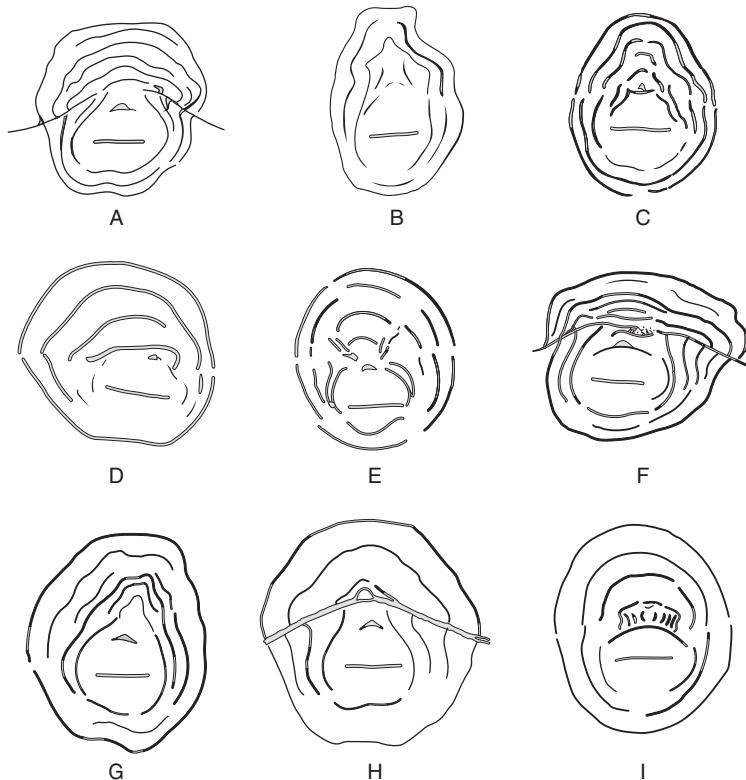


Fig. 3.13. Root-knot nematode perineal patterns. A: *Meloidogyne arenaria*. B: *M. artiellia*. C: *M. chitwoodi/M. fallax*. D: *M. exigua*. E: *M. graminicola*. F: *M. hapla*. G: *M. incognita*. H: *M. javanica*. I: *M. naasi*. (Redrawn after Jepson, 1987.)

Isozymes. Esterase M2-VF1 type and malate dehydrogenase N1b type.

Hosts. Detected on *Brassica napus*, *B. oleracea*, *Cicer arietinum*, *Vicia sativa*, *Avena sativa* and *Triticum vulgare*. Experimental studies indicate that nearly all members of the Brassicaceae, Fabaceae and Poaceae are good hosts for *M. artiellia*.

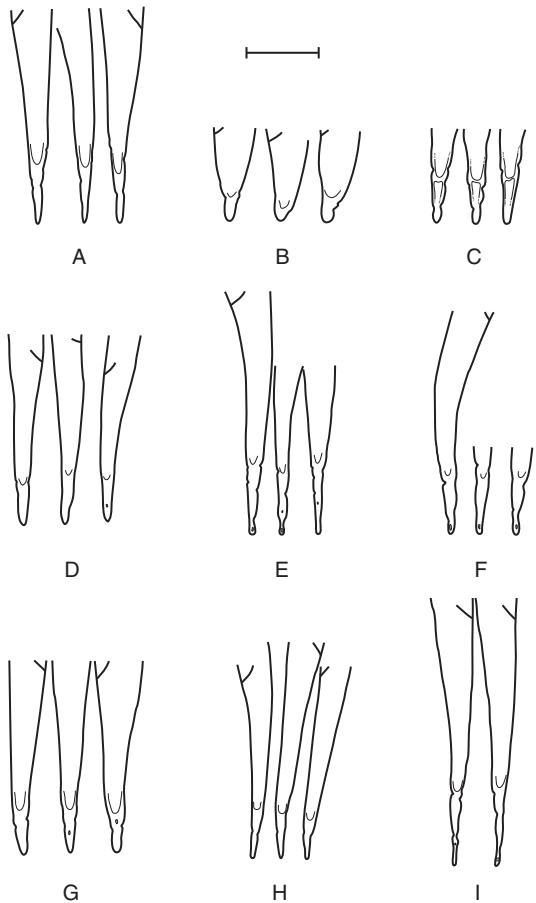


Fig. 3.14. Root-knot nematode second-stage juvenile tails.

A: *Meloidogyne arenaria*. B: *M. artiellia*. C: *M. chitwoodi/M. fallax*. D: *M. exigua*. E: *M. graminicola*. F: *M. hapla*. G: *M. incognita*. H: *M. javanica*. I: *M. naasi* (scale bar = 20 µm). (Redrawn after Jepson, 1987).

Distribution. In Europe reported from Belgium, France, Greece, Italy, Spain and UK. Outside Europe it has been found in Israel and Syria.

Remarks. The J2 are relatively small and can be overlooked easily in soil samples. Usually this species induces very small galls, sometimes with lateral root proliferation.

3.9.3. *Meloidogyne chitwoodi*

Female. Body rounded with short neck and slight posterior protuberance. Stylet 11–14 (13) µm long, knobs small, oval to irregularly shaped and backwardly sloping. S-E pore just below stylet knob level. Perineal pattern rounded to oval, striae relatively coarse and lateral lines weakly visible.

Male. Head not offset from the body. Head cap rounded, labial disc not elevated, lateral lips present. Stylet 17–19 (18) µm long, knobs small, oval to irregular shaped and backwardly sloping. DGO to stylet knobs: 2.5–4.0 µm.

Second-stage juvenile. Body 360–420 µm long. Hemizonid anterior and adjacent to the S-E pore. Tail conical and 40–45 µm long; hyaline tail part 9–12 µm long, anterior region clearly delimited, tail tip bluntly rounded.

Isozymes. Esterase S1 type and malate dehydrogenase N1a type.

Hosts. Able to parasitize many mono- and dicotyledonous hosts, including economically important crops such as potato (tubers), wheat and maize. Usually it induces relatively small galls.

Distribution. A major pest in the Pacific Northwest region of the USA, also known from eight other states in the USA and Mexico. Also reported from Europe (Belgium, France, Germany, Portugal, Switzerland and The Netherlands), Russia, South Africa, Australia and South America (Argentina).

Remarks. Listed in many countries as a quarantine organism.

3.9.4. *Meloidogyne enterolobii*

Female. Body pear-shaped without posterior protuberance. Stylet 13–18 (15) µm long, knobs oval, anteriorly often indented, slightly sloping backward to offset. Perineal pattern oval shaped, striae mostly fine, dorsal arch rounded to square, weak lateral line(s) sometimes present. General pattern shape as in *M. incognita*.

Male. Head slightly offset from the body. Head cap high and rounded, head region slightly offset, not annulated, lateral lips usually not present. Stylet 20–24 (22) µm long, knobs large, ovoid to rounded, slightly sloping backwards. DGO to stylet knobs: 3.5–6 µm.

Second-stage juvenile. Body 380–460 µm long. Hemizonid anterior and adjacent to the S-E pore. Tail slender and 45–60 µm long; anterior hyaline tail part not clearly delimited, usually nearly straight and parallel, tapering to rounded tip.

Isozymes. Esterase VS1-S1 type and malate dehydrogenase N1a type.

Distribution. Recorded from Africa, Asia, North and South America and found in Europe (glasshouses).

Remarks. This species belongs to the so-called *M. incognita* group, and is well known for its resistance-breaking behaviour. *Meloidogyne mayaguensis* is a junior synonym for *M. enterolobii*.

3.9.5. *Meloidogyne exigua* (type species)

Female. Body rounded, relatively small with short neck. Stylet 12–15 (14) µm long, knobs small, rounded and offset. Perineal pattern rounded to oval with coarse striae, lateral lines absent.

Male. Head not offset from the body. Head cap rounded, labial disk not elevated, lateral lips usually present. Stylet 15–19 (18) µm long, knobs small, rounded and offset. DGO to stylet knobs: 2–4 µm.

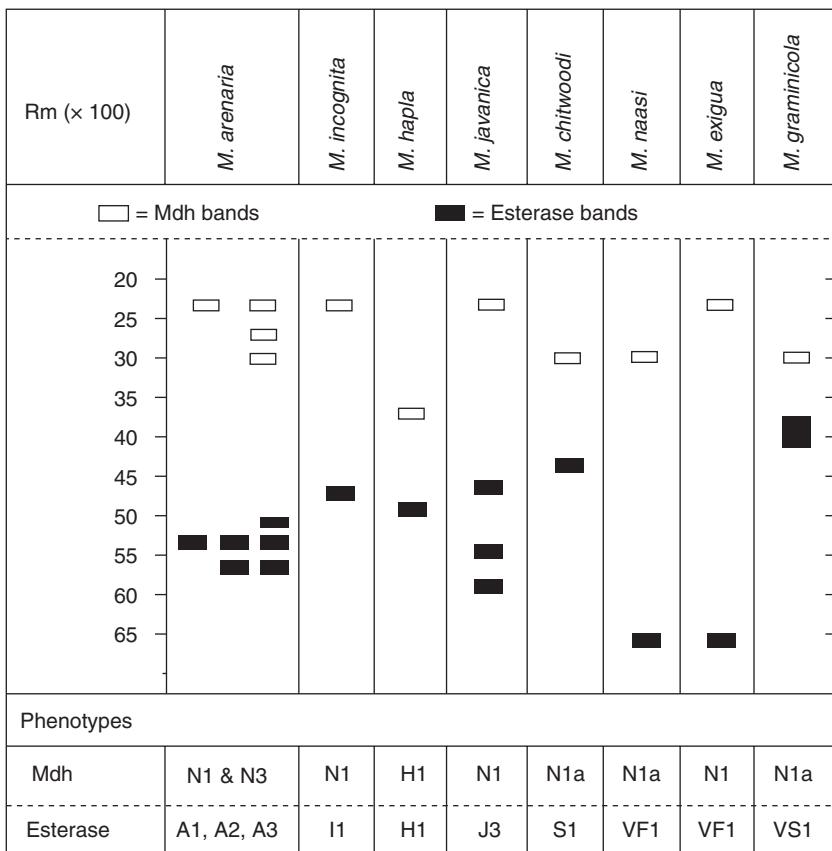


Fig. 3.15. Malate dehydrogenase (Mdh) and esterase isozyme phenotypes of several species of *Meloidogyne*. Rm: relative movement. (From Eisenback and Hirschmann Triantaphyllou (1991), plus information on codes for Mdh and esterase.)

Second-stage juveniles. Body 330–360 μm long. Hemizonid anterior and adjacent to the S-E pore. Tail conical and 42–48 μm in length; hyaline tail part 12–14 μm long, narrow constricting annulations often present near anterior region, tail tip bluntly rounded.

Isozymes. Esterase VF1 type and malate dehydrogenase N1 type.

Hosts. The main host is coffee; a number of other dicotyledonous hosts are also known.

Distribution. Mainly known from South America, Central America and the Caribbean; incidentally reported from Europe and Asia.

Remarks. On coffee, this species usually induces elongated galls. Other root-knot nematode species described from coffee are: *M. africana*, *M. arabicida*, *M. coffeicola*, *M. decalineata*, *M. konaensis*, *M. megadora* and *M. paranaensis*. Also *M. arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, *M. kikuyensis*, *M. enterolobii* and *M. oteifae* have been reported from coffee.

3.9.6. *Meloidogyne fallax*

Female. Body rounded with short neck and slight posterior protuberance. Stylet 13.5–15.5 (14.5) µm long; knobs large and rounded, slightly sloping posteriorly. S–E pore just below stylet knob level. Perineal pattern rounded to oval, striae relatively coarse and lateral lines weakly visible.

Male. Head slightly offset from the body. Head cap rounded, labial disc elevated, lateral lips present. Stylet 19–21 (20) µm long, knobs large, rounded and offset. DGO to stylet knobs: 3–6 µm.

Second-stage juvenile. Body 380–440 µm long. Hemizonid at S–E pore level. Tail conical and 40–45 µm in length; hyaline tail part 12–16 µm long, anterior region clearly delimited, tail tip bluntly rounded.

Isozymes. Esterase ‘0’ type (after prolonged staining a weak F3 type appears) and malate dehydrogenase N1b type.

Hosts. Like *M. chitwoodi* able to parasitize many mono- and dicotyledonous hosts, including economically important crops like potato (tubers) and wheat. *Meloidogyne fallax* and *M. chitwoodi* have similar hosts but species specificity has also been found. Usually induces relatively small galls.

Distribution. Known from Europe (Belgium, France, Germany, Switzerland and The Netherlands), South Africa, Australia and New Zealand (widely distributed).

Remarks. Closely related to *M. chitwoodi* and also listed in many countries as a quarantine organism.

3.9.7. *Meloidogyne graminicola*

Female. Body elongated with slight posterior protuberance. Stylet 12.5–15 (13.5) µm in length, knobs ovoid and offset. Perineal pattern rounded to slightly oval with smooth striae, without lateral lines.

Male. Head not offset from the body. Head cap rounded, labial disc not elevated, lateral lips usually present. Stylet 15.5–19.5 (17.5) µm long, knobs ovoid and offset. DGO to stylet knobs: 3–4 µm.

Second-stage juvenile. Body 410–480 µm long. Hemizonid anterior and adjacent to S–E pore. Tail slender and 60–80 µm long, small hyaline tail part 16–25 µm long. Tail tip finely rounded.

Isozymes. Esterase VS1 and malate dehydrogenase N1c type.

Hosts. Rice is the most important host, but many grasses and dicotyledonous weeds are known as good hosts.

Distribution. Common species found in all major rice-growing areas.

Remarks. Galls relatively large, often terminal and curved at root tips. Other root-knot nematodes described from rice are: *M. hainanensis*, *M. lini*, *M. oryzae*, *M. salasi* and *M. triticioryzae*. Also *M. arenaria*, *M. incognita* and *M. javanica* have been reported from rice.

3.9.8. *Meloidogyne hapla*

Female. Body pear-shaped without posterior protuberance. Stylet ranging in length from 13 to 17 (15) μm , knobs relatively small, rounded and offset. Perineal pattern with fine striae, rounded with low dorsal arch, punctuations usually present above the anus, lateral lines present.

Male. Head clearly offset from the body. Head cap rounded, labial disc usually not elevated, lateral lips present. Stylet 19–22 (20.5) μm long; knobs relatively small, rounded and offset. DGO to stylet knobs: 4–5 μm long.

Second-stage juvenile. Body 360–500 μm long. Hemizonid anterior not adjacent to the S–E pore. Tail slender and 48–70 μm in length; hyaline tail part often irregular shaped, 12–19 μm long; anterior region not clearly delimited, tail tip finely rounded.

Isozymes. Esterase H1 type and malate dehydrogenase H1 type.

Hosts. Many dicotyledonous hosts are known including important food crops and ornamentals. Records of monocotyledonous hosts are rare.

Distribution. Recorded worldwide from temperate regions; also found in tropical and subtropical regions at higher altitudes.

Remarks. Induces relatively small galls, often with secondary roots. Undoubtedly morphologically the most variable species of the genus.

3.9.9. *Meloidogyne incognita*

Female. Body pear-shaped without posterior protuberance. Stylet ranging in length from 15 to 16 μm , knobs rounded and offset. Perineal pattern usually with relatively high dorsal arch and without lateral lines.

Male. Head not offset from the body. Head cap with elevated labial disc, usually without lateral lips, head region often with incomplete head annulations. Stylet 23–26 (24.5) μm long, knobs rounded to oval shaped and offset. DGO to stylet knobs: 2–4 μm long.

Second-stage juvenile. Body 350–450 μm long. Hemizonid anterior or adjacent to the S–E pore. Tail slender and 43–65 μm in length; hyaline tail part 6–14 μm long, anterior region clearly delimited, tail tip rounded.

Isozymes. Esterase I1 type and malate dehydrogenase N1 type.

Hosts. Able to reproduce on many monocotyledonous and dicotyledonous plants.

Distribution. *Meloidogyne incognita*, together with *M. arenaria* and *M. javanica*, are the most common root-knot nematodes. Known worldwide, restricted in the temperate regions to greenhouses.

Remarks. Induces usually large galls. Some agriculturally important populations, initially identified as *M. incognita*, have been described as new species, i.e. *M. brasiliensis*, *M. enterolobii*, *M. hispanica*, *M. floridensis* and *M. microtyla*. It is very likely that more of these relatively important *M. incognita*-like populations will be described in the near future, making identification in this *M. incognita* group more complex.

3.9.10. *Meloidogyne javanica*

Female. Body pear-shaped without posterior protuberance. Stylet ranging in length from 14 to 18 (16) μm , knobs ovoid and offset. Perineal pattern rounded with distinct lateral lines.

Male. Head not offset from the body. Head cap rounded and offset, usually labial disc not elevated and lateral lips not present. Stylet 19–23.5 (20.5) μm long, knobs well developed, ovoid and offset. DGO to stylet knobs: 3–5.5 μm .

Second-stage juvenile. Body 400–560 μm long. Hemizonid anterior and adjacent to the S–E pore. Tail slender and 47–60 μm in length; hyaline tail part 9–18 μm long, tail tip finely rounded.

Isozymes. Esterase J3 type and malate dehydrogenase N1 type.

Hosts. Able to reproduce on many monocotyledonous and dicotyledonous plants.

Distribution. Belongs together with *M. arenaria* and *M. incognita* to the most common root-knot nematodes. It is known worldwide, but restricted in the temperate regions to greenhouses.

Remarks. Usually induces relatively large galls.

3.9.11. *Meloidogyne naasi*

Female. Body rounded with slight posterior protuberance. Stylet ranging in length from 13 to 15 (14) μm ; knobs ovoid and backwardly sloping. S–E pore near stylet knob level. Perineal pattern rounded to oval with prominent phasmids, relatively coarse striae, without lateral lines.

Male. Head offset from the body, labial disc not elevated and lateral lips not present. Stylet 16–18.5 (18) μm long, knob shape as in females. DGO to stylet knobs: 2.5–3.5 μm . Small vesicles are usually present in the anterior lumen lining of the metacorpus.

Second-stage juvenile. Ranging in length from 410 to 460 μm . Anterior metacorpial lumen lining with several (2–10) small vesicles. Hemizonid anterior adjacent to the S–E pore. Tail 60–75 μm in length and slender; hyaline tail part 16–25 μm in length with a fine rounded tail tip.

Isozymes. Esterase VF1 type and malate dehydrogenase N1a type.

Hosts. Reported from several grasses (including golf course and lawn grasses) and cereals. Rarely reported from dicotyledonous plants. Galls are relatively small, sometimes terminal, hooked or with lateral roots. Females usually completely embedded within the root tissue.

Distribution. Widely distributed in Europe; also reported from North and South America and occasionally from Asia.

Remarks. The metacorpial vesicles of males and J2 can be observed easily in fresh material; in permanent slides they are hardly or not visible. These vesicles are also present in *M. sasseri* J2 but are smaller compared with *M. naasi*.

3.10. Biochemical and Molecular Identification

As with other genera, characterization of species of *Meloidogyne* includes the use of biochemical and molecular techniques. These are discussed in detail in Chapter 2, so only a brief mention is included here.

3.10.1. Protein electrophoresis

Esbenshade and Triantaphyllou (1985) described a method to identify females of several *Meloidogyne* species with isozyme electrophoresis. In particular, esterase (EC 3.1.1.1) and malate dehydrogenase (EC 1.1.1.37) isozyme patterns discriminate most species clearly and, therefore, are used for routine identification of root-knot nematodes (Fig. 3.15). It has become a relatively simple exercise to run electrophoresis with precast polyacrylamide gels on semi-automatic units. These systems are very sensitive as only a single young egg-laying female is needed; therefore, they are useful for early detection of species mixtures (Karssen *et al.*, 1995). Additionally, 27 isozymes have been used for a robust study of the enzymatic relationship and evolution within the genus *Meloidogyne* (Esbenshade and Triantaphyllou, 1987).

Two-dimensional gel electrophoresis has also been used to study the total soluble protein patterns of root-knot nematodes. It was used with *M. hapla*, *M. chitwoodi* and *M. fallax* and confirmed these species as distinct biological groups. Antisera have also been used to discriminate *M. chitwoodi* and *M. fallax* from other root-knot nematodes, using a combination of two-dimensional gel electrophoresis, internal amino acid sequencing and serology.

3.10.2. PCR

Another powerful method to identify *Meloidogyne* species is the use of the polymerase chain reaction (PCR). PCR amplification and restriction fragment length polymorphism (RFLP) of the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA) are applied in many laboratories to differentiate common root-knot nematodes. Another rapid PCR identification method is based on amplification of the rDNA intergenic spacer (IGS) regions without the need for restriction enzyme digestion. In addition, species-specific primers have been developed for some root-knot nematode species and are based on unique sequences within ribosomal IGS or designed from species-specific randomly amplified polymorphic DNA (RAPD) fragments (see Blok and Powers (2009) for a review of biochemical and molecular identification of *Meloidogyne* spp.). Satellite DNA probes and amplified fragment length polymorphisms (AFLPs) have also been used successfully to separate root-knot nematodes. RAPD was used to examine the molecular diversity between populations of some *Meloidogyne* species. The first phylogenetic analysis, based on 18S rDNA sequences of 13 species, was published by De Ley *et al.* (2002). A schematic overview of the phylogeny of the Meloidogynidae derived from SSU rDNA sequence data of many more species is shown in Chapter 2.

3.11. Interactions with Other Plant Pathogens

In nature, plants are rarely exposed to the influence of a single pathogen. Roots usually host a great number of microorganisms whose action is often combined to induce damage. *Meloidogyne* frequently plays a major role in disease interactions (Kahn, 1993).

3.11.1. *Meloidogyne* as a wounding agent

Nematode parasitism of plants is associated with wounding their hosts. For a long time wounding was considered of paramount importance in facilitating the entry of fungal pathogens, with wounds acting as a passage or portal. However, the wound-facilitation concept in the fungus–nematode interaction, especially between *Fusarium* and *Meloidogyne*, was later disproved. Modification of host substrates was found to be primarily responsible for such interactions. There is evidence that predisposition to infection by *Fusarium* wilt fungi occurs through translocatable metabolites produced at the site of nematode infection.

Several studies indicated that root-knot nematodes cause wounds that facilitate entry of bacteria and that modify the host tissue to enrich the substrate nutritionally to the advantage of the bacteria. The role of *Meloidogyne* as a wounding agent has been observed for several combinations of bacterium and nematode; a well-known example of this kind of interaction is the combination of *Ralstonia solanacearum* and *M. incognita* on tobacco. The interaction between these two pathogens may be synergistic because the wilt symptoms are more severe when nematodes and bacteria are inoculated together than when bacteria alone are inoculated on artificially wounded roots. *Meloidogyne*-modified tissue acts as a more favourable substrate for bacteria; the nematode causes physiological changes in the host substrate. On tomato cultivars susceptible or resistant to *Clavibacter michiganensis* subsp. *michiganensis*, the presence of *M. incognita* increased the bacterial canker only when the nematode was inoculated before the bacterium (Moura *et al.*, 1975). Such an interaction did not occur when both pathogens were inoculated simultaneously or the bacterium was inoculated before the nematode.

3.11.2. Effects of *Meloidogyne* on host susceptibility

Root-knot nematode infection of *Fusarium*-infected plants increases the wilt symptom expression and death rate of the plants. Evidence indicates that the root diffusates from root-knot infected plants stimulate the fungus in the rhizosphere. These diffusates also suppress actinomycetes, which are antagonists of *Fusarium*. It seems that the nature of interactions between root-knot nematodes and *Fusarium* wilt fungi is not physical, by the nematode puncturing the plant root, but is physiological.

The giant cells induced by *Meloidogyne* juveniles remain in a state of high metabolic activity through the continuous stimulation by the nematode. They are probably the major site of interaction between the nematode and *Fusarium*. The giant cells remain in a continuous juvenile state, which delays maturation and suberization of

other vascular tissues, and thus *Fusarium* successfully penetrates and establishes in the xylem elements. Inhibition of tylose formation by root-knot nematodes has also been suggested as a possible mechanism for increased wilt severity (Webster, 1985).

Like wilt fungi, root-rot fungi are capable of causing disease on their own and have their own inherent mechanism of root penetration. The role of *Meloidogyne* in root-rot diseases in general is related to assisting the fungal pathogen in its pathogenesis and increasing host susceptibility. The invasion tracks formed by penetrating juveniles of root-knot nematodes provide a better substratum for the establishment of the fungal pathogens. Physiological alterations, which ensure better nutrient availability for the penetrated fungal pathogen, are the key factor of the synergistic damage caused to the host.

Fungi that are non-pathogenic to a host plant may become pathogenic in the presence of nematodes. Root-knot nematodes possess outstanding abilities to cause physiological changes in plants that can induce susceptibility to attack by fungi present in the rhizosphere, whether pathogenic or non-pathogenic.

Root-knot nematodes break the monogenic *Fusarium* wilt resistance in tomato cultivars. Histopathological changes in the host, caused by nematode infection, apparently are responsible for rendering the gene(s) for resistance ineffective and as a result the host is not able to express the resistant reaction. By contrast, there are reports which indicate that *Fusarium* wilt resistance in cultivars of cabbage, peas and other crops, possessing a single gene for resistance against wilt fungi, is not altered by root-knot nematode infection. The multigenic resistance to *Fusarium* wilt in cotton is less effective when plants are infected by *Meloidogyne* but is not broken.

3.11.3. Interactions of *Meloidogyne* with other nematodes

Plant-parasitic nematodes frequently parasitize plants in mixed populations of two or more genera and species. Interaction may enhance or inhibit nematode reproduction, which is ultimately the driving force in interspecific competition. Competition is usually strongest between organisms that are most alike with respect to their physiological demands on the host (Eisenback, 1993).

Ectoparasites feeding on the preferred penetration sites of the infective J2 of *Meloidogyne* may be a limiting factor in the success of the latter. Ectoparasites may damage the root system and thus indirectly reduce the number of feeding sites available for *Meloidogyne*. By contrast, *Meloidogyne* can suppress ectoparasites even though they are separated by plant tissue, probably by physiological mechanisms. Sometimes *Meloidogyne* and ectoparasitic nematodes are mutually antagonistic. However, interactions between these two groups may be beneficial for one or both species.

Interactions between migratory endoparasitic nematodes and *Meloidogyne* species may be time dependent, i.e. inhibition of the development of one of the components of the system by the other may disappear after some time. Interaction between these two groups may also depend on the sequence of their infection. In experimental studies, the mutual effect of *Meloidogyne* and migratory endoparasitic nematodes depends on whether both groups are inoculated simultaneously or one after the other (Chitamber and Raski, 1984). In addition, the change in physiology of the host plants by *Meloidogyne* affects the suitability of the host for migratory endoparasites; root-knot nematodes produce a translocatable factor that inhibits the reproduction

of *Pratylenchus* species (Estores and Chen, 1970). The effect of host suitability on nematode interactions is nematode density-dependent. Interspecific competition with *Radopholus similis* rendered frequency of populations and population levels of *M. incognita* very low in banana plantations (Moens *et al.*, 2006).

Two or more root-knot nematode species are commonly found in the same field, root system or gall. Factors other than competition for feeding sites may be important in the domination of a particular species. Temperature and other climatic factors may be important because certain species may be better adapted to cooler temperatures, whereas others are more common in warmer climates. Also plant resistance to one of the *Meloidogyne* species may influence the interaction and even persistence in the soil.

3.12. Management and Control

Management systems are built around key pests (organisms that cause significant reduction in crop yield every year unless some pest control action is taken). *Meloidogyne* species frequently attain that status, even in the presence of other plant pathogens. The basic objective in any pest management is to increase both quantity and quality of crop yield. With respect to *Meloidogyne*, increasing yield quality is sometimes the most important objective. Successful management starts with a correct identification of the *Meloidogyne* problem.

3.12.1. Prevention

Meloidogyne species are not found in seed but may be present in vegetative planting material such as corms, bulbs or roots. Obviously, planting materials are possible sources for passive dispersal. In plant parts, numbers of root-knot nematodes can be reduced by chemicals or hot water treatment; however, it is often better to discard infected material. It is recommended to use only certified nematode-free plants from reliable nurseries or seedlings produced in *Meloidogyne*-free seedbeds. Root-knot nematodes may also be disseminated via farm machinery or vehicles to which contaminated soil sticks. Cleaning machinery before and after use is recommended, but generally not practised. To reduce risks via international trade, several root-knot nematode species have been listed as quarantine organisms (see Chapter 12).

3.12.2. Crop rotation

Meloidogyne species are obligate and specialized parasites and each has species and cultivars of plants where the susceptibility ranges from highly susceptible to immune. Populations of *Meloidogyne* in a field without host plants will become non-infective and die of starvation. In crop rotations for controlling *Meloidogyne* species, susceptible crops are rotated with immune or resistant crops. Susceptible hosts of the four most common species, *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*, are numerous and belong to many plant families. However, rotations have been developed and several Gramineae are effective in reducing these *Meloidogyne* species. Other widely

distributed species (e.g. *M. naasi* and *M. graminis*) have hosts which belong to only a few plant families, whilst other species (e.g. *M. partityla*, *M. kralli* and *M. ichinohei*) have a very restricted host range. With these species, the development of effective rotations for control is easier. The North Carolina Differential Host Test (Hartman and Sasser, 1985) not only separates the four most common species, it further distinguishes four races of *M. incognita* and two races of *M. arenaria*. Host assays separate the known two races of *M. chitwoodi* and their respective pathotypes (Brown *et al.*, 2009). Despite statistically significant morphological variation among adult females of *M. chitwoodi* from the USA, morphometrics were not able to distinguish between isolates representing these races and pathotypes, and molecular traits, determined by nuclear ribosomal genes, were stable across all isolates (Humphreys-Pereira and Elling, 2013). Crop rotations have been developed in relation to the presence or absence of these races. However, caution should be exercised in applying the results obtained in one location to another, as different populations of the same species of *Meloidogyne* may react differently on the same plant species (Moens *et al.*, 2009).

Trap crops allow the infective J2 to enter the roots but due to antagonistic plant responses *Meloidogyne* fail to complete their life cycle. The effect of *Tagetes* species on populations of *Meloidogyne* species is highly variable, depending on the combination of species and cultivar of *Tagetes* and the species and race of nematode. It appears that reduction of *M. incognita* by marigold (*Tagetes patula*) is due primarily to an antagonistic or trap crop effect. J2 enter roots but there is neither giant cell formation nor a hypersensitive reaction. Rocket (arugula) (*Eruca sativa*) has shown potential as a trap crop for *M. hapla*.

Fallow periods can be included in crop rotations and have been shown to be successful in vegetable production. However, lack of income and detrimental effects from soil erosion limit the use of fallowing. Finally, one should always be aware that *Meloidogyne* species can reproduce on many weeds, and the presence of weeds in the field can compromise the success of rotations.

3.12.3. Cultural methods and physical control

Root destruction and organic amendments can be practised to reduce the number and impact of root-knot nematodes (see Chapter 14). Removing infected roots after harvest reduces root-knot soil infestation. Organic amendments, in addition to their positive influence on the soil physical structure and water holding capacity, have the capability of reducing nematode population densities to varying degrees. Control of *Meloidogyne* is rarely the primary reason for their use (Nyczepir and Thomas, 2009). However, brassicaceous amendments with a high glucosinolate level can enhance nematicidal effects.

Where water is abundant and fields are level, it is sometimes possible to control *Meloidogyne* species by flooding land to a depth of 10 cm or more for several months. Flooding does not necessarily kill the eggs and juveniles of root-knot nematodes by drowning; it inhibits infection and reproduction on any plant that grows while the field is flooded.

In some climates, *Meloidogyne* populations can be reduced by ploughing at intervals of 2–4 weeks during the dry season. This exposes eggs and juveniles to desiccation and many in the upper layers of soil are killed.

Frost killing, steaming and solarization can be used to reduce root-knot nematode numbers. *Meloidogyne* infestations of glasshouse soil can be reduced by exposing them to very low temperatures for a sufficient period of time. Steaming often fails to give satisfactory results for root-knot control, especially when survivors in the deeper layers of soil can build up infestations. Soil solarization (Gaur and Perry, 1991) is only adaptable to regions where sufficient solar energy is available for long periods of time.

3.12.4. Resistance

Resistant cultivars inhibit *Meloidogyne* reproduction. The reduction in nematode population density may permit the growing of susceptible crops more often in the rotation scheme. Cultivars resistant to root-knot nematode species are known for woody and non-woody plants, and for perennial and annual plants of various families. Their resistance is monogenic or polygenic. Sometimes they are resistant to a single *Meloidogyne* species (coffee and *M. exigua*); other cultivars show resistance to several species (tomato and *M. arenaria*, *M. incognita* and *M. javanica*). The Mi-1 gene conferring resistance in tomato to root-knot nematodes is inactivated at soil temperatures above 28°C. Resistance ‘breaking’ biotypes or Mi-virulent isolates of *Meloidogyne* have been reported in many areas of the world. Heat-stable resistance genes have been found in tomato. The use of resistant plants to combat *Meloidogyne* species and the associated molecular studies on resistance are detailed in Chapters 14 and 15.

3.12.5. Biological control

Many natural enemies attack *Meloidogyne* species in soil and reduce their populations. However, the number of organisms that can be used as biological control agents is restricted. Research on biological control agents has progressed beyond the initial stages and some are being field tested or even commercialized; however, none is widely used (see Chapter 13 and Hallmann *et al.*, 2009 for a comprehensive review of biological control of *Meloidogyne* spp.).

Endospores of the bacterium *Pasteuria penetrans* adhere to the cuticle of different nematodes and demonstrate some specificity against root-knot nematodes; they can survive in air-dried soil and are little affected by a range of nematicides. Amongst the many fungal antagonists for species of *Meloidogyne*, *Purpureocillium* (= *Paecilomyces*) *lilacinus* and *Pochonia chlamydospora* are the most studied for their control potential. *Purpureocillium lilacinus* is cosmopolitan but is present particularly in warmer regions. This facultative nematode egg parasite is primarily a saprophyte, being able to compete for and use a wide range of common substrates in soil. Strains of *P. lilacinus* have been developed for control use. *Pochonia chlamydospora* is widely distributed and a facultative parasite of the eggs of sedentary nematodes, including *Meloidogyne* spp. The fungus survives as a saprophyte in the absence of a host. It can be used as a biocontrol agent in integrated pest management strategies; the effect of the fungus on the nematode depends on the type of crop. Populations of this fungus may build up in soil and have beneficial effects in regulation of root-knot nematode populations.

3.12.6. Chemical control

Nematicides (see Chapter 16) are effective against *Meloidogyne* and can give good economic returns on high-value crops; however, in low-yielding crops chemical control is uneconomical. Treatment may not be sufficient for a year and re-treatment will usually be required the following year if plants susceptible to root-knot nematodes are to be grown. Fumigants are commonly applied as pre-plant treatments to reduce nematode numbers. In addition to broad-spectrum fumigants, non-fumigant nematicides have been shown to be at least moderately effective for controlling root-knot nematodes. They are applied to the soil as granular or liquid formulations. As they are not toxic to plants, they are the only chemical options for established plants.

4

Cyst Nematodes*

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* A revision of Turner, S.J. and Rowe, J.A. (2006) Cyst nematodes. In: Perry, R.N. and Moens, M. (eds) *Plant Nematology*, 1st edn. CAB International, Wallingford, UK.

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4.1. Introduction to Cyst Nematodes

The cyst nematodes are a major group of plant-parasitic nematodes and of great economic importance in many countries throughout the world. They cause considerable yield losses to many important crops, including cereals, rice, potatoes and soybean, with the most economically important species occurring within the genera *Heterodera* and *Globodera* (Table 4.1). *Heterodera* contains by far the largest number of species (Table 4.2), although several other cyst-forming species have been described within other genera (Table 4.3). Eight genera, *Heterodera* (82 species), *Globodera* (12 species), *Cactodera* (13 species), *Dolichodera* (1 species), *Paradolichodera* (1 species), *Betulodera* (1 species), *Punctodera* (4 species) and *Vittatidera* (1 species), and a total of 114 valid species are presently recognized within this nematode group (Subbotin *et al.*, 2010a,b). The classification of cyst nematodes is given in Box 4.1. Cyst nematodes were originally considered to be largely a pest of temperate regions but many cyst nematodes are now known to be present in tropical and subtropical regions (Evans and Rowe, 1998).

It is impossible to evaluate the total economic losses caused by cyst nematodes throughout the world as many environmental, biological and cultural factors cause yield losses to crops, although some specific examples of losses exist. Potato cyst nematodes (PCN) have been well studied within Europe, and overall losses are estimated at about 9% of potato production; however, in other regions of the world, or when no control strategies are employed, total losses can occur. This range could equally be applied to all major crops that are hosts to cyst nematodes.

All cyst nematodes feed within the root system of their hosts and are characterized by the tanning and drying (cutinization) of the body wall of the sedentary adult female following fertilization and production of embryonated eggs (see Section 4.2). The resultant cyst allows the succeeding generation to survive for extended periods until a suitable host is growing in the near vicinity. It is this ability to persist for many years in the soil in the absence of a host that contributes to the economic importance of this group in agricultural situations.

Table 4.1. Cyst nematodes of major economic importance. (Adapted from Evans and Rowe, 1998 and Subbotin *et al.*, 2010a,b.)

Genus	Species	Main crops affected	Region
<i>Globodera</i>	<i>pallida</i>	Potato, tomato, eggplant	Temperate
	<i>rostochiensis</i>	Potato, tomato, eggplant	Temperate
	<i>tabacum</i>	Tobacco, tomato	Temperate
<i>Heterodera</i>	<i>avenae</i>	Wheat, barley, oat, maize	Temperate
	<i>filipjevi</i>	Wheat, barley, oat, maize	Temperate
	<i>cajani</i>	Cowpea, pea, Phaseolus bean, pigeon pea, sesame, soybean, sweetcorn	Tropical
	<i>cruciferae</i>	Brussels sprout, broccoli, cabbage, cauliflower, radish, kohlrabi, pea, rape	Temperate
	<i>glycines</i>	Adzuki bean, broad bean, French bean, hyacinth bean, kidney bean, moth bean, mung bean, navy bean, rice bean, snap bean, soybean, blackgram, cowpea, sesame, white lupin, yellow lupin	Temperate
	<i>goettingiana</i>	Broad bean, chickpea, lentil, pea, white lupin, yellow lupin, white clover	Temperate
<i>Heterodera</i>	<i>latipons</i>	Barley, oat, rye	Temperate
	<i>oryzicola</i>	Rice, banana and plantain	Tropical
	<i>sacchari</i>	Rice, sugarcane	Tropical
	<i>schachtii</i>	Adzuki bean, beet, broccoli, Brussels sprout, cabbage, cauliflower, celery, chickpea, chicory, Chinese cabbage, cowpea, dill, kale, kohlrabi, lentil, pea, radish, rape, rhubarb, rutabaga, spinach, tomato, turnip, yellow lupin	Temperate
	<i>sorghii</i>	Sorghum, maize, rice	Tropical
	<i>trifolii</i>	Carnation, chickpea, cucumber, gherkin, pea, pumpkin, red clover, rhubarb, spinach, squash, tomato, white clover, white lupin, zucchini	Temperate
<i>Heterodera</i>	<i>zeae</i>	Maize, barley, rice, sorghum, wheat	Tropical

4.2. Life Cycle and Behaviour

The life cycle of cyst nematodes is shown in Fig. 4.1. After gastrulation, the embryo extends in length within the eggshell and movement begins, then folds develop in the embryo. After the first moult, the stylet forms at the anterior end of the second-stage juvenile (J2). This is the dormant stage of the life cycle and, depending on the species and environmental conditions, the J2 can remain within the protective cyst for many years (see Chapter 7 for a discussion of dormancy as a survival strategy). The eggshell containing the J2 consists of three layers in cyst nematodes: the outer lipoprotein layer derived from the vitelline layers of the fertilized oocyte, the middle chitinous layer, which provides the eggshell with its structural strength, and the innermost lipid layer, which represents the main permeability barrier. The active part of the life cycle starts when the J2 hatches out of the egg, having used its stylet to cut a slit in the eggshell; the hatching process of cyst nematodes is discussed in detail in Section 7.6.5.

Table 4.2. Cyst species (82) of the genus *Heterodera* Schmidt, 1871.
(Adapted from Subbotin *et al.*, 2010b.)

Species	Main host plant family	Species	Main host plant family
<i>africana</i>	Poaceae	<i>lespedezae</i>	Fabaceae
<i>agrostis</i>	Poaceae	<i>leuceilyma</i>	Poaceae
<i>amygdali</i>	Rosaceae	<i>litoralis</i>	Amaranthaceae
<i>arenaria</i>	Poaceae	<i>longicolla</i>	Poaceae
<i>aucklandica</i>	Poaceae	<i>mani</i>	Poaceae
<i>australis</i>	Poaceae	<i>medicaginis</i>	Fabaceae
<i>avenae</i>	Poaceae	<i>mediterranea</i>	Anarcadiaceae
<i>axonopi</i>	Poaceae	<i>menthae</i>	Lamiaceae
<i>bamboosi</i>	Poaceae	<i>mothi</i>	Cyperaceae
<i>bergeniae</i>	Saxifragaceae	<i>orientalis</i>	Poaceae
<i>betae</i>	Amaranthaceae	<i>oryzae</i>	Poaceae
<i>bifenestra</i>	Poaceae	<i>oryzicola</i>	Poaceae
<i>cajani</i>	Fabaceae	<i>pakistanensis</i>	Poaceae
<i>canadensis</i>	Cyperaceae	<i>persica</i>	Apiaceae
<i>cardiolata</i>	Poaceae	<i>phragmitidis</i>	Poaceae
<i>carotae</i>	Apiaceae	<i>plantaginis</i>	Plantaginaceae
<i>ciceri</i>	Fabaceae	<i>pratensis</i>	Poaceae
<i>cireae</i>	Onagraceae	<i>raskii</i>	Cyperaceae
<i>cruciferae</i>	Brassicaceae	<i>ripae</i>	Poaceae
<i>cyperi</i>	Cyperaceae	<i>riparia</i>	Urticaceae
<i>daverti</i>	Fabaceae	<i>rosii</i>	Polygonaceae
<i>delvii</i>	Poaceae	<i>sacchari</i>	Poaceae
<i>elachista</i>	Poaceae	<i>sacchariphila</i>	Poaceae
<i>fengi</i>	Poaceae	<i>salixophila</i>	Salicaceae
<i>fici</i>	Moraceae	<i>schachtii*</i>	Amaranthaceae
<i>filipjevi</i>	Poaceae	<i>scutellariae</i>	Lamiaceae
<i>galeopsidis</i>	Lamiaceae	<i>sinensis</i>	Poaceae
<i>gambiensis</i>	Poaceae	<i>skohensis</i>	Poaceae
<i>glycines</i>	Fabaceae	<i>sonchophila</i>	Asteraceae
<i>glycyrrhizae</i>	Fabaceae	<i>sorghii</i>	Poaceae
<i>goettingiana</i>	Fabaceae	<i>spinicauda</i>	Poaceae
<i>goldenii</i>	Poacea	<i>spiraeae</i>	Rosaceae
<i>graminis</i>	Poacea	<i>swarupi</i>	Poaceae
<i>graminophila</i>	Poacea	<i>trifolii</i>	Fabaceae
<i>hainanensis</i>	Poacea	<i>turangae</i>	Salicaceae
<i>hordecalis</i>	Poacea	<i>turcomanica</i>	Amaranthaceae
<i>humuli</i>	Cannabaceae	<i>urtica</i>	Urticaceae
<i>johanseni</i>	Brassicaceae	<i>ustinovi</i>	Poaceae
<i>kirjanovae</i>	Betulaceae	<i>uzbekistanica</i>	Salicaceae
<i>koreana</i>	Poaceae	<i>vallicola</i>	Ulmaceae
<i>latipons</i>	Poaceae	<i>zeae</i>	Poaceae

*Type species.

Hatching represents the end of dormancy. Cyst nematodes exhibit diapause and quiescence, the two types of dormancy (Jones *et al.*, 1998; Chapter 7). Diapause, a state of arrested development whereby hatching does not occur until specific requirements, including a time component, have been satisfied, enables the J2 to overcome

Table 4.3. Cyst-forming species of genera other than *Heterodera*.
(Adapted from Subbotin *et al.*, 2010a.)

Genus (number of species)	Species	Host-plant family
<i>Betulodera</i> (1)	<i>betulae*</i>	Betulaceae
<i>Cactodera</i> (13)	<i>acnidae</i> <i>amaranthi</i> <i>cacti*</i> <i>eremica</i> <i>estonica</i> <i>evansi</i> <i>galinsogae</i> <i>milleri</i> <i>radicale</i> <i>rosae</i> <i>salina</i> <i>thornei</i> <i>weissi</i> <i>fluvialis*</i>	Amaranthaceae Amaranthaceae Cactaceae Amaranthaceae Polygonaceae Caryophyllaceae Asteraceae Amaranthaceae unknown Poaceae Amaranthaceae Portulaceae Polygonaceae Poaceae Asteraceae Solanaceae Unknown Solanaceae Solanaceae Rosaceae Solanaceae Asteraceae Solanaceae Solanaceae Onagraceae Cyperaceae Poaceae Poaceae Poaceae Poaceae
<i>Dolichodera</i> (1)		
<i>Globodera</i> (12)	<i>artemisiae</i> <i>bravoae</i> <i>capensis</i> <i>ellingtoniae</i> <i>leptonepia</i> <i>mali</i> <i>mexicana</i> <i>millefolii</i> <i>pallida</i> <i>rostochiensis*</i> <i>tabacum</i> <i>zelandica</i> <i>tenuissima*</i> <i>chalcoensis</i> <i>matadorensis</i> <i>punctata*</i> <i>stonei</i> <i>zeaphila*</i>	
<i>Paradolichodera</i> (1)		
<i>Punctodera</i> (4)		
<i>Vittatidera</i> (1)		

*Type species.

environmental conditions that are unfavourable for hatch, such as extreme temperatures or drought. The extent of the diapause varies but several cyst nematodes, e.g. *Globodera rostochiensis* and *Heterodera avenae*, show obligate diapause during their first season of development. In *G. rostochiensis* and *G. pallida*, diapause is terminated in late spring, when the combination of rising soil temperature and adequate soil moisture is conducive for infection of the new potato crop. The duration of obligate diapause is affected by the photoperiod experienced by the infected plant, with unhatched J2 from plants grown under continuous light showing no obligate diapause. Facultative diapause is initiated by external factors, such as various environmental factors, from the second season onwards. Once diapause is completed, the J2 may enter into a quiescence state, which requires various environmental cues to effect further development

Box 4.1. Classification of the cyst nematodes.

Phylum: Nematoda Potts, 1932
Class: Chromadorea Inglis, 1983
Subclass: Chromadoria Pearse, 1942
Order: Rhabditida Chitwood, 1933
Suborder: Tylenchina Thorne, 1949
Infraorder: Tylenchomorpha De Ley & Blaxter, 2002
Superfamily: Tylenchoidae Örley, 1880
Family: Hoplolaimidae Filipjev, 1934
Subfamily: Heteroderinae Filipjev & Schuurmans Stekhoven, 1941
Genera:
 Heterodera Schmidt, 1871 (Type Genus)
 Globodera Skarbilovich, 1959
 Punctodera Mulvey & Stone, 1976
 Cactodera Krall & Krall, 1978
 Dolichodera Mulvey & Ebsary, 1980
 Betulodera Sturhan, 2002
 Paradolichodera Sturhan, Wouts & Subbotin, 2007
 Vittatidera Bernard, Handoo, Powers, Donald & Heinz, 2010

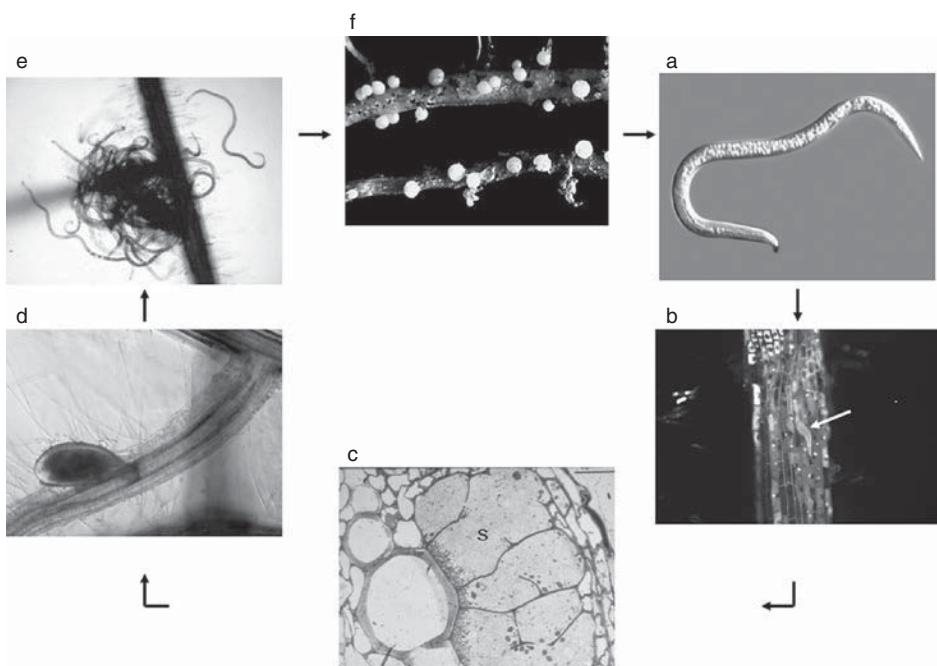


Fig. 4.1. Life cycle of a cyst nematode. Cysts contain up to 400 eggs, each one containing a second-stage juvenile (J2). After hatch (a), the J2 moves through the soil, invades a host root (arrowed) (b) and moves through the root to establish a feeding site (syncytium) (c) on which it feeds and develops. Juveniles develop either into females, which become saccate and rupture the root (d), or to vermiform males, which leave the root, locate the female and mate (e). The female then dies to form the cyst (f).

of the life cycle. In temperate regions this usually occurs with an increase in soil temperature together with specific hatching stimuli produced by the host root system, termed root diffusate or root exudate. Whilst all species hatch in large numbers in response to appropriate host root diffusates, cyst nematodes can be classified into three broad categories based on their hatching responses to water: (i) low J2 water hatch (*G. rostochiensis*, *G. pallida*, *H. cruciferae*, *H. carotae*, *H. goettingiana*, *H. humuli*); (ii) moderate J2 water hatch (*H. trifolii*, *H. galeopsidis*, *H. glycines*); and (iii) high J2 water hatch (*H. schachtii*, *H. avenae*).

Cyst nematodes exhibit considerable variation in optimum temperature for hatch; for example, *G. pallida* is adapted to lower temperatures than *G. rostochiensis* (16°C and 20°C, respectively). Low optimum temperatures for hatching are characteristic of cyst nematodes that can invade during winter or early spring, such as *H. cruciferae*. As expected, nematodes adapted to warmer climates exhibit higher temperature optima, e.g. 30°C for *H. zea*. Soil type can also affect rates of hatch. In general, coarse-textured soils favour hatching and subsequent invasion of root systems, providing suitable conditions for aeration and nematode migration. Maximum hatch usually occurs in soil at field capacity, whilst drought and waterlogging inhibit hatch.

Once hatched out of the eggshell, the J2 then leaves the cyst via either of the natural openings of the cyst, i.e. the fenestral region or the neck where the female's head has broken away. The J2 released into the soil will begin to search for a suitable host, relying primarily on gradients of chemicals released by the host's root system (see Chapter 8). As a survival strategy not all juveniles hatch out at the same time. A proportion of J2 are retained either within the cyst body and/or in external egg masses. *Globodera* species do not produce egg sacs but occasionally exude a small droplet of moisture, whilst egg sac production varies between *Heterodera* species and in individual species according to environmental conditions, e.g. *H. glycines* produces more eggs in egg masses under favourable conditions.

The J2 enters the root system of its host, usually directly behind the growing root tip, and then migrates to the pericycle and proceeds to select a suitable cell with which to form a feeding site (see Chapter 9). The hollow mouth stylet pierces a cell wall, being careful not to bridge the plasmalemma until a feeding tube is formed. Saliva from the pharyngeal glands is then injected and the cell contents are withdrawn into the nematodes by the action of the pharyngeal pump. The feeding tube acts as a particle filter to stop large molecules being ingested. This specific interaction induces enlargement of root cells and breakdown of their walls to form a large syncytial 'transfer cell' with dense, granular cytoplasm. The transfer cell develops cell-wall ingrowths adjacent to the conducting tissue, which greatly increase the internal surface area, facilitating the passage of nutrients into the syncytium. Provided that the J2 are able to stimulate the host plant to induce and maintain syncytia of sufficient size to receive all the nutrients they require, juveniles develop into both male and female adults. This stage of the life cycle will take approximately 7 days depending on the temperature, and the second moult to third-stage juvenile (J3) will then take place. The J3 has a well-developed genital primordial and rectum; the male has a single testis and the female has paired ovaries. The female at this point is about 0.4 mm long and its shape is becoming globular to facilitate the rapid growth of the developing ovaries. At the fourth moult, the female ruptures the root cortex and the formation of the vulva gives access to the reproductive system, which is being taken over by the formation of eggs.

Males develop at a similar rate in the same root as the females. They too emerge at the fourth moult but are still wrapped in the third-stage cuticle on emergence. Males are non-feeding, free-living and live for only a short time in the soil. The males are attracted to females, which exude sex pheromones (see Chapter 8) and may be the subject of multiple mating. After mating, the embryos develop within the egg as far as the formation of the J2 while still within the female's body. As indicated earlier, the female then dies and her cuticle tans to form a tough protective cyst containing several hundred embryonated eggs, the number depending on species and prevailing environmental conditions. In some of the *Heterodera* species an egg sac is exuded outside the cone region of the cyst. Eventually the cysts become detached from the roots as the plant dies and remain dormant in the soil until the next suitable host grows in the vicinity (Turner and Evans, 1998).

The time taken to complete the life cycle, from egg to egg, of a cyst nematode varies depending upon the co-evolution of the species with its host range and the environmental conditions. Typical life cycles are completed in about 30 days but this may be reduced in warmer climates; for example, *H. oryzicola* completes a life cycle in 23 days at 27°C, *H. glycines* 21 days at 25°C, whilst the temperate species *H. trifolii* requires 31 days at 20°C but 45 days at 15.5°C.

The number of generations per year also varies between cyst nematode species. Generally, as the soil temperature increases so does the number of generations, up to an upper threshold for each species. Under standard field conditions most temperate species of cyst nematodes will complete one or two generations, corresponding to the natural life cycle of its host combined with the length of the optimal temperature range. However, in tropical regions where favourable environmental conditions are more constant throughout the year, multiple generations are usual, with up to 11 generations being reported for *H. oryzicola*.

4.3. General Morphology of the Subfamily Heteroderinae

Nematode species of the subfamily Heteroderinae have similar gross morphology and are often distinguished from each other only by small details. The adult female (or cyst) and J2 are of the greatest importance in diagnosis as they are the stages most often found in soil extracts. Morphological identification using only the juveniles is not reliable and should be avoided, although they are the stage most likely to be obtained in soil extracts. If only juveniles are found in the soil it should be sampled again for females or cysts.

Mature females. The mature females are swollen into a spherical, sub-spherical or lemon shape to contain the developing ovaries (as well as the developing eggs) within the body cavity. Within some specimens, J2 can also be found. An egg sac may be extruded from the body but this depends on the species. In the mature female of the cyst nematode genera, annulations are restricted to the head region. The stylet and pharynx are strongly developed, with a prominent median bulb, and lie in the anterior part of the body, which forms a 'neck'. Posterior to the excretory pore, the swelling of the body is greatly developed and the excretory pore lies at the base of the neck. In most of the genera, the vulva is at the opposite pole of the body to the neck. The vulval slit runs transversely.

Cysts. Cysts are formed by the polyphenol oxidase tanning of the female cuticle, and they retain the female shape (Fig. 4.2A and B). The surface of the cyst is covered by a pattern of ridges derived from the pattern on the female cuticle. A thin-walled area surrounds the vulva and the cuticle can be lost, forming an opening, the fenestra (see Fig. 4.3 for the basic structures of terminal regions used for diagnosis). The fenestration (presence or absence; shape) is used in the diagnosis of genera (Fig. 4.4). Other measurements from the cysts are used in diagnosis of genera and species (Fig. 4.5).

Eggs. Eggs of most species fall within a similar size range and similar length:breadth ratios, i.e. length (L) = 86–134 µm; width (W) = 36–52 µm; L/W ratio = 2.1:2.6. In general, eggs are usually unornamented and are not a reliable stage for diagnostic purposes. However, the eggs from species of *Cactodera* can be used for diagnostic purposes; in some species, such as *C. cacti* and *C. milleri*, the eggshell is covered with small punctuations that resemble microvilli, whereas other members of the group, such as *C. weissi* and *C. amaranthi*, have smooth cuticles.

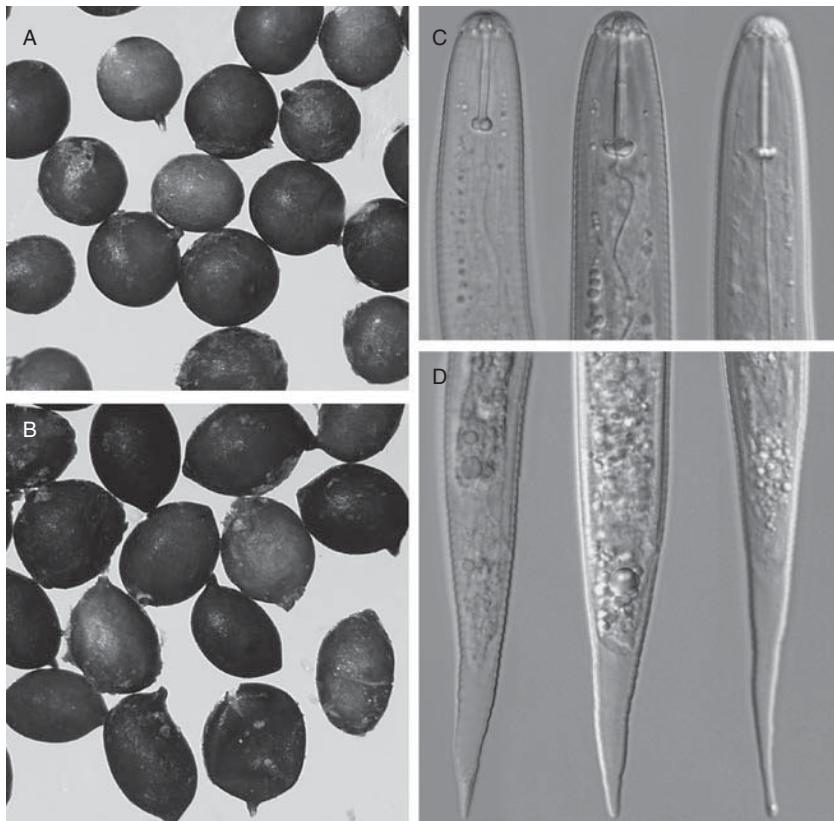


Fig. 4.2. Cyst and second-stage juvenile (J2) characteristics A: Cysts of *Globodera*. B: Cysts of *Heterodera*. C: Anterior regions of J2 of *G. rostochiensis*, *H. schachtii*, *Punctodera punctata*. D: Posterior region of J2 of *G. rostochiensis*, *H. schachtii*, *Punctodera punctata*.

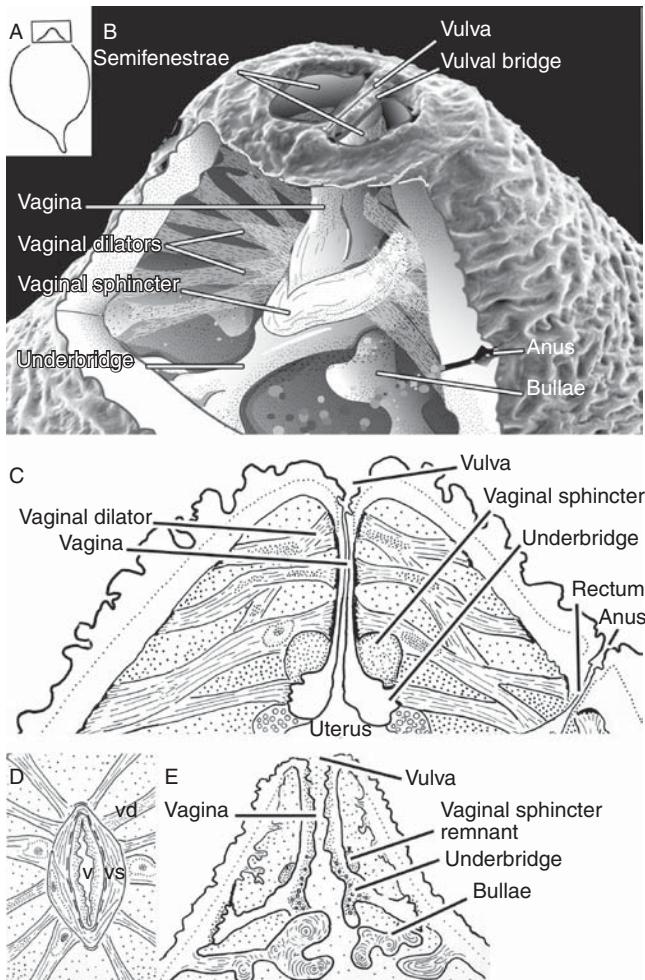


Fig. 4.3. Drawings depicting basic structures of terminal regions of cyst nematodes used for diagnosis. A: Overview of female. Box indicates terminal region as enlarged in B and C. B: Ventrolateral 3D view with cutaway showing internal structures. C: Right lateral view of *Heteroderma schachtii*. D: Transverse view through vulva (v) at level of vaginal sphincter (vs) and vaginal dilators (vd) of *H. schachtii*. E: Dorsal view of mature cyst. Main features for diagnosis are fenestrae type and measurements, underbridge and vulval features. (After Subbotin *et al.*, 2010a.)

Second-stage juveniles. J2 are vermiform, with an offset, dome-shaped head and conical tail tapering to a point. On death, the body assumes a gentle curve with the ventral surface concave so that the nematode lies on its side. The cuticle is regularly annulated with the lateral fields running from near the head to the tail; the number of incisures may be reduced anteriorly and posteriorly. The head skeleton, stylet and pharynx are well developed, the latter occupying approximately one-third of the body length (Fig. 4.2C). The median bulb is rounded in shape with a prominent valve. The pharyngeal glands overlap the intestine ventrally and subventrally; the single dorsal pharyngeal gland nucleus is more prominent than, and anterior to, the two

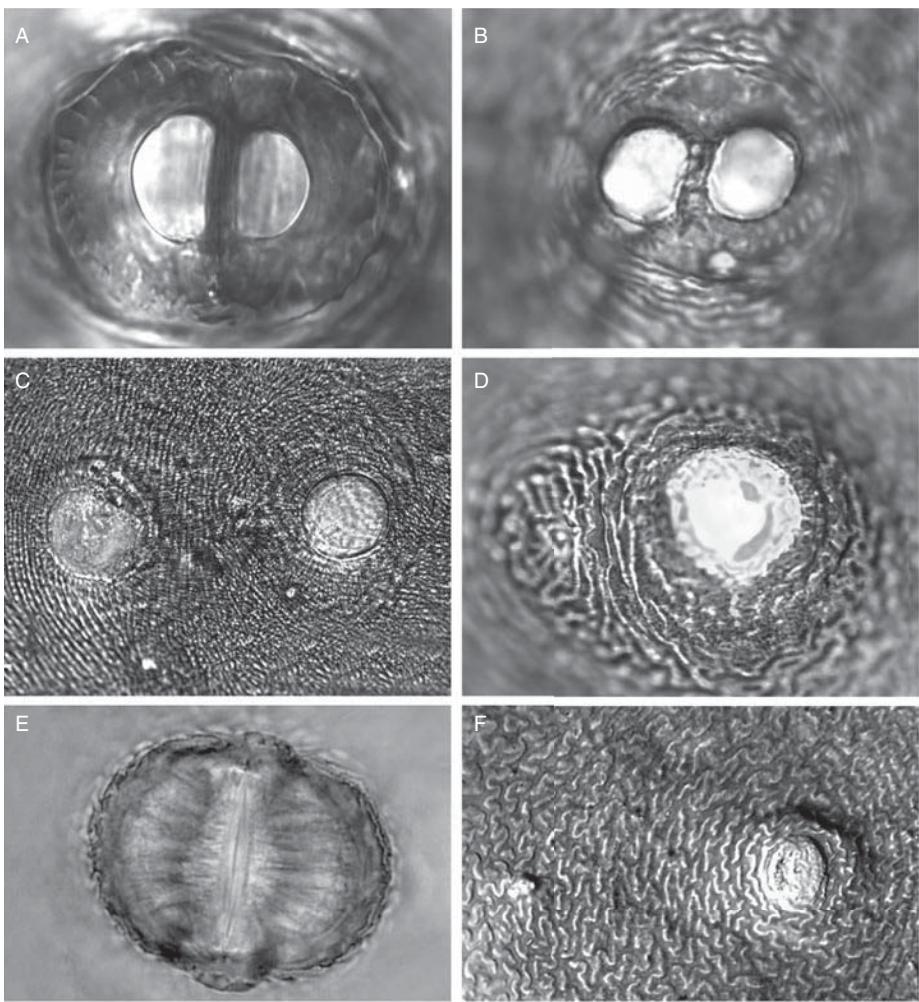


Fig. 4.4. Fenestration of cyst nematodes. The word fenestra (meaning window) refers to the thin-walled area on the vulval cone or perineal area of mature cysts. In young cysts the fenestral area is membranous but later decays, leaving a hole in the cyst wall. There are three main types of fenestration: circumfenestrate, bifenate and ambifenate. The fenestration shown by a particular cyst is an important feature in identification.
 A: *Heterodera glycines* (ambifenate). B: *H. avenae* (bifenate). C: *Punctodera punctata* (circumfenestrate). D: *Cactodera cacti* (circumfenestrate). E: *H. orientalis* (no fenestration). F: *Globodera rostochiensis* (circumfenestrate). (After Subbotin *et al.*, 2010a, with modifications and courtesy of V.N. Chizhov.)

subventral gland nuclei. The excretory pore is clearly visible on the ventral surface opposite the pharyngeal glands with hemizonid anterior to it. The anus may be marked by a small notch or step in the cuticle and the tail has a clear tip, the hyaline portion (Figs 4.2D and 4.6). Phasmids are visible in some species as small refractive points lying laterally on the tail surface, usually within the lateral field. Measurements from the J2 are used in diagnosis of genera and species (Fig. 4.6).

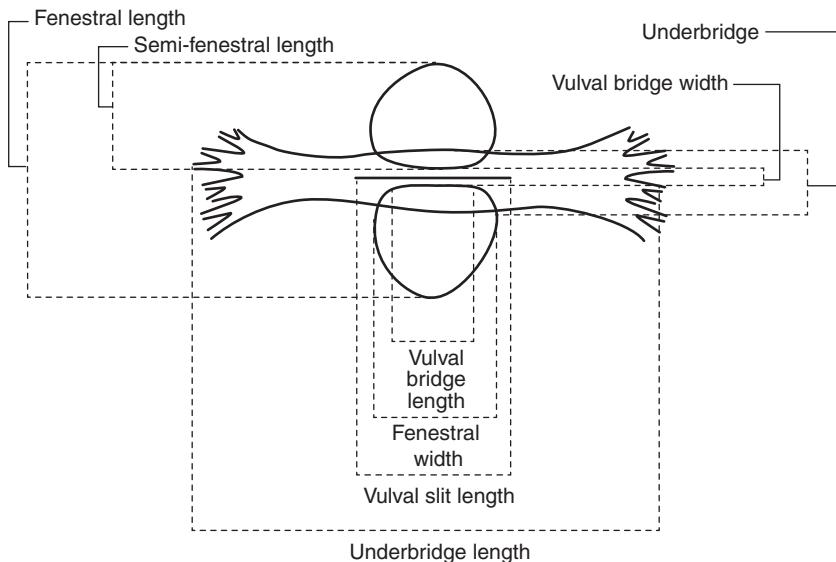


Fig. 4.5. Measurements of the fenestral area (fenestrae and underbridge) of a cyst, important for species and genus diagnostics.

4.4. Genera and Principal Species

4.4.1. Genus *Heterodera* Schmidt, 1871

Females and cysts are usually lemon-shaped, the neck protruding from the anterior end and at the opposite pole the posterior usually ending in a cone (Fig. 4.7). The cone carries the fenestra that internally is associated with the reproductive organs and, from the exterior, will provide diagnostic features used for identification. Some members of this genus, such as *H. schachtii*, have a very high conspicuous cone and others, such as *H. cruciferae*, have much lower cones. The mature female containing eggs dies, causing her cuticle to tan and dry out, thus protecting the eggs until the invasive J2 hatch. The cyst can range in colour from light brown to dark brown or almost black. The cuticle surface displays folds and ridges often in specific patterns, e.g. zigzag or parallel, which are helpful in the diagnosis of species. *Heterodera* cysts may or may not have an underbridge. Bullae may also be present and both features are very diagnostic of the groups contained within this genus. To fully investigate the posterior part of the cyst (perinea), it must first be mounted on a slide, ideally containing 5–10 additional perineal patterns for further detailed analysis. Molecular and morphological data support division of most *Heterodera* species into several groups: *Afenestrata*, *Avenae*, *Cyperi*, *Goettingiana*, *Humuli*, *Sacchari* and *Schachtii*. Key features for study include the formation of the fenestra. These are classified as without fenestration (*Afenestrata* group), ambifenestrata (two openings divided by a narrow vulval bridge) or bifenestrata (two openings separated by a much wider vulval bridge). The length of the vulval slit varies. In the *Avenae* group it is very short at 8–10 µm, whereas members of the *Schachtii* group have a much longer slit averaging 65 µm in length.

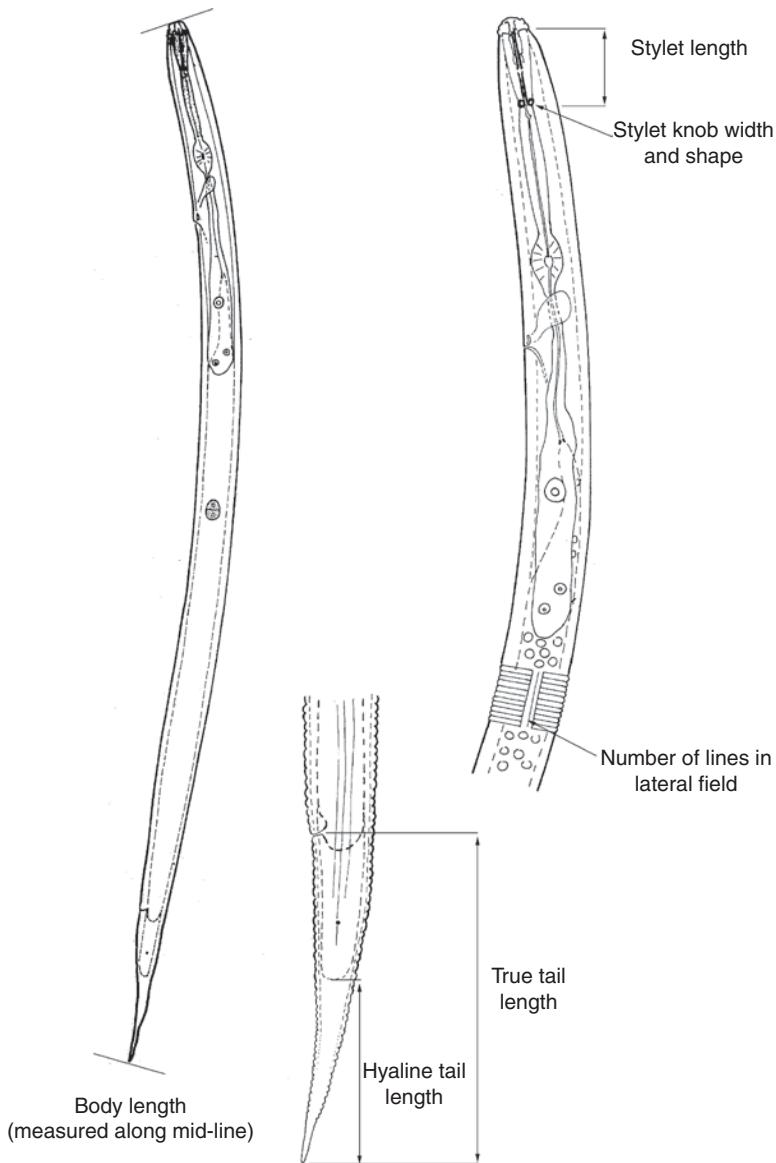


Fig. 4.6. Measurements of second-stage juvenile, important for species and genus diagnostics.

The J2 are also used for diagnosis together with the cyst features. The stylet length and the position and shape of the basal knobs are important features. The number of lateral fields is usually three or four. The number of head annules present, the width of the body at the excretory pore and the anus, and the length of both the true tail, i.e. from the anus to the tail tip, and the hyaline tail length are diagnostic (Fig. 4.6).

The type species of the genus is *H. schachtii*.

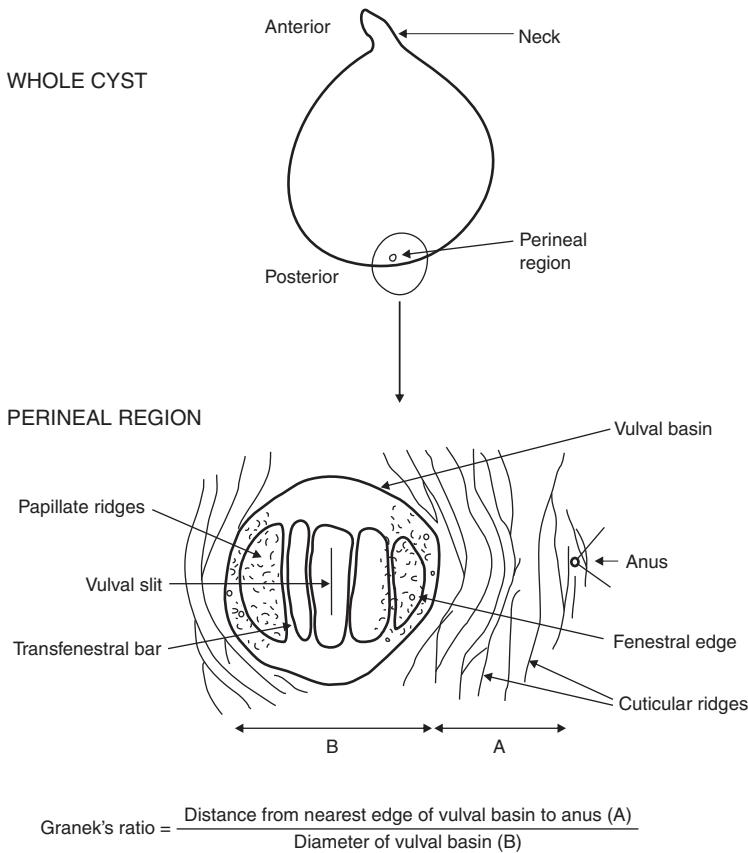


Fig. 4.7. The whole cyst shape and vulval area details for calculation of Granek's ratio.

4.4.1.1. Cereal cyst nematode, *Heterodera avenae* Wollenweber, 1924

The cereal cyst nematode was first recorded by Kühn (1874) as a parasite of cereals in Germany and was later found in other countries. *Heterodera avenae* is now found in most wheat-growing regions of the world. The cereal cyst nematode is an important pest of cereals and is the principal nematode species on temperate cereals. In Europe, more than 50% of the fields in major cereal-growing areas are infected by this nematode (Rivoal and Cook, 1993), with annual yield losses reaching £3 million (Nicol and Rivoal, 2008). At least \$US3.4 million is estimated to be lost annually in wheat production in the states of Idaho, Oregon, and Washington because of cereal cyst nematodes. The yield losses it causes on wheat range from 15–20% in Pakistan, and 40–92% on wheat and 17–77% on barley in Saudi Arabia. In China yield losses of wheat crops induced by this nematode can reach 70%. Hosts of *H. avenae* include species of cereals and grasses from the following genera: *Agropyron*, *Agrostis*, *Alopecurus*, *Anisantha*, *Arrhenatherum*, *Avena*, *Brachypodium*, *Bromus*, *Dactylis*, *Echinochloa*, *Festuca*, *Hordeum*, *Koeleria*, *Lolium*, *Phalaris*, *Phleum*, *Poa*, *Polypogon*, *Secale*, *Setaria*, *Sorghum*, *Trisetum*, *Triticum*, *Vulpia*, *Zerna* and *Zea* (Williams and Siddiqi, 1972). *Heterodera avenae* has only one

generation per year, with J2 hatch from the eggs determined largely by temperature (Rivoal and Cook, 1993).

DESCRIPTION

Cysts: L = 518–801 µm; W = 432–744 µm; L/W ratio = 0.8–1.8; fenestral length = 32–55 µm; vulval slit = 7–12 µm.

Male: L = 1020–1590 µm; stylet = 27–33 µm; spicules = 33–38 µm; gubernaculum = 10–13 µm.

J2: L = 505–598 µm; stylet = 24–27.5 µm; hyaline region = 34–50 µm; tail = 52–79 µm. Cyst lemon-shaped, with prominent neck and vulval cone. Subcrystalline layer conspicuous, sloughing off with formation of dark brown cyst. Bifenestrated, bullae prominent, crowded beneath vulval cone. J2 vermiform, with a sharply pointed tail. Stylet well developed, with large, anteriorly flattened to concave basal knobs.

Heterodera avenae belongs to the *Avenae* group and to the *H. avenae* complex (also called Cereal Cyst Nematodes (CCN)). It differs from species it closely resembles (*H. australis*, *H. aucklandica*, *H. riparia*, *H. pratensis* and *H. arenaria*) by morphometrical characters of J2 and cysts and by PCR-ITS-RFLP and IEF of proteins. Based on the ITS rRNA gene sequences and PCR-ITS-RFLP all world populations of *H. avenae* can be divided into several types: (i) *H. avenae*, European and North American populations, type A; (ii) *H. avenae*, Asian and African populations, type B; (iii) *H. avenae*, several French populations; and (iv) *H. 'avenae'* from China, type C.

4.4.1.2. Sugar beet cyst nematode, *Heterodera schachtii* Schmidt, 1871

The sugar beet cyst nematode (Fig. 4.8) has been recognized as a plant pathogen since 1859 when it was associated with stunted and declining sugar beet in Germany. In the following years it became recognized as a pest of great importance in beet-growing areas of several European countries. *Heterodera schachtii* is found in all major sugar beet production areas of the world, favouring temperate regions but apparently tolerating a broad range of climates. It is widespread in Europe, the USA and Canada (Baldwin and Mundo-Ocampo, 1991). Annual yield loss in EU countries based upon world market sugar prices was estimated in 1999 at up to €90 million (Müller, 1999). The optimum temperature for development is around 25°C. In some climates, three to five generations may complete development on sugar beet in one season (Franklin, 1972). *Heterodera schachtii* was described from the host *Beta vulgaris* and it parasitizes mainly plants of the families Amaranthaceae (Caryophyllales) (many species of *Beta* and *Chenopodium*) and Brassicaceae (Brassicales) (*Brassica oleracea*, *B. napus*, *B. rapa*, *Raphanus sativus* and many others including a diversity of common weeds) (Franklin, 1972). Some plants from Polygonaceae, Scrophulariaceae, Caryophyllaceae and Solanaceae are susceptible to nematode infection.

DESCRIPTION

Cysts: L = 480–960 µm; W = 396–696 µm; L/W ratio = 0.9–2.0; fenestral length = 28–48 µm; vulval slit = 33–54 µm.

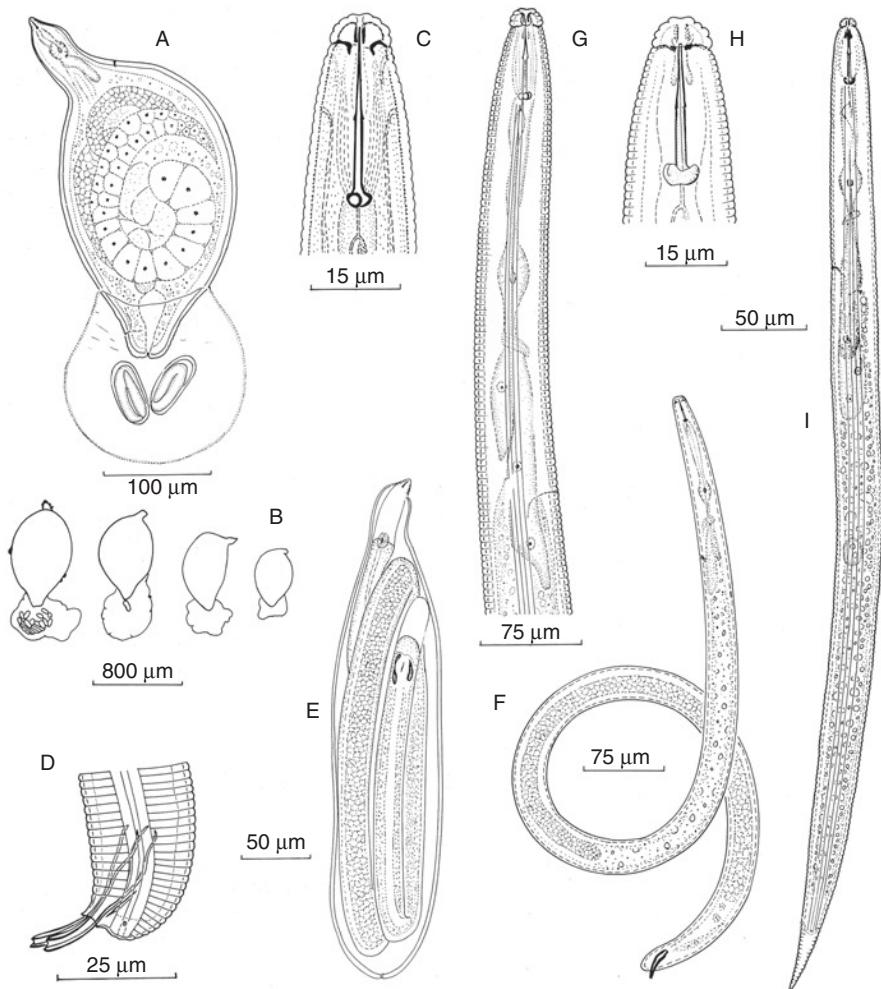


Fig. 4.8. *Heterodera schachtii*. A: Adult female with egg sac. B: Cysts and egg sacs. C: Anterior region of male. D: Male tail. E: Fourth-stage male moulting. F: Adult male. G: Male pharyngeal region. H: Anterior region of J2. I: J2. (After Franklin, 1972.)

Male: L = 1038–1638 μm ; stylet = 27–30 μm ; spicules = 27–39 μm ; gubernaculum = 10–11 μm .

J2: L = 400–512 μm ; stylet = 23–28 μm ; hyaline region = 17–33 μm ; tail = 40–56 μm (Fig. 4.8).

Cyst colour light to dark brown. Ambifenestrate, within cone, remnants of vagina attached to side walls by underbridge and a number of irregularly arranged, dark brown molar-shaped bullae situated a short distance beneath the vulval bridge. J2 labial region offset, hemispherical, with four indistinct annuli. Stylet moderately heavy with

prominent, forwardly-directed knobs. Tail acutely conical with rounded tip, distinct hyaline terminal section 1–1.25 stylet lengths long.

Heterodera schachtii belongs to the *Schachtii* group and is distinguished from closely related species (*H. trifolii*, *H. glycines*, *H. betae* and others) by a combination of morphological and morphometric characteristics. PCR-ITS-RFLP profile generated by *MvaI* is unique for *H. schachtii*. Diagnostics of this species using PCR with species-specific primers has also been developed.

4.4.1.3. Soybean cyst nematode, *Heterodera glycines* Ichinohe, 1952

A cyst nematode parasitizing soybean plants, *Glycine max* and causing ‘yellow dwarf’ symptoms was recorded from Shirakawa, Fukushima Prefecture, Japan, in 1915. Ichinohe (1952) was the first to make careful morphological comparisons with other *Heterodera* species and to give a specific name and brief description of this nematode. In Japan, yield losses have been estimated at 10–70% (Ichinohe, 1988). Presently, *H. glycines* occurs in most countries of the world where soybean is produced. In a study of losses predicted in ten soybean-producing countries together accounting for 97% of the world crop, *H. glycines* appeared to be the most important constraint (losses of 8,969,400 t) on yield and estimated at \$US1960 million (Wrather *et al.*, 2001). In these countries, total yield losses attributed to *H. glycines* were greater than those for any other pest of the crop. *Heterodera glycines* is widely distributed throughout the north-central USA where different maturity groups with the same source of resistance to *H. glycines* are grown. State surveys in the region report from 14 to 63% of fields are infested with *H. glycines*.

Heterodera glycines has a broad host range, especially Fabaceae, but also on other families. More than 66 weed species of nine families are suitable hosts. Riggs (1992) provided a list of non-fabaceous hosts comprising 63 species in 50 genera from 22 families (e.g. Boraginaceae, Capparaceae, Caryophyllaceae, Chenopodiaceae, Brassicaceae, Lamiaceae, Fabaceae, Scrophulariaceae, Solanaceae). In field conditions, *H. glycines* was also found in several other plants, including henbit (*Lamium amplexicaule*), purple deadnettle (*Lamium purpureum*), mouse-ear chickweed (*Cerastium holosteoides*) and common chickweed (*Stellaria media*) (Riggs, 1992).

Three to five generations develop during the cropping season. Optimum temperature is 23–28°C; development stops below 14°C and above 34°C. In the absence of a host, J2 and eggs in cysts may remain viable in soil for 6–8 years.

Heterodera glycines disturbs root growth, interferes with nodulation and causes early yellowing of soybean plants. The above-ground symptoms of damage on individual plants and appearance of infested fields are usually not sufficiently specific to allow direct identification. Infected plants are predisposed to *Fusarium* wilt. Sudden death syndrome is a soil-borne disease of soybean caused by the fungus *Fusarium solani* in association with *H. glycines*.

DESCRIPTION

Cysts: L = 340–920 µm; W = 200–688 µm; L/W ratio = 1.0–2.4; fenestral length = 35–72 µm; vulval slit = 36–60 µm.

Male: L = 911–1400 µm; stylet = 24–27 µm; spicules = 28–45 µm; gubernaculum = 8–13 µm.

J2: L = 345–504 µm; stylet = 21–25 µm; hyaline region = 18–36 µm; tail = 35–59 µm.

Cyst mainly lemon-shaped, sometimes round with a protruding neck and cone. Ambifenestrate, bullae prominent, located at or anterior to underbridge, extending into vulval cone from interior of body wall cuticle. Shape varying from round to finger-like, round bullae differently sized, finger-like bullae of variable length and thickness. Underbridge well developed. J2 body vermiform with regularly annulated cuticle. Stylet robust with anteriorly protruding knobs. Tail tapering uniformly to a finely rounded terminus.

Heterodera glycines belongs to the *Schachtii* group and is distinguished from similar species (*H. medicaginis*, *H. schachtii*, *H. trifolii*, *H. daverti* and others) by a combination of morphological and morphometric characteristics. It differs from *H. schachtii* by the shape of the stylet knobs of J2 (slightly convex vs moderately or strongly concave), shorter average J2 stylet and longer average fenestral length.

4.4.1.4. Pea cyst nematode, *Heterodera goettingiana* Liebscher, 1892

In 1890, G. Liebscher reported infection and yield loss of pea (*Pisum sativum*) and vetch (*Vicia sativa*) by nematodes identified as *H. schachtii* at fields of the Agricultural Institute at Göttingen, Germany. Two years later he described this species as *H. goettingiana*. Several researchers have reported diseases of peas, primarily in European countries, caused by this nematode; however, little was known about biology and pathogenicity of *H. goettingiana* until the mid-1900s (Franklin, 1951). Infected pea fields show sharply delineated patches with dwarfed, poorly branched and yellowing plants that die prematurely. Infected plants either fail to flower or flower too early. The root system is poorly developed. Development takes 3–15 weeks depending on soil temperature and moisture as well as host species. One or two generations occur during the growing season in the UK, and three generations may develop in southern Italy. Cysts with eggs can remain viable in the absence of a host for 12 years.

DESCRIPTION

Cysts: L = 400–780 µm; W = 310–540 µm; L/W ratio = 1.3–2.2; fenestral length = 43–71 µm; vulval slit = 43–61 µm.

Male: L = 1270 µm; stylet = 27 µm; spicules = 27 µm; gubernaculum = 12 µm. (No ranges available.)

J2: L = 408–519 µm; stylet = 23–26 µm; hyaline region = 27–38 µm; tail = 54–74 µm.

Cyst lemon-shaped with light to dark brown cyst wall. Subcrystalline layer not visible. Vulval cone ambifenestrate. In some old cysts, vulval bridge ruptured, fenestrae joining to form a large oval fenestrum. Bullae absent, although bullae-like structures and vulval denticles present. Underbridge weak. J2 body vermiform, curved ventrally after fixation. Labial region hemispherical, with 2–5 annuli, slightly offset from body. Lateral field with four incisures, not areolated. Stylet knobs rounded, slightly projecting anteriorly. Tail tapering uniformly to a finely rounded terminus.

Heterodera goettingiana belongs to the *Goettingiana* group. It differs from several other representatives of the *Goettingiana* group (*H. cruciferae*, *H. carotae*, *H. circeae*, *H. scutellariae* and *H. persica*) by longer average J2 body, longer tail and longer hyaline region.

4.4.1.5. Mediterranean cereal cyst nematode *Heterodera latipons* Franklin, 1969

In the early 1960s, a cyst nematode similar to *H. avenae* was detected in Israel and Libya on the roots of stunted wheat and barley plants. It was morphologically studied and described by Franklin (1969) as a new species under the name *H. latipons*, based on characteristics of the Israel population. This nematode was later recorded in many countries, mainly from the Mediterranean and the Orient. *Heterodera latipons* often occurs in mixed populations with *H. avenae* in cereal cropping systems. Hosts include barley (*Hordeum vulgare*), oat (*Avena sativa*), rye (*Secale cereale*), *Phalaris minor*, *P. paradoxa* and *Elytrigia repens*. *Triticum durum* was considered to be a poor host of this nematode. Yield losses as high as 50% were reported on barley in Cyprus. In Syria, the nematode causes average yield losses of 20 and 30% in barley and durum wheat, respectively, and the nematode was more damaging under water stress conditions. Moreover, damage is more severe in fields infested concomitantly with *H. latipons* and the fungus *Bipolaris sorokiniana*, the causal agent of the common root rot and seedling blight of barley, i.e. the presence of the nematode increases the aggressiveness of the fungus (Scholz, 2001). In all areas studied, *H. latipons* completed only one life cycle during the growing season (Mor *et al.*, 1992).

DESCRIPTION

Cysts: L = 300–700 µm; W = 320–560 µm; L/W ratio = 0.6–1.7; fenestral length = 52–76 µm; vulval slit = 6–11 µm.

Male: L = 960–1406 µm; stylet = 22–29 µm; spicules = 32–36 µm; gubernaculum = 8 µm.

J2: L = 401–559 µm; stylet = 22–25 µm; hyaline region = 20–36 µm; tail = 42–68 µm.

Cysts are dark to mid-brown covered with white subcrystalline layer. Bifenestratae, semifenestrae separated by a distance greater than fenestral width, vulval slit short. Strong underbridge with pronounced thickening in middle and with ends splayed. Bullae usually absent, sometimes present at underbridge level. J2 body slightly curved dorsoventrally when killed by heat. Stylet with well-developed, anteriorly concave knobs.

Heterodera latipons belongs to the *Avenae* group and closely resembles *H. hordecalis* and *H. turcomanica*. These nematodes share similar circular semifenestrae separated by a distance longer than the semifenestra diameter and a rather typical underbridge but with a pronounced enlargement underlying the vulval slit. The most important differentiating character between *H. latipons* and *H. hordecalis* is the vulval slit, which in *H. latipons* is much shorter.

4.4.2. Genus *Globodera* Skarbilovich, 1959

Mature females and cysts are spheroid, lacking terminal cone. Vulval area is circumfenestrate. Vulva located in a cavity beneath outline of body, vulval slit <15 µm. There is no anal fenestra. Vaginal remnants, underbridge and bullae rarely present

(Fig. 4.7). Cuticle with distinct D layer (Cordero and Baldwin, 1990). All eggs retained in body, egg sac absent. Colouration is helpful in making a diagnosis of species, especially separation of the PCN species *G. rostochiensis* and *G. pallida*. Egg surface is smooth. Male lateral field with four lines, spicules >30 µm, distally pointed. J2 has four incisures in lateral field. Tail conical, pointed, phasmids punctiform. *En face* pattern typically with six separate lips, sometimes with fusion of adjacent sub-medial lips. The type species of the genus is *G. rostochiensis*.

PCN include two species, *G. rostochiensis* and *G. pallida*, which have been reported from many countries and are considered to be one of the most economically important pests of potato. Molecular analysis, however, indicates presence of several still undescribed species of PCN. It is now generally accepted that PCN are native to South America (Grenier *et al.*, 2010), where it is the principal pest of Andean potato crops. *Globodera rostochiensis* and *G. pallida* are differently distributed in the Andes. Factors that may be responsible include day length, temperature, altitude, rainfall or the interaction of any of them with the host potato. Human activities over centuries may also have influenced distribution. In South America, these species are mainly found between 2000 m and 4000 m above sea level, with the heaviest infestations between 2900 m and 3800 m above sea level. The two species occupy different zones in the Andes. The demarcation line between the two species is near 15.6°S. With few exceptions, populations north of this line are mainly *G. pallida*. Those from areas around Lake Titicaca and further south are predominantly *G. rostochiensis* with few *G. pallida* or mixtures of both species. The most southerly population, from the east side of the Andes in Bolivia, are mixtures of *G. rostochiensis* and *G. pallida*. These two species have been introduced in many parts of the world, particularly to Europe, and also to the USA, Canada, New Zealand and numerous other countries where potatoes are grown.

4.4.2.1. Golden potato cyst nematode *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959

The golden cyst nematode associated with potato plants, *Solanum tuberosum*, from Rostock, Germany was first reported in 1881 and was considered to be *H. schachtii*, this being the only known species of cyst nematode at that time. During the early 1900s, the PCN became more widely known throughout Europe and was described in 1923. In temperate regions, *G. rostochiensis* usually completes only one generation, although a second generation may be initiated but not completed; J2 hatched from first-generation eggs, but they were unable to reach the adult stage. In sub-tropical regions two generations might occur. Development of one generation requires 6–10 weeks. The J2 can go into diapause and remain viable for many years, hatching continuing for 25 or more years. Heavily infected plants become yellow and stunted. Infected plants have reduced root systems, which are abnormally branched and brownish in colour. Symptoms in the field first appear in small patches. At low nematode densities tuber sizes are reduced, whereas at higher densities both number and size of tubers can be reduced. At eight and 64 eggs g⁻¹ of soil, yield losses of about 20 and 70%, respectively, can be expected. Seinhorst (1982) and Elston *et al.* (1991) proposed models that described the relationships of PCN population densities before planting with potato yield and post-harvest nematode

populations (see Chapter 10). The damaging effect of PCN is not only determined by nematode density, but also by such factors as cultivar, crop husbandry and environmental conditions. PCN are responsible for annual potato tuber losses of up to 9% in Europe. Information on economic importance in some South American countries is scarce or unavailable although yield losses in Bolivia and Peru have been estimated to be around US\$13,000,000 and US\$128,000,000, respectively (Franco and Gonzalez, 2010).

Hosts include potato, *Solanum tuberosum* (Solanaceae, Solanales), tomato, *S. lycopersicum* and eggplant, *S. melongena*. Other hosts include many *Solanum* spp., *Datura* spp., *Hyoscyamus niger*, *Nicotiana acuminata*, *Physalis* spp., *Physochlaina orientalis*, *Salpiglossis* spp., *Capsicum annuum* and *Saracha jaltomata*.

DESCRIPTION

Cysts: L = 450–990 µm; W = 250–810 µm; L/W ratio = 0.9–1.8; fenestral diameter = 14–21 µm; number of ridges between anus and fenestra = 16–22; Granek's ratio = 2.3–7.0.

Male: L = 960–1406 µm; stylet = 22–29 µm; spicules = 32–36 µm; gubernaculum = 8 µm.

J2: L = 366–502 µm; stylet = 19–23 µm; DGO = 2.4–6.7 µm; hyaline region = 18–30 µm; tail = 37–57 µm.

Female colour changing from white to yellow to light golden as female matures to cyst stage. Cyst brown, ovate to spherical in shape with protruding neck, circumfенestrate (Fig. 4.3F), abullate. Fenestra circular, anus conspicuous at apex of a V-shaped subsurface cuticular mark. J2 body tapering at both extremities but more at posterior end. Stylet well developed, with prominent rounded knobs as viewed laterally. Lateral fields with four lines extending for most of body length. Tail tapering to small, rounded terminus.

Globodera rostochiensis is morphologically similar to *G. pallida* and *G. tabacum*. It differs from *G. pallida* by yellow or gold vs cream coloured maturing females, higher number of ridges between the vulva and anus, larger mean for Granek's ratio, stylet knob shape, shorter average stylet length and rounded vs more pointed J2 tail terminus (Box 4.2).

4.4.2.2. Pale potato cyst nematode, *Globodera pallida* Stone, 1973

The pale PCN, *G. pallida*, is considered to be a major pest of potato crops in cool temperate climates. It is reported from several countries in Europe, Asia, Africa and South America. In Central and North America *G. pallida* has been reported in Panama, the USA and Canada, but in the last two countries *Globodera* species on potato have a rather restricted distribution with small infested areas, because of rigorous phytosanitary regulations and seed potato certification programmes, compared to the widespread infestations found in European countries. Recently, mtDNA analysis has been used to study genetic relationships among Peruvian populations of *G. pallida*, thus identifying the origin of Western European populations of this species (Picard *et al.*, 2007; Plantard *et al.*, 2008). Using the mtDNA gene, cytochrome b (*cytb*) sequences and microsatellite loci, Plantard *et al.* (2008) showed

Box 4.2. Morphometric comparison between *Globodera rostochiensis* and *G. pallida* (measurements in μm ; values for second-stage juveniles are ranges compiled from various sources).

Second-stage juvenile characters

	Body length	Stylet length	Stylet knob shape	Tail length	Hyaline region tail length
<i>G. rostochiensis</i>	366–502	19–23	Rounded	37–57	18–30
<i>G. pallida</i>	380–533	23–25	Robust; square hooked	40–57	20–31

Cyst characters

	Distance from anus to vulval basin; mean (range)	Fenestral diameter	Number of ridges between anus and vulval basin	Granek's ratio
<i>G. rostochiensis</i>	66.0 (50.0–77.0)	Usually <19.0	16–22	2.3–7.0
<i>G. pallida</i>	45.0 (35.0–55.0)	Usually >17.0	7–17	1.2–3.6

that the *G. pallida* presently distributed in Europe derived from a single restricted area in the extreme south of Peru, located between the north shore of Lake Titicaca and Cusco. *Globodera pallida* develops one generation for a vegetation season. This species is adapted to cool temperatures and is able to hatch earlier in the year and develop at 2°C cooler than *G. rostochiensis* (Langeslag *et al.*, 1982). The symptoms of attack by *G. pallida* are similar to those for *G. rostochiensis* and the damage threshold is 1–2 eggs g^{-1} soil. Hosts includes potato (*S. tuberosum*), eggplant (*S. melongena*), tomato (*S. lycopersicum*), many other species of *Solanum*, and black henbane (*Hyoscyamus niger*).

DESCRIPTION

Cysts: L = 420–748 μm ; W = 400–685 μm ; fenestral diameter = 17.5–25 μm ; number of ridges between anus and fenestra = 7–17; Granek's ratio = 1.2–3.6.

Male: L = 1198 μm ; stylet = 27 μm ; spicules = 36 μm ; gubernaculum = 11 μm .

J2: L = 380–533 μm ; stylet = 22.5–25 μm ; DGO = 2.7–5 μm ; hyaline region = 20–31 μm ; tail = 40–57 μm .

Female is white in colour, some populations passing, after 4–6 weeks, through a cream stage, turning glossy brown when dead. Cyst vulval region intact or fenestrated

with single circumfenestrate (Fig. 4.3D) opening occupying all or part of vulval basin, abullate. J2 lateral field with four incisures but with three anteriorly and posteriorly, occasionally completely areolated. Stylet well developed, basal knobs with distinct anterior projection as viewed laterally. Tail tapering uniformly with a finely rounded point, hyaline region forming about half of tail region.

Globodera pallida is most closely related to *G. rostochiensis* and *G. tabacum*. It differs from *G. rostochiensis* by cream-coloured females vs yellow or gold, smaller number of ridges between the vulva and anus, smaller mean for Granek's ratio, stylet knob shape, longer stylet length, tail terminus and presence of refractive bodies on hyaline part of tail (usually 4–7 refractive bodies vs absence) in J2.

4.4.2.3. Tobacco cyst nematode *Globodera tabacum* (Lownsbery & Lownsbery, 1954) Skarbilovich, 1959

Globodera tabacum is considered as a serious and important pest of shade and broad-leaf tobacco. It is recorded from several countries in Europe, Asia, Africa, South and North America. *Globodera tabacum* is a polytypic species containing the following subspecies: *G. tabacum tabacum* (Lownsbery & Lownsbery, 1954), *G. tabacum virginiae* (Miller and Gray, 1968) and *G. tabacum solanacearum* (Miller & Gray, 1972). All three subspecies develop on tobacco and horse nettle (*Solanum carolinense*), but otherwise differ in host preference. *Globodera tabacum* parasitizes *Nicotiana tabacum*, *S. carolinense*, tomato and other species of the genera *Nicotiana* and *Solanum*, as well as *Atropa belladonna*, *Hyoscyamus niger*, *Nicandra physalodes* and *Capsicum annuum*. Two or more generations usually occur. Infected tobacco plants have small root systems and above-ground symptoms are similar to those associated with severe root-knot and lesion nematode infestations. Nematode infection is often associated with increased damage from bacterial wilt, black shank. Farmers in Virginia, USA, have recorded complete crop failures, but losses generally average 15%. A high density of nematode populations early in the growing season can reduce flue-cured tobacco yield by 25–50%, although tobacco may escape significant losses from moderate populations, especially under favourable growing conditions.

DESCRIPTION

Cysts: L = 337–937 µm; W = 232–812 µm; L/W ratio = 0.9–1.5; fenestral diameter = 13–36 µm; number of ridges between anus and fenestra = 5–15; Granek's ratio = 1.4–4.2.

Male: L = 710–1450 µm; stylet = 24–29 µm; spicules = 26–35 µm; gubernaculum = 9–12 µm.

J2: L = 458–621 µm; stylet = 20–27 µm; DGO = 4.3–9 µm; hyaline region = 17–35 µm; tail = 34–64 µm.

Female body ovate to spherical with elongate neck, white, becoming yellow. Cyst light shiny brown, circumfenestrate, abullate. J2 with well-developed rounded basal knobs. Terminus of tail finely rounded.

Globodera tabacum differs from *G. rostochiensis* by J2 with longer mean values of body length, mean stylet and by cysts with smaller mean number of cuticular ridges.

It differs from *G. mexicana* by J2 with longer mean body length and from *G. pallida* by cysts with a smaller mean number of cuticular ridges and by J2 with longer mean body length.

4.4.3. Genus *Punctodera* Mulvey & Stone, 1976

Mature females and cysts are spherical, pear-shaped or ovoid, with short projecting neck and heavy subcrystalline layer. Cuticle reticulate, subcuticle with punctations. D-layer present. Terminal region not cone-shaped; cyst light to dark brown. Vulval slit extremely short (<5 µm), anus at a short distance from vulval fenestra. Circumfenestrata, fenestra surrounding vulva 16–40 µm (approx. 20 µm in type species) in diameter, anus offset toward ventral margin of fenestra, an anal fenestra of similar shape and size to vulval fenestra present. Underbridge and perineal papilla-like tubercles absent. Bullae present or absent. Egg retained in body, no egg sac. Males vermiform, less than 1.5 mm long. DGO 2.6–4.6 µm. Spicules 31–33 µm long, distally pointed. Tail less than 0.5 anal body diameter long, cloacal lips not forming a tube. J2 body length is 0.35–0.49 mm, stylet 24–26 µm and tail conical, 63–78 µm long, hyaline region in type species 38–41 µm long. Lateral field with four incisures. Phasmid openings punctiform, without a lens-like structure. Parasites of monocotyledonous plants. The type species of the genus is *Punctodera punctata*.

4.4.3.1. Grass cyst nematode, *Punctodera punctata* (Thorne, 1928) Mulvey & Stone, 1976

This species was described by Thorne based on specimens from heavily infected wheat roots from a field in the Humboldt area, Saskatchewan, Canada. Several further attempts to collect topotype specimens failed. Subsequently, *P. punctata* was also reported as a common species infecting grasses from Europe, the USA and Canada. However, all attempts to infect wheat or other cereals by these nematodes failed to give any positive results. Several authors suggested that *P. punctata* might represent a complex of several closely related species. Many grasses are good hosts of this nematode. Only a single generation occurs each year.

DESCRIPTION

Female and cysts: L = 330–901 µm; W = 170–720 µm; L/W ratio = 1.2–3.0; vulval fenestral diameter = 16–33 µm; anal fenestral diameter = 33 (25.2–42.0) µm.

Male: L = 910–1270 µm; stylet = 26–33 µm; spicules = 28–36 µm; gubernaculum = 8–10 µm.

J2: L = 520–643 µm; stylet = 23–32 µm; DGO = 3.5–6.5 µm; hyaline region = 38–64 µm; tail = 63–93 µm.

Females and cysts are ovoid, pear- or flask-shaped without vulval cone, white. Vulva slit approx. 4 µm long, bordered by thickened ridges, set in a subcircular translucent area of cuticle. Anal slit less than 4 µm long, positioned towards ventral side of a similar subcircular area. Newly formed cysts with conspicuous subcrystalline layer.

J2 with well-developed projecting anteriorly basal knobs. Conspicuous hyaline region at least twice as long as stylet, distal third of tail tapering, ending in a rounded point.

Punctodera punctata differs from other *Punctodera* species by the pear-shaped cysts and the absence of bullae.

4.4.4. Genus *Cactodera* Krall & Krall, 1978

Mature females and cysts are lemon-shaped to spherical, with posterior protuberance. Vulva terminal, vulval slit <30 µm, fenestra circumfenestrate. Anus without fenestration. Bullae and underbridge absent, vulval denticles usually present. Cuticle with D-layer. The eggs are usually retained within the cyst body. The eggshell surface may be covered by tiny punctations. The surface structure of eggs is important diagnostically within *Cactodera* as some species have smooth surfaced eggs, e.g. *Cactodera weissi* and *C. amaranthi*, whilst others, such as *C. milleri*, *C. eremica* and *C. thornei*, are punctuated. J2 have a lateral field with four incisures, phasmid openings punctiform. The type species of the genus is *C. cacti*.

4.4.4.1 *Cactus cyst nematode*, *Cactodera cacti* (Filipjev & Schuurmans Stekhoven, 1941) Krall & Krall, 1978

A cyst nematode infecting cacti, *Discocactus akkermannii* and *Cereus speciosus*, both of which were expressing declining symptoms, was first recorded and described from Maartensdijk, near Utrecht, The Netherlands. The cactus cyst nematode is distributed worldwide, mainly on plants of the family Cactaceae grown in glasshouses as ornamentals. The dispersal of *C. cacti* from native regions in the Americas is beyond doubt associated with the international trade of infested ornamental cactus plants around the world. The cactus cyst nematode has been associated with or found to infect plants belonging to three families: Cactaceae (Caryophyllales): *Cereus*, *Cleistocactus*, *Coryphantha*, *Discocactus*, *Echinocactus*, *Echinopsis*, *Echinocereus*, *Epiphyllum*, *Gymnocalycium*, *Hatiora*, *Heliocereus*, *Hylocereus*, *Leuchtenbergia*, *Mammillaria*, *Melocactus*, *Notocactus*, *Nopalea*, *Notocactus*, *Opuntia*, *Oreocereus*, *Rebutia*, *Rhipsalis*, *Schlumbergera*, *Selenicereus*, *Thelocactus*; Umbelliferae (order Apiales): *Apium*; and Euphorbiaceae (order Malpighiales): *Euphorbia*. Infected plants may exhibit various symptoms including branched roots and increased numbers of rootlets. Plants become reddish-brown to yellow in colour, wilted and stunted, with reduced flower production and shortening of the flowering period. With high infection the host may die.

DESCRIPTION

Cysts: L = 328–780 µm; W = 240–598 µm; L/W ratio = 1.1–2.0; fenestral diameter = 16–48 µm.

Male: L = 910–1113 µm; stylet = 22–29 µm; spicules = 30–37 µm; gubernaculum = 10–15 µm.

J2: L = 344–584 µm; stylet = 21–26 µm; hyaline region = 12–21 µm; tail = 34–60 µm.

Female body lemon-shaped to almost spherical, pearly white, yellow or golden, maturing to light brown. Cyst usually lemon-shaped, but may be rounded with protruding neck and vulva, light or medium brown, sometimes reddish-brown. Vulval denticles generally present, visible beneath fenestral surface. Cone tops abullate, circumfenestrate. J2 vermiform, body tapering anteriorly and posteriorly. Tail tapering, with hyaline region often shorter than stylet. Eggshells heavily punctate as seen under optical microscope with oil immersion.

Cactodera cacti resembles *C. weissi*, *C. acnidae*, *C. milleri* and *C. galinsogae*. It differs from *C. weissi* and *C. acnidae* in having eggshells heavily punctate vs shells without visible markings, and J2 with larger stylet. It differs from J2 of *C. galinsogae* by a longer tail and from *C. milleri* by cysts with a larger fenestral diameter.

4.4.5. Genus *Dolichodera* Mulvey & Ebsary, 1980

Females and cysts: body elongate to oval, without terminal protuberance, white, swollen part 400–500 µm long, 140–270 µm wide, 2–2.8 times as long as wide, neck moderately long. Cuticle not annulated but with fine irregular striae. Vulval area terminal or just subterminal, circumfenestrate, fenestra approx. 20 µm in diameter, bullae present, perineal tubercles absent. Anus pore-like, lacking a fenestra, located 10–13 µm dorsal to vulval fenestral margin. Cyst with several large bullae. Perineal tubercles absent. Vulva circumfenestrate, underbridge absent. Anus lacking fenestra. Male not found. J2 with long tail (95–120 µm). Lateral field with three incisures, inner one faint. Labial region hemispherical, offset, with two annuli. Tail tip narrowly rounded. Phasmid openings lacking a lens-like ampulla, located about one anal body diameter posterior to anus. The type species of the genus is *Dolichodera fluvialis* Mulvey & Ebsary, 1980, parasitizing *Spartina pectinata*.

4.4.6. Genus *Betulodera* Sturhan, 2002

Cysts are lemon-shaped, pear-shaped or spheroid with insignificant, obtuse vulval cone. Cyst wall thick, with irregular network-like pattern, D-layer absent (no punctations in inner, deeper layers of cyst wall), subcrystalline layer heavily developed. Vulva terminal, surrounded by circumfenestration, vulval slit short (<10 µm), underbridge absent, denticles occasionally present, anus without fenestration. Male body twisted, no cloacal tube, spicules with bifid distal tips, phasmid openings punctiform. J2 has lateral field with three incisures, phasmid openings punctiform, without lens-like structure, labial region with three or four labial annuli and labial disc fused with submedial lips. The type and only species: *Betulodera betulae* (Hirschmann & Riggs, 1969) Sturhan, 2002.

4.4.7. Genus *Paradolichodera* Sturhan, Wouts & Subbotin, 2007

Mature female and cyst are elongate to ovoid, with rounded posterior end. Cuticle transparent, with faint transverse striations on anterior part of body and posteriorly

mostly with faint irregular ridges superimposed on distinct punctations. Cuticle turning yellowish to light brown on death, covered by a subcrystalline-like film. Eggs retained in body, egg sac not observed. Labial disc squarish. Stylet well developed. Vulva terminal or subterminal, vulval slit short, circumfenestrate. Anus lacking fenestration. Male body not twisted, lateral field with four incisures. Cloacal tube present, spicules rounded at tip. Phasmids lacking. J2 long, extremely slender for family, lateral fields indistinct. Stylet short ($<20\text{ }\mu\text{m}$). Dorsal gland orifice located more than half stylet length posterior to stylet base, pharyngeal glands long, filling body cavity. Tail long, slender, phasmid openings punctiform. Type and only species: *Paradolichodera tenuissima* Sturhan, Wouts & Subbotin, 2007.

4.4.8. Genus *Vittatidera* Bernard, Handoo, Powers, Donald & Heinz, 2010

Cysts are orange-brown to brown, lemon-shaped with short necks and vulval cone. Vulval aperture circular to rhomboid, circumfenestrate, with irregular denticle-like protuberances around the periphery of orifice. Bullae, vulval bridge and vulval under-bridge absent. It resembles representatives of the genera *Cactodera* and *Betulodera* in having lemon-shaped cysts and circumfenestrate vulval area. Male variable length, stylet knobs rounded. J2 having conoid tail with narrowly rounded tip, phasmid apertures pore-like. Stylet length $<18\text{ }\mu\text{m}$. Stylet knobs rounded. Lateral field with four incisures. Eggshell smooth. The type and only species is *Vittatidera zeaphila* Bernard, Handoo, Powers, Donald & Heinz, 2010, parasitizing maize in north-western Tennessee, USA.

4.5. Pathotypes and Races

As resistant varieties were increasingly developed as a means of controlling cyst nematodes in several major crops (see Chapter 14), it became apparent that genetic variation existed within populations able to overcome such resistance (Cook and Rivoal, 1998). This led to the growing realization that within cyst nematode species that are morphologically identical, distinct virulent strains occur. Various pathotype schemes for the major cyst nematodes were proposed, with ‘pathotype’ being regarded as a group of individual nematodes with common gene(s) for (a)virulence and differing from gene or gene combinations found in other groups. Three cyst nematode groups have been extensively studied and pathotype schemes proposed, which are all based on the ability (or inability) of populations within each species to reproduce on a range of ‘differential’ host plants; these three groups are PCN (*G. rostochiensis* and *G. pallida*), cereal cyst nematode (*H. avenae*) and the soybean cyst nematode (*H. glycines*).

Two pathotype schemes for *G. rostochiensis* and *G. pallida* were proposed in 1977 that described the virulence of populations from Europe and South America (Table 4.4). In the pathotype/differential clone interactions, susceptible (+) indicated a multiplication rate (P_f/P_i) >1.0 , and resistant (-) indicated a $P_f/P_i <1.0$, where P_i and P_f are the initial and final population sizes, respectively. This standardized disparate national schemes, especially those used within European countries, but it soon became clear that environmental influences and the extensive heterogeneity of

Table 4.4. Pathotype groups of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. (Adapted from Cook and Noel, 2002.)

Species and accession	Ploidy, resistance gene	Species			<i>G. rostochiensis</i>			<i>G. pallida</i>						
		<i>Globodera</i> species virulence group ^a		Ro1	Ro3	Ro5	Pa1		—		—		Pa2/3	
		European pathotypes ^b		Ro1	Ro4	Ro2	Ro3	Ro5	Pa1		—		Pa2	
		South American pathotypes ^c		R1A	R1B	R2A	R3A	—	P1A	P1B	P2A	P3A	P4A	P5A
<i>Solanum tuberosum</i> ssp. <i>tuberosum</i>	4x, (minor)	+/-	+	+	+	+	+	+	+	+	+	+	+	+
<i>S. tuberosum</i> ssp. <i>andigena</i> CPC 1673	4x, H1 on chromosome 5	—	—	+	+	+	+	+	"	"	"	+	+	"
<i>S. kurtzianum</i> KTT 60.21.19	2x, K1 K2 A and B	—	(+)	—	(+)	(+)	+	+	+	+	+	+	+	+
<i>S. vernei</i> GLKS 58.1642.4	2x, quantitative	—	+	—	—	+	+	+	+	+	—	+	+	+
<i>S. vernei</i> Vt 62.33.3	2x, quantitative	—	—	—	—	+	—	+	—	—	—	—	+	+
ex. <i>S. multidissectum</i> hybrid P55/7	2x, 1 + polygenes H2	+	+	+	+	+	—	-/+	—	+	+	+	+	+
<i>S. t. ssp. andigena</i> CIP 280090.10	H3 + polygenes Quantitative	+	"	"	"	"	(-)	"	"	"	(-)	(-)	"	
<i>S. vernei</i> hybrid 69.1377/94	2x, polygenes	—	—	—	—	—	—	"	"	"	"	"	"	"
<i>S. vernei</i> hybrid 63.346/19	2x, polygenes	—	—	—	—	—	+	"	"	"	"	+	+	"
<i>S. spegazzinii</i>	2x, Fa = H1	—	—	+	+	+	"	"	"	"	"	"	"	"
<i>S. spegazzinii</i>	2x, Fb + 2 minor	+	—	+	—	—	"	"	"	"	"	"	"	"
	Glo1 on chromosome 7	(-)	+	+	+	+	"	"	"	"	"	"	"	"

^aTrudgill (1985); ^bKort *et al.* (1977); ^cCanto-Saenz and de Scurrall (1977).

Note: + = compatible interaction: nematode multiplication, potato susceptible; — = incompatible interaction: nematode no multiplication, potato resistant; () = partial or uncertain interaction; " = no information.

some populations, especially those of *G. pallida*, caused problems. Populations in the centres of origin of the two species in South America are more heterogeneous in virulence characteristics than those introduced and dispersed in the rest of the world. Some populations are relatively homozygous for virulence, e.g. Ro1 (R1A) and Pa1 (P1A). Others, such as most *G. pallida* populations, are heterogeneous and give varying results; thus, these populations cannot reliably be described as pathotypes and are increasingly referred to as virulence groupings within pathotypes.

The pathotype scheme for cereal cyst nematodes (*H. avenae*) is based on their multiplication on host differentials of barley, oats and wheat crops, with the major division into three pathotype groups based upon reactions of the barley cultivars with known resistance genes (*Rha1*, *Rha2*, *Rha3*). Each pathotype group is further subdivided by their reactions on other differentials. Resistance is defined as fewer than 5% new females on susceptible controls (Table 4.5). As with the PCN pathotype schemes, because the genetics of field populations are largely unknown and variability exists within them, the term 'virulence phenotype' has been proposed. Evidence suggests that the different species of cereal cyst nematodes (*H. avenae*, *H. filipjevi* and *H. australis*) have populations with different virulence phenotypes. There is limited evidence for loss of effectiveness of resistance genes used in widely grown cultivars and part of the reason for this may be that endemic biological control develops when cereals are intensively cultivated in moist temperate soils and, therefore, selection pressures for virulent strains are reduced.

Differences in virulence between soybean cyst nematode (*H. glycines*) populations are referred to as races rather than pathotypes. Such differences were recognized during breeding programmes in the USA for resistant soybean varieties. Using four soybean differentials (Pickett, Peking, PI 88788 and PI 90763), 16 races were characterized (Table 4.6). A resistant response (avirulence) is defined as a Female Index of <10% of that obtained on the susceptible cultivar Lee. Although the soybean cyst nematode race scheme predicts whether a cultivar will control the nematode population within a field in a particular season, as with other schemes, it cannot predict the consequences of selection pressure with a heterogeneous population.

Despite the limitations of the various pathotype/race schemes for cyst nematodes, providing their limitations are recognized, they continue to give a useful indication of the virulence characteristics of particular nematode gene pools. As such, they can provide critical information necessary for effective management and the emergence of new virulent strains.

4.6. Biochemical and Molecular Diagnosis

Biochemical approaches such as gel electrophoresis for separating protein and enzyme profiles have shown great potential for helping to identify cyst nematodes. Isoelectric focusing (IEF) is currently used in nematology laboratories for routine diagnostics of *G. pallida* and *G. rostochiensis*. By comparison with the biochemical approaches, analysis of DNA for diagnostics has several advantages. The main DNA region targeted for diagnostics of cyst nematodes is the internal transcribed spacer 1 (ITS1) and internal transcribed spacer 2 (ITS2), which are situated between 18S and 5.8S and 5.8S and 28S rRNA genes, respectively. Genes of mitochondrial DNA (mtDNA), with their relatively higher rate of mutations relative to rRNA genes, have

Table 4.5. Pathotype groups of cereal cyst nematodes, *Heterodera avenae*, *H. filipjevi* and *H. australis*. (Adapted from Cook and Rivoal, 1998.)

Species	<i>H. avenae</i>							<i>H. australis</i>		<i>H. filipjevi</i>	
	Pathotype group							Ha2 group		Ha3 group	
Pathotype	Ha11	Ha21	Ha31	Ha41	Ha51	Ha61	Ha71	Ha12	Ha13	Ha23	Ha33
Different species and cultivar											
<i>Barley</i>											
Varde	+	"	"	+	"	+	+	+	+	+	+
Emir	+	+	"	+	"	-	+	+	+	+	+
Ortolan/Drost	-	-	-	-	-	-	-	+	+	+	+
Morocco	-	-	-	-	-	-	-	-	-	-	-
Siri	-	-	-	+	+	+	-	-	+	+	+
KVL 191	-	-	-	"	+	+	+	-	"	"	"
Bajo Aragon	-	"	"	-	"	-	-	-	+	+	+
Herta	+	+	-	"	-	"	-	+	+	"	"
Martin 403	-	"	"	-	"	-	-	-	-	+	+
Dalmatische	(-)	"	"	+	"	-	(+)	+	+	(-)	+
La Estanzuela	"	"	"	"	"	"	+	"	"	(-)	"
Hartian 43	-	"	"	"	"	"	-	-	"	-	+
<i>Oat</i>											
Nidar	+	"	"	(+)	"	+	-	+	+	+	+
Sol II	+	-	-	-	-	+	-	+	+	+	+
Pura Hybrid BS1	-	-	"	-	-	-	-	-	+	-	+
<i>Avena sterilis</i> 1376	-	-	"	-	-	-	-	-	-	-	-
Silva	(-)	"	"	-	"	(-)	-	(-)	(-)	(-)	+
IGV.H. 72-646	-	"	"	-	"	-	-	-	+	+	+
<i>Wheat</i>											
Capa	+	+	"	+	"	+	+	+	+	+	+
AUS10894	-	"	"	-	"	-	+	-	(-)	+	+
Loros	-	-	"	-	"	(-)	-	-	(-)	+	+
Psathias	"	"	"	+	"	"	"	+	+	+	-
Iskamish K-2-light	+	"	"	-	"	(-)	"	+	+	+	+

Note: + = susceptible; - = resistant (<5% new females compared to numbers on susceptible control); (-) = intermediate; " = no information

Table 4.6. Races of soybean cyst nematode, *Heterodera glycines*. (Adapted from Cook and Rivoal, 1998.)

Differential cultivar	Race	3	6	13	9	1	5	11	2	8	10	12	14	7	15	1	4
	Virulence phenotype	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pickett		—	+	—	+	—	+	—	+	—	+	—	+	—	+	—	+
Peking		—	—	+	+	—	—	+	+	—	—	+	+	—	—	+	+
PI 88788		—	—	—	—	+	+	+	+	—	—	—	—	+	+	+	+
PI 90763		—	—	—	—	—	—	—	—	+	+	+	+	+	+	+	+

Note: — resistant (female index <10% cultivar Lee); + susceptible (female index >10% that of susceptible control cultivar Lee).

great potential for diagnostics of races and populations of cyst nematodes. PCR-RFLP and PCR with species-specific primer(s) presently are used for diagnostics of many cyst nematode species (Subbotin *et al.*, 2010a,b; see Chapter 2).

4.7. Interactions with Other Plant Pathogens

As with all plant-parasitic nematodes, the mechanism of feeding on plant tissue results in wounding and provision of entry sites for other pathogens (Barker and McGawley, 1998). However, more specific associations, which can result in either synergistic or antagonistic responses by the host plant, demonstrate that more complex interactions have evolved. Most investigations of the interrelationships of cyst nematodes and other plant parasites have focused on those with fungi, especially those causing wilt and root rot (Table 4.7). A number of cyst nematode species interact with *Fusarium* wilt species, causing the wilt disease to be more severe than in the absence of the nematode, e.g. *G. tabacum* on tobacco, *H. cajani* on pigeon pea and *H. glycines* on soybean. Generally, these interactions involve synergism with regard to disease development but often result in restricted nematode reproduction because of the associated root damage. Only limited examples of interactions between cyst nematodes and root-rot fungi (*Rhizoctonia* spp.) have been documented, but those that have generally show an enhancement of the disease in the presence of the nematode, for example *G. rostochiensis* on potato, *H. avenae* on wheat, *H. glycines* on soybean and *H. schachtii* on sugar beet. Although such associations usually have an adverse effect on the host plant, the interaction of *H. cajani* with *Rhizoctonia bataticola* suppresses the associated damage caused by the fungus.

The economic effect of these interactions varies but their effect can be important with major high-value crops such as soybean. A major disease of soybean is ‘sudden death syndrome’ (SDS) caused by *Fusarium solani*, which is sometimes associated with the presence of *H. glycines*. Although experimental data indicate that the nematode is not necessary for the development of SDS, field observations have shown that soybean cultivars resistant to *H. glycines* were less affected by SDS than susceptible ones. The decreased *H. glycines* population levels correspond to the restriction in root and shoot growth attributable to the additive root damage by the fungus and nematode.

Table 4.7. Summary of interrelationships of selected cyst nematodes of the genera *Globodera* and *Heterodera* and plant-pathogenic fungi. (Adapted from Barker and McGawley, 1998.)

Nematode	Associated fungi	Host	Comments
<i>G. rostochiensis</i>	<i>Rhizoctonia solani</i>	Potato	Yield loss, but only small interaction effect
	<i>Pyrenopeziza lycopersici</i>	Tomato	Fungus probably prevents syncytium formation by the nematode
<i>G. rostochiensis</i> and <i>G. pallida</i>	<i>Verticillium dahliae</i>	Potato	Results in 'early dying disease'
<i>G. tabacum</i>	<i>Fusarium oxysporum</i> <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> , <i>Verticillium albo-atrum</i>	Tobacco Tomato	Wilt disease enhanced More <i>Verticillium</i> but less <i>Fusarium</i> wilt in the presence than absence of <i>G. tabacum</i>
<i>H. avenae</i>	<i>Rhizoctonia solani</i> <i>Gaeumannomyces graminis</i>	Wheat	In combination, greater reduction in tillering, height, weight, root number and length than with either alone
<i>H. cajani</i>	<i>Fusarium udum</i> <i>Rhizoctonia bataticola</i>	Pigeon pea	Wilt enhanced
<i>H. glycines</i>	<i>Rhizoctonia solani</i> <i>Calonectria crotalariae</i> <i>Phytophthora megasperma</i> var. <i>sojae</i> <i>Fusarium oxysporum</i> <i>Fusarium solani</i> <i>Macrophomina phaseolina</i>	Cowpea Soybean	Damage suppressed Limited nematode reproduction Enhanced activity of both parasites Increased seedling disease (additive) Increased wilt Variable; increased foliar symptoms of fungus; suppressed nematode reproduction Synergism
<i>H. oryzicola</i> <i>H. schachtii</i>	<i>Sclerotium rolfsii</i> <i>Pythium ultimum</i> <i>Pythium aphanidermatum</i> <i>Pythium solani</i> <i>Rhizoctonia solani</i>	Rice Sugar beet	Variable, but usually synergistic Damage was less when fungus present; fungus inhibited nematode invasion Synergistic or damping off when in combination Additive Synergism Synergism, especially at high inoculum levels

The relationship of cyst nematodes with mycorrhizal fungi has focused on the vesicular-arbuscular mycorrhizal fungi, *Glomus* spp. This group is important as it may be useful in enhancing crop yields on nematode-infested soil by rendering nematode-susceptible plants more tolerant to these pathogens or through the suppression of nematode infections and reproduction. Although most research has focused on root-knot nematodes, observations of interactions of *H. glycines*,

H. cajani and *G. rostochiensis* with various *Glomus* species have been shown. For example, *Glomus fasciculatum* suppresses *H. glycines* on soybean and *H. cajani* on cowpea where the fungus may actually parasitize the nematode eggs.

Nematode communities usually contain many species that potentially may interact with each other. In most instances, where cyst nematodes are present, they are antagonistic to other plant-parasitic species. Examples of this include *G. tabacum* and *Pratylenchus penetrans* on tobacco, *H. avenae* and *P. neglectus* on wheat, *H. cajani* and *Helicotylenchus retusus* in pigeon pea, and *H. glycines* and *Meloidogyne incognita* on soybean. Very few examples of neutral or stimulatory responses between cyst and other nematode species have been documented. The potential interactions between cyst nematodes and bacteria, insects and other pests have received only limited study. One notable exception is that of *G. pallida* and the bacteria *Ralstonia solanacearum* on potato, in which the nematode enhances damage caused by the associated wilt.

Thus, cyst nematodes show a wide range of important interrelationships with associated organisms in a wide range of habitats. As such, full evaluations of host-parasite relationships should be undertaken in the presence of other pathogens likely to be present in their natural habitat. Such evaluations are needed for effective management strategies.

4.8. Management

The cyst nematodes that cause major damage to cultivated crops are mainly those species within the genera *Globodera* and *Heterodera* and these present a unique problem in their management (Riggs and Schuster, 1998). Many or all of the eggs are produced inside the female body that, upon death, becomes a cyst with a hardened protective wall. This structure is resistant to invasion by potential parasites and protects the eggs inside from rapid desiccation, enhancing their ability to remain dormant for many years. In many cases (e.g. *Globodera* spp.) substantial hatch will only occur in the presence of a hatching factor produced by a potential host (see Section 4.3 and Chapter 7), so that any management strategy must be effective over a period of years or usable year after year. However, unlike root-knot nematodes (Chapter 3), cyst nematodes have a relatively narrow host range, making appropriate crop rotation a viable option in certain situations.

4.8.1. Prevention

Fundamental to the prevention of cyst nematodes spreading into non-infested regions is the use of certified planting material, and strict legislation for those commodities being traded both internationally and locally. This tactic has been the mainstay for controlling several major pests such as *G. rostochiensis*, *G. pallida* and *H. schachtii* (Chapter 12). Efficient management and containment of an infestation may be compromised by the ease with which cysts can be dispersed by, for example, wind, in small aggregates of soil, on small roots attached to other parts of plants, by flood water run-off, or by adhering to machinery or animals passing through infested land. General hygiene practices should be adopted in higher risk situations when the pest is known to be present in the locality. Such measures would include cleaning machinery both before and after use, restricting movement of soil outside the field boundary and construction of natural wind breaks.

4.8.2. Crop rotation

Where host range is limited, crop rotation has proved an important component in managing cyst nematode levels. Alternative non-host crops can safely be cultivated, during which time a combination of spontaneous hatch and natural mortality will reduce the field population to below threshold levels (Chapter 11). Cyst nematodes that have only one to three cultivated host plants include *G. rostochiensis*, *G. pallida* (potato, eggplant and tomato), *H. avenae* (oat, barley and wheat), *H. ziae* (cultivated and wild maize) and *H. carotae* (cultivated and wild carrot). Even those cyst nematodes with broader host ranges, such as *H. schachtii* and *H. glycines*, have relatively few cultivated hosts, facilitating the potential for control by use of rotations.

4.8.3. Resistance

Cultivar resistance remains the most economical practice for managing cyst nematodes (Chapter 14), although these are not always available. Resistance of major crop hosts to *G. rostochiensis*, *G. pallida*, *G. tabacum tabacum*, *G. tabacum solanacearum*, *H. avenae*, *H. glycines*, *H. schachtii* and *H. cajani* have been found and attempts made to incorporate it into commercial cultivars. Only low level, or no, resistance is known in the major crop hosts of *H. cruciferae*, *H. oryzae*, *H. sacchari* and *H. oryzicola*. However, in many cases resistance is found only in wild species, with the accompanying inherent difficulties of transferring into commercial cultivars (Riggs and Schuster, 1998). The inappropriate continuous planting of resistant cultivars is now known to increase selection pressure for virulent populations (e.g. potatoes and *G. rostochiensis* and *G. pallida*), limiting the durability of resistance in some cultivars, or resulting in the increase of other nematode problems (e.g. cereal cyst nematode with the associated build-up of *Pratylenchus neglectus*).

4.8.4. Biological control

Cyst nematodes would appear to be the perfect target for the use of biological agents in their management. Eggs of cyst nematodes are contained either inside the female's body/cyst or in a gelatinous sac, so they should be very susceptible to parasitism by fungi or bacteria in the rhizosphere. Numerous studies using nematophagous fungi and bacteria against economically important cyst nematodes have been undertaken with varying degrees of success and failure. However, the best example of suppressiveness of soils toward cyst nematodes is in the control of *H. avenae* by the fungi *Nematophtora gynophila* and *Pochonia chlamydosporium* (Chapter 14).

4.8.5. Chemical control

Nematicides have been very effective in controlling cyst nematodes (Whitehead, 1998; see Chapter 16) but several of the most effective have now been withdrawn because of health and safety concerns. The effectiveness of nematicides is reduced by their biological degradation by soil organisms, which may be increased by the multiple use

of a nematicide. Examples of cyst nematodes that produce only one or two generations a year, and for which nematicides have been extensively used as a management strategy, are *G. rostochiensis* and *G. pallida* on potatoes, *H. goettingiana* on peas and *H. avenae* on cereals. However, those species that produce several generations in a year, such as *H. glycines*, appear to be more difficult to control.

4.8.6. Integrated management

The repeated use of a single control measure is likely to fail, sooner or later, from selection of virulent biotypes, accelerated microbial degradation of nematicide, or the selection of populations better able to overcome such diverse management programmes; in general, selection of individuals unaffected by any control measure that may be applied. The potential for managing cyst nematodes by combining two or more control strategies in an integrated programme has been widely demonstrated. Usually some level of crop rotation is practised, alongside additional measures. The advantages of this approach include the use of partially effective strategies and protection of highly effective ones that are vulnerable to nematode adaptation or environmental risk; examples include integrated control of *G. rostochiensis* and *G. pallida* in Europe, and *H. glycines* in the USA (Roberts, 1993).

5

Migratory Endoparasitic Nematodes*

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5.1. Introduction to Migratory Endoparasitic Nematodes

All life stages of migratory endoparasitic nematodes can be found within plant tissues. Unlike sedentary endoparasites, the nematodes in this group do not induce permanent feeding sites, but instead feed and reproduce while migrating between or through plant cells. The symptoms caused by migratory endoparasites vary. Some species are known for enzymatic degradation of host tissues, others for inducing hormonal imbalances that cause galling, swelling and other tissue distortions, and some cause visible lesions when phenolic compounds are released in and around wounded cells. Species in three nematode families are adapted to migratory endoparasitism. Those in the Pratylenchidae inhabit primarily cortical cells in roots and other below-ground plant parts. Species in the Anguinidae and Aphelenchoididae are unique among nematodes in parasitizing above-ground parts of plants, often exhibiting impressive survival and dispersal behaviours that permit them to live within stems, leaves, buds and seeds (see Chapter 7).

5.2. The Pratylenchids: Lesion, Burrowing and Rice Root Nematodes

Pratylenchus (lesion nematodes), *Radopholus* (burrowing nematodes) and *Hirschmanniella* (rice root nematodes) are among 11 genera in the family Pratylenchidae, and belong to the subfamilies Pratylenchinae, Radopholinae and Hirschmanniellinae, respectively (Chapter 1; Box 5.1). All are obligate plant parasites, but economically important species of these subfamilies are known only in the three genera treated here. Quite a large number of morphological features differentiate these taxa but two broad groups can be distinguished. In *Pratylenchus*, *Apratylenchus*, *Hirschmanniella* and *Zygotylenchus* there is no pronounced sexual dimorphism and the pharyngeal glands overlap the intestine ventrally, whereas in *Radopholus* and the remaining genera (*Apratylenchoides*, *Achlysiella*, *Pratylenchoides*, *Hoplotylus*, *Zygradus*), male and female anterior morphology differs distinctly and the pharyngeal overlap is dorsal (Fig. 5.1). These morphological differences suggest that the family is polyphyletic. Molecular analyses have also shown a closer relationship between *Radopholus similis* and ectoparasitic and cyst-forming species in the Hoplolaimidae. Morphological similarities in the genera *Pratylenchus* and *Radopholus* probably resulted from convergence, due to their similar feeding behaviours.

Two other subfamilies occur in the Pratylenchidae, each with a single genus. Although the juvenile, male and vermiform female stages of *Nacobbus* (Nacobbinae) behave as migratory endoparasites, the group is conventionally treated among the sedentary plant-parasitic nematodes because the females eventually establish permanent feeding sites within induced root galls (Manzanilla-López *et al.*, 2002). Similarly, the immature stages and males of a newly described genus, *Apratylenchus* (Apratylenchinae), resemble those of *Pratylenchus*; however, the mature female posterior assumes a distinctive club-shaped form. Two species of *Apratylenchus* were described from roots of coffee in Vietnam but their economic significance is unknown.

Box 5.1. Classification of the subfamilies Pratylenchinae, Radopholinae and Hirschmanniellinae

- Phylum Nematoda Potts, 1932
- Class Chromadorea Inglis, 1983
- Subclass Chromadoria Pearse, 1942
- Order Rhabditida Chitwood, 1933
- Suborder Tylenchina Thorne, 1949
- Infraorder Tylenchomorpha De Ley & Blaxter, 2002
- Superfamily Tylenchoidea Örley, 1880
- Family Pratylenchidae Thorne, 1949
- Subfamily Pratylenchinae Thorne, 1949
 - Genus *Pratylenchus* Filipjev, 1936
- Subfamily Radopholinae Allen & Sher, 1967
 - Genus *Radopholus* Thorne, 1949
- Subfamily Hirschmanniellinae Fotedar & Handoo, 1978
 - Genus *Hirschmanniella* Luc & Goodey, 1964

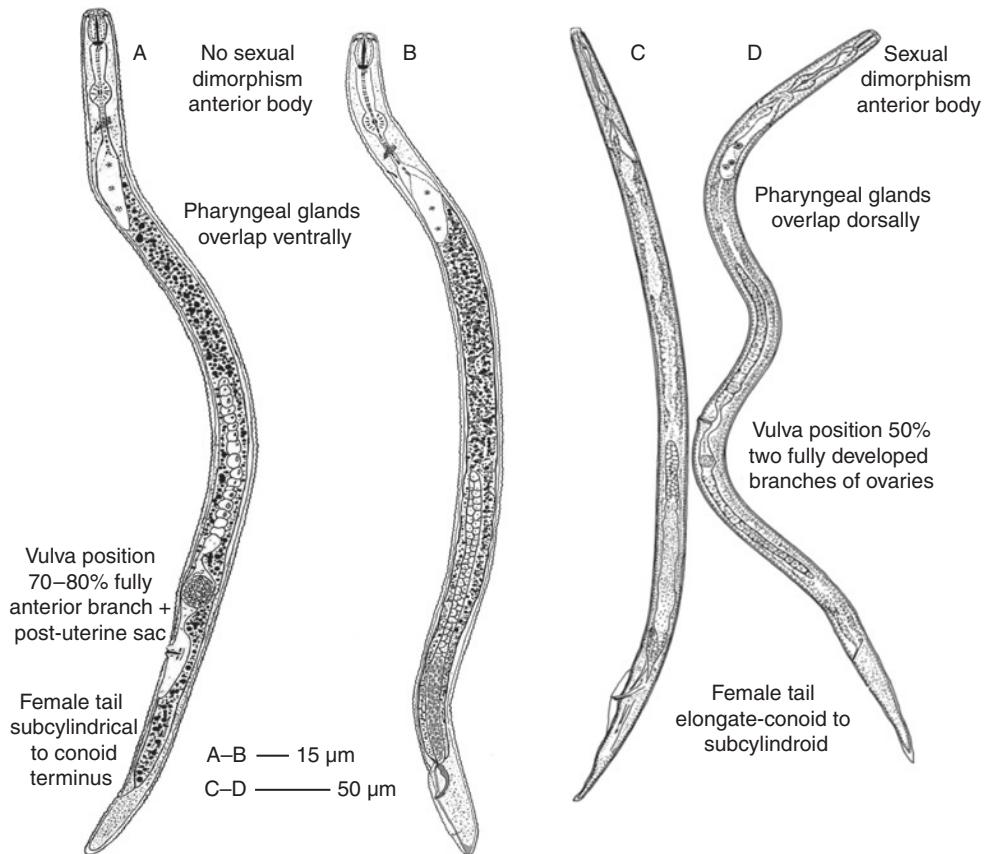


Fig. 5.1. Entire female and male body of *Pratylenchus dunensis* (A, B) and *Radopholus arabocoffeae* (C, D) showing differences in pharyngeal glands overlapping the intestine, in sclerotized lip region, in stylet morphology between male and female, in position of the vulva and in tail shape. (A, B after de le Peña *et al.*, 2006; C, D after Trinh *et al.*, 2004.)

5.2.1. Lesion nematodes, *Pratylenchus* spp.

Among the pratylenchids, *Pratylenchus* is easily the most cosmopolitan genus in terms of the variety of habitats occupied. The genus may also have the broadest host range among plant-parasitic nematodes. *Pratylenchus* is worldwide in distribution and while most species are of no or little economic importance, others are responsible for substantial yield losses in many agronomic and horticultural crops. Indeed, next to root-knot and cyst nematodes, *Pratylenchus* spp. cause the greatest crop damage by nematodes worldwide (Castillo and Vovlas, 2007).

5.2.1.1. Morphology and identification

Species of *Pratylenchus* (from Luc, 1987) are stout, small to medium size nematodes with body length less than 0.9 mm and rarely strongly motile. The stylet and lips are

heavily sclerotized. The lip region is usually not (or only slightly) offset from the body and is low and flattened anteriorly, with two, three or occasionally four labial annuli. The pharyngeal glands overlap the intestine ventrally for a medium distance and there is no sexual dimorphism in the anterior part of the body. The vulva is posterior and females are monoprodelphic with an anterior genital branch that may or may not contain a developed spermatheca and a posterior branch that is reduced to a post-uterine sac. In species with males, the male tail is pointed with caudal alae extending to the tip. The gubernaculum is simple and does not protrude.

Of the more than 70 species in the genus, a large number are inadequately described. Some are known to be species complexes. *Pratylenchus* exhibits relatively little interspecific morphological variation when viewed with the light microscope. Some characters, such as tail shape, can be quite variable within a species. This makes it easy to recognize members of the genus but difficult to differentiate between species. The ranges of dimensions of most characters overlap between species, so it is often necessary to examine at least ten specimens to sample the range of morphometric and morphological variation in a population. The face morphology when viewed with SEM is often useful to differentiate otherwise similar species of *Pratylenchus* (Fig. 5.2). Some characteristics for several of the more economically important species of *Pratylenchus* are given in Table 5.1.

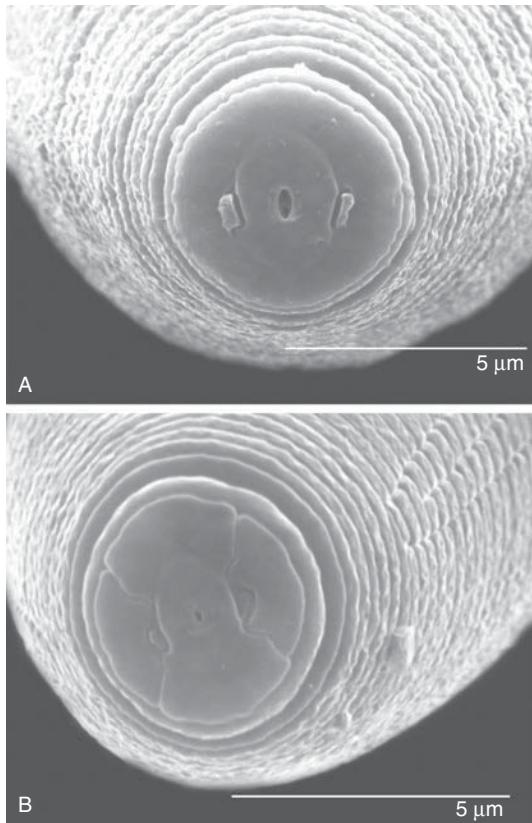


Fig. 5.2. Scanning electron micrographs of first head annule face views showing a smooth pattern in *Pratylenchus coffeae* (A) isolated from citrus in Florida, and a divided pattern in *P. pseudocoffeae* (B) isolated from *Aster elliotii* in Florida.

Table 5.1. Some commonly encountered species of lesion nematodes, their geographic ranges, some of the crops they damage, and morphological characters useful for their identification. (From Loof, 1991, and other sources.)

Species	Head annules	Males present	Face pattern	Additional characters	Geographic distribution and selected economically important hosts
<i>Pratylenchus coffeae</i>	2	Yes	Smooth	Tails of <i>P. coffeae</i> bluntly rounded, truncate or indented vs tail narrowly rounded for <i>P. loosi</i> .	Pantropic and subtropic. Banana, plantain, coffee, citrus, yam and many other crops.
<i>P. loosi</i>	2	Yes	Smooth		South Asia and Japan. Tea, coffee, citrus.
<i>P. brachyurus</i>	2	No	Smooth	Very posterior vulva and tail with smooth rounded or truncate terminus.	Pantropic and subtropic. Peanut, potato, pineapple, peach, soybean, tobacco, coffee, rubber.
<i>P. neglectus</i>	2	No	Divided	<i>P. neglectus</i> vulva is usually more posterior (81–86%) than that of <i>P. scribneri</i> (72–80%).	Temperate and subtropical regions of most continents. Cereals, turf, crucifers, legumes, strawberry, tobacco, mint, maize.
<i>P. scribneri</i>	2	No	Divided		Widely distributed in subtropical and warmer temperate regions. Maize, tomato, sugar beet, onion, soybean, potato, bean, ornamentals.
<i>P. penetrans</i>	3	Yes	Divided	Smooth rounded tail terminus in <i>P. penetrans</i> vs rounded annulated tail terminus for <i>P. fallax</i> .	Cosmopolitan, chiefly temperate. Very wide host range. Fruit trees, conifers, ornamentals, potato, vegetables, maize, forage crops, sugar beet, fern, among many others.
<i>P. fallax</i>	3	Yes	Divided		Europe and Japan. Cereals, fruit trees, ornamentals, forage crops, strawberry.
<i>P. thornei</i>	3	No	Divided	Long vulva–anus distance and tail bluntly rounded or truncate.	Widespread in subtropical and warmer temperate regions. Cereals, legumes, fruit trees, woody and herbaceous ornamentals.
<i>P. zeae</i>	3	No	Smooth	Vulva 65–76% and tail tapered with sub-acute terminus in <i>P. zeae</i> vs vulva 78–86% and crenate tail for <i>P. crenatus</i> .	Pantropic and subtropic. Cereals, forage crops, turf, sugar cane, tobacco, peanut.
<i>P. crenatus</i>	3	No	Smooth		Temperate, but occasionally subtropics and elevated tropics. Cereals, forage grasses, carrots.
<i>P. vulnus</i>	3–4	Yes	Divided	Tail is dorsally sinuate before terminus, vulva 73–76% in <i>P. goodeyi</i> vs narrowly rounded to sub-acute tail and vulva at 77–82% in <i>P. vulnus</i> .	Worldwide subtropical and Mediterranean climates. Mostly woody plants including rose, deciduous fruit and nut, citrus seedlings.
<i>P. goodeyi</i>	4	Yes	Divided		East Africa, Canary Islands, Greece and Australia. Banana.

Note: Partial DNA sequences for all of these species are available from Genbank (<http://www.ncbi.nlm.nih.gov/gquery/gquery.fcgi>).

5.2.1.2. Life cycle and behaviour

Parthenogenesis appears to be the mode of reproduction in about half of the known *Pratylenchus* species, based on absence of males and absence of sperm in the female spermatheca. Obligatory amphimixis (see Chapter 7) has been demonstrated in several of the remaining species. Eggs are oviposited at rates up to two per day, mainly in root tissue but also in soil along the root surface. Eggs are clustered in root tissue due to the gregarious behaviour of lesion nematodes. Eggs can also function in survival between hosts. *Pratylenchus penetrans* second-stage juveniles remain unhatched and quiescent, protected within eggs in the soil until stimulated to hatch by diffusates from roots, especially young host plant roots. This behaviour also reduces the amount of time that juveniles actively deplete energy reserves in search of food. All life stages of lesion nematode species can be isolated from both soil and roots, and the proportion of the population in the soil is distinctly seasonal in regions where temperature or moisture regulates root availability. As lesion nematodes readily migrate to fresh cortical tissue when resources become limiting, higher numbers of nematodes are often recovered from feeder roots that appear healthy than from diseased roots, suberized roots or soil.

Juvenile lesion nematodes sometimes feed ectoparasitically on plant root hairs, although cell death seldom occurs without repeated feeding. More commonly, all vermiform stages penetrate into and reside in the root cortex, but usually do not penetrate or feed on the endodermis or central stele. The nematodes are attracted to the zone of elongation or to root junctures. Root penetration attracts additional nematodes to the same entry site, resulting in clusters of nematodes within the root tissue. For amphimictic species this behaviour aids in locating a mate. Nematodes rupture cell walls by repeated stylet thrusting, after which they migrate through the cell or pause to feed. When feeding, the nematode secretes salivary enzymes for a short time before ingesting the cytoplasm. Feeding time may be brief during migration or may last for several hours. Cell death occurs when nematodes migrate through a cell and following a long feeding bout, but not following brief feeding. Migration and long feeding bouts alternate with resting phases of several hours, during which the nematode coils within a cell. Between temperatures of 17°C and 30°C *P. penetrans* completed its life cycle in 548 degree days (base 5.1°C; 22–46 days) on clover roots. In general, tropical species at elevated temperatures complete a life cycle in 3–4 weeks, whereas temperate species at cooler temperatures require 5–7 weeks.

5.2.1.3. Host reaction

Brown to reddish necrotic lesions parallel to the root axis and eventual secondary decomposition characterize roots infected by lesion nematodes. The presence and extent of root lesions vary with the plant and nematode species, depending on the amount of polyphenol deposition caused by nematode damage. These symptoms differ from those caused by migratory stages of sedentary endoparasitic nematodes that travel between and through cortical cells without feeding until a permanent feeding site is established. By contrast, *Pratylenchus* species tend to migrate through the cortical cells, feeding frequently and causing extensive necrosis and visible lesions. Cells adjacent to those fed upon for long periods may also die. The propensity of migratory

endoparasites to move continuously in and out of roots also increases entry points for secondary invaders. Heavily infected root systems are reduced in size and may exhibit additional symptoms such as ‘witch’s broom’, irregular root swelling and stunted rootlets. Symptoms in tubers, such as potato, range from scabby or sunken lesions (caused by *P. scribneri*) to warty protuberances (*P. penetrans*). In stored yam tubers, *P. coffeae* causes dry rot in the surface tissues.

Above-ground symptoms result from root dysfunction – stunted, chlorotic plants appear in patches in the field. In woody perennials, these symptoms are associated with young trees when orchards are replanted on sites where the nematodes, along with other pathogens, have increased over many years. Symptoms that develop on older trees include canopy thinning, stem dieback and reduced fruit yield.

Despite the wide host range of *Pratylenchus*, host status varies greatly, often in proportion to nematode virulence. For virulent host–nematode combinations, such as *P. coffeae* on citrus, population densities can exceed 10,000 nematodes g⁻¹ of roots. By contrast, *P. brachyurus* is widely associated with citrus, but population densities are less than 10% those of *P. coffeae* and the nematode causes little if any economic loss.

5.2.1.4. Dispersal

Endoparasitic nematodes are readily dispersed by movement of infected propagative plant material. Within fields, the nematodes move actively through soil when host roots are available and are passively moved with irrigation or precipitation run-off. Anhydrobiotic survival during dry periods permits the airborne dispersal of some *Pratylenchus* species during windstorms.

5.2.1.5. Ecology, host range and distribution

The geographic distribution of *Pratylenchus* species has been described as zonal (De Waele and Elsen, 2002). Species tend to occur worldwide in climates with suitable temperature ranges. Thus, *P. coffeae*, *P. loosi*, *P. goodeyi*, *P. brachyurus* and *P. zeae* are found throughout the tropics (Table 5.1). Most other economically important species occur in zones encompassed by temperate, subtropical or cooler (elevated) tropical regions. A few, such as *P. fallax* and *P. crenatus*, are prevalent only in temperate zones. The distribution and population development within a zone is affected by plant host suitability and physical factors such as soil texture and rainfall patterns.

Lesion nematode species characteristically reproduce on a wide range of hosts. *Pratylenchus penetrans* and *P. coffeae* have more than 350 and 130 known hosts, respectively. Most plants serve as hosts to some species in the genus, but the economic importance of lesion nematodes has been characterized for relatively few crops and regions.

Multiple species of lesion nematode pathogenic to cereals are often present in fields and can reach damaging levels when cereals are grown intensively. In northern Europe, intensive maize cropping increases the likelihood of damage to winter wheat by *P. penetrans*, *P. crenatus* and *P. fallax*. Yield of winter wheat in the north-west USA is inversely related to cropping intensity and to numbers of *P. neglectus*, which may

have a greater impact on yield than even soil moisture. Widespread damage to cereals by lesion nematodes in Australia has fostered variety screening and the discovery of wheat cultivars tolerant to *P. neglectus* and *P. thornei* that can increase grain yield by up to a third. Lesion nematodes are also the most important nematode pathogens of maize in the USA maize belt, where grain losses of more than 1 tonne ha⁻¹ occur in heavily infested fields. Among many species that damage maize, *P. scribneri*, *P. penetrans* and *P. hexincisus* are important in temperate zones and *P. zae* and *P. brachyurus* are most frequently encountered in warmer regions.

Pratylenchus affects harvestable below-ground plant parts directly and also by compromising the feeder root system. High numbers of *P. penetrans* can severely reduce potato yield; in addition, the marketable tubers are blemished by scabby or shallow sunken lesions. *Pratylenchus coffeae* is a pest of yam in the Americas and the Pacific Islands but, interestingly, not in Africa. Reduced vine growth and yield reduction are usually the result of planting infested seed tubers. However, soil populations are sufficient to reduce the quality of tubers due to dry rot that continues to develop during storage. Peanut in Australia, India and the southern regions of the USA and Africa are widely infected by *P. brachyurus*, which infects root, pegs and pods. In addition to reducing plant size, nematode infection of pegs reduces pod and seed development and pod infection results in necrotic lesions that can serve as entry points for secondary invaders that further reduce seed yield or quality.

Pratylenchus coffeae, *P. goodeyi* and *P. speijeri* are the primary lesion nematode pests of banana and plantain. The nematodes cause plant stunting and delayed maturity, but their major economic impact is a reduction of the plantation life and the ability to cause extensive toppling of the mature, fruit-bearing plants during storms. In eastern and central Africa, *P. goodeyi* is often absent at lower altitudes where *Radopholus similis* is the dominant nematode parasite of *Musa* spp. However, *P. goodeyi* predominates in the cooler, higher altitudes of Africa and the Canary Islands. *Pratylenchus coffeae* attacks *Musa* spp. throughout the tropics, causing greater losses in some regions than others. *Pratylenchus speijeri*, a species especially virulent to the staple food crop plantain in West Africa, was distinguished from *P. coffeae* based on molecular phylogenetic relationships.

Damage to roots of woody perennials by *Pratylenchus* spp. can reduce tree vigour and yield, but more importantly the nematodes contribute with other soil-borne pathogens to orchard replant problems. *Pratylenchus penetrans* is a worldwide pathogen of pome and stone fruit and nut trees. The geographical range of *P. vulnus* is very similar but damage is restricted to warmer climates. *Pratylenchus coffeae* is the most common lesion nematode associated with coffee decline worldwide, but in West Africa, Brazil and Peru, *P. brachyurus* is widespread on coffee and in Central America coffee is attacked by a complex of described and undescribed species, all formerly considered to be *P. coffeae*. Similarly, citrus is a host and is severely damaged by *P. coffeae* in some, but not all, regions where both occur (Fig. 5.3).

5.2.1.6. Molecular diagnosis

DNA sequences are often critical to species determination and identification (Fig. 5.4). Restriction fragment length polymorphisms (RFLPs) of portions of the rDNA internal transcribed spacers (ITS) discriminated multiple populations of 18 *Pratylenchus*



Fig. 5.3. Citrus tree condition in a Florida orchard infested by *Pratylenchus coffeae* showing typical early decline symptoms of mature trees and the need for extensive replacement of non-profitable trees. Similar symptoms are caused by *Radopholus similis* in Florida citrus groves.

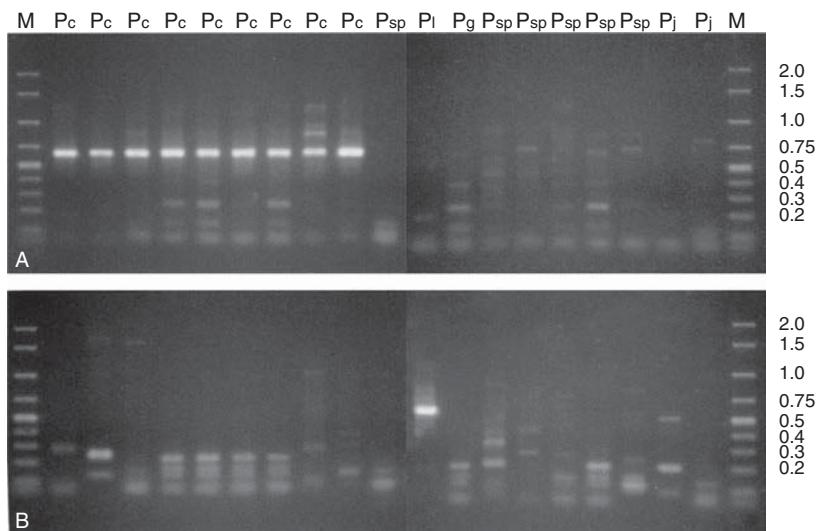


Fig. 5.4. Amplification of rDNA spacer regions of closely related *Pratylenchus* species from around the world. Populations of *P. coffeae* (Pc), *P. loosi* (Pl), *P. gutierrezi* (Pg), *P. jaehni* (Pj) and undescribed *Pratylenchus* species (Psp) were identified based on morphology and genetic sequences. DNA from these populations was then amplified using primers developed for populations of *P. coffeae* (panel A) and *P. loosi* (panel B) in Japan. Molecular weights (number of base pairs) are also indicated (M). (Adapted from Duncan *et al.*, 1999.)

species. Whereas populations of *P. goodeyi* and *P. vulnus* showed no intraspecific variation by this method, several putative *P. coffeae* populations were readily differentiated by combinations of restriction enzymes. Sequence analysis of a portion of the 28S rDNA gene revealed that half of 14 populations of *P. coffeae* from five continents are phylogenetic species, each possessing characteristics that are unique among the populations. Consequently, newly described species that differ from *P. coffeae* in Java (the species type locality) based variously on reproductive incompatibility, minor morphological differences and molecular phylogeny include *P. jaehni* from citrus in Brazil, *P. speijeri* from plantain in Ghana, and two species from native plants in Florida, USA. Presumably, additional species await description among the *P. coffeae* complex. Primers that selectively amplify rDNA of either *P. coffeae* or *P. loosi* were developed (Uehara *et al.*, 1998) and demonstrated the usefulness of molecular probes to discriminate between morphologically cryptic species in this genus. Species-specific, molecular probes are used in real-time PCR to quantify *P. neglectus* and *P. thornei* in soil samples, allowing grain growers in Australia to make management decisions before planting.

5.2.1.7. Interaction with other pathogens

Infection by lesion nematodes predisposes roots to numerous primary and secondary pathogens. Some *Pratylenchus* species appear to damage a host only in the presence of disease organisms, e.g. *P. brachyurus* and *Fusarium oxysporum* on cotton. Others, such as *P. penetrans* and *Rhizoctonia fragariae* on strawberry, do not interact but rather cause additive crop damage. The best-known interaction involves *Verticillium dahliae* in the disease syndrome called ‘potato early dying’. Although the mechanism is unknown, the disease will occur at population densities below damaging levels for either pathogen individually (synergism). *Pratylenchus penetrans* and *P. thornei* can induce potato early dying, whereas *P. crenatus* and *P. neglectus* cannot. *Pratylenchus scribneri*, a species widely distributed throughout tropical and temperate regions, interacts with *V. dahliae* only at elevated temperatures.

5.2.1.8. Management and control

Sanitation is important to reduce damage by species of *Pratylenchus*. Citrus nurseries in Florida, South Africa, Brazil and elsewhere have either mandatory or voluntary programmes to certify that young trees are free of nematode pathogens such as *P. coffeae* and *P. jaehni*. Pruning tissue with *Pratylenchus* lesions from banana and plantain corms followed by hot water treatment before planting greatly increases productivity, even in infested fields (Fig. 5.5).

Crop rotation is sometimes used to manage damage by lesion nematodes. In Australia, wheat that is susceptible to *P. thornei* can be rotated with several non-susceptible crops. However, the wide host ranges of most lesion nematodes can make successful crop rotation difficult. Numbers of *P. thornei* and *P. neglectus* were little affected by rotations of grains and commonly grown broadleaf crops in the north-western USA, whereas use of a summer fallow in the rotation decreased nematode numbers and increased grain yields. The use of sunn hemp (*Crotalaria* spp., notably



Fig. 5.5. Uprooted banana plants with reduced root systems caused by *Radopholus similis* (photograph courtesy of P. Quénéhervé) and (inset) banana corms before and after paring of the nematode-infected tissue (photograph courtesy of D. De Waele).

Crotalaria spectabilis) as a leguminous rotation crop to increase soil fertility in sub-Saharan Africa is compromised by reports that it increases numbers of *P. zeae* to levels that can damage subsequent maize crops. Marigolds (*Tagetes patula*) produce a nematicidal chemical, thiophene α-terthienyl, and have been widely reported to provide effective control of *Pratylenchus* spp. when used as a cover crop (Pudasaini *et al.*, 2006a).

Germplasm resistant to lesion nematodes is less available than for sedentary endoparasites whose complex host–parasite relationships are more readily disrupted by simple changes in the plant genome. Nevertheless, resistant germplasm has been identified in a number of important crops such as banana, potato and sweet potato, various cereals and forage crops, strawberry and some rootstocks of woody plants. Occasionally, varieties exhibit resistance to more than one nematode group. Several sweet potato cultivars resistant to *P. coffeae* are also resistant to the root-knot nematode *Meloidogyne incognita* and potato clones that are less susceptible to *P. penetrans* (quantitative resistance) are also resistant to some species and races of potato cyst nematode. Conversely, resistance in a cultivar can vary among *Pratylenchus* species. Some wheat cultivars resistant to *P. thornei* in Australia are damaged by *P. neglectus*. Variation of cultivar resistance to different populations of the same species of *Pratylenchus* is also fairly common and further complicates the use of this tactic. Functional genomic approaches to developing lesion nematode resistant crops are promising. Analyses of the *P. coffeae* and *P. thornei* transcriptomes have revealed potential parasitism genes that can be targeted, and reduced reproduction in *P. thornei* and *P. neglectus* was achieved using double-stranded RNA to silence genes (RNAi) in these nematodes.

Soil fumigation and use of nematicides can increase yield in many crops attacked by lesion nematodes but due to the high cost and environmental concerns, chemical control is generally restricted to high-value crops such as coffee and banana. Where available, use of resistance is often as profitable as and more durable than chemical control.

5.2.2. Burrowing nematodes, *Radopholus* spp.

Radopholus similis is the only one of more than 30 species in the genus recognized as a pathogen of widespread economic importance. *Radopholus* is probably indigenous to either the Indo-Malayan or Australasia regions, which are rich in burrowing nematode species and include the centres of origin of many of the important hosts of burrowing nematodes. Several new species of local importance have been described in the past two decades. *Radopholus citri* and *R. bridgei* were found to be pathogens of citrus and turmeric, respectively, in Java. *Radopholus musicola* was described from northern Australia where it damages banana. Although *R. similis* has not been detected in Vietnam, *R. duriophilus*, *R. arabocoffeae* and *R. daklakensis* were all discovered there in association with declining durian (*Durio zibethinus*), Arabica coffee and Robusta coffee, respectively. By contrast, *R. similis* is widespread throughout the tropics where it is a serious limiting factor in the production of many crops. The pantropic distribution of the species probably resulted from the widespread movement of infected banana corms from Southeast Asia (Marin *et al.*, 1998).

5.2.2.1. Morphology and identification

As described by Loof (1991) and Luc (1987), the genus is characterized by strong sexual dimorphism. For the female *R. similis*, the body is slender and ranges in length from 0.53 to 0.88 mm. The head is strongly sclerotized internally, composed of three to four annules and not offset. The short, stout stylet has well-developed knobs. The pharyngeal glands overlap the intestine dorsally. The vulva is post-equatorial, both branches of the ovaries are fully developed (some species in the genus have a post-uterine sac), and the round spermatheca contains rod-shaped sperm (identified as spermatids). The tail terminus is almost pointed and striated. The male head is strongly offset, knob-shaped, unsclerotized, with four lobes and reduced lateral sectors. Male characters are useful to differentiate several *Radopholus* species from *R. similis*. For example, the main feature that distinguishes *R. similis* from *R. citri* is a thin stylet with reduced knobs in males of the former and a strongly developed stylet conus in males of the latter, while *R. musicola* differs from *R. similis* and *R. bridgei* for having a small stylet, but with well-developed knobs.

5.2.2.2. Life cycle and behaviour

The behaviour and life cycle of burrowing nematodes are, in the main, very like that of lesion nematodes. An interesting difference is that whilst burrowing nematodes

normally reproduce sexually, *R. similis* females that do not mate after a period of time can reproduce as hermaphrodites. A further difference is that mature male burrowing nematodes do not feed. Males may comprise 0–50% of the population and differences in reproductive capacity between some populations is inversely related to the male:female sex ratio.

Burrowing nematodes infect at or near the root tip and reside almost exclusively in the root cortex, although they also damage the stele in banana. Migration and feeding behaviour is like that of *Pratylenchus*. As with most migratory endoparasites, the nematode remains within the root until forced by overcrowding and decay to migrate. Population development is host-dependent. Within roots, mature females begin to lay eggs at an average rate of nearly 2 day⁻¹ on citrus and 5 day⁻¹ on banana. At optimum temperature, juveniles may hatch in 2–3 days on some hosts or up to three times longer in others. The life cycle on citrus can be completed in 18–20 days under optimum conditions. Most populations of *R. similis* reproduce best at intermediate (25°C) or high (30°C), rather than lower (15–20°C) temperatures. Populations introduced into European parks and nurseries appear to have adapted to temperate conditions, reproducing at temperatures too low for most tropical populations. In Florida citrus groves, the optimum temperatures occur longest each year in the deeper soil horizons where root infection is greatest. Temperature extremes in the surface soils are nearer the limits for development of *R. similis*, which may explain low population development in surface roots. The nematode does not have a known resting stage, so recurring moisture deficits typical of surface soils may also inhibit development near the soil surface. The tendency of *R. similis* to inhabit deeper soil horizons in Florida affects its pest status and its management options (see Section 5.2.2.8).

Soil texture affects *R. similis* population growth and virulence. Population growth on banana is greater in coarse sand than in finer soils. The nematode is more pathogenic to citrus in sandy than loamy soils in controlled pot studies and in the field. Movement from tree to tree is also greatest in coarse textured soil.

Radopholus similis is the only plant-parasitic nematode in which a symbiosis has been detected with a *Wolbachia*-like bacterium. *Wolbachia* are widely associated with insects and filarial nematodes. The bacterium appears to supply essential nutrients to filarial nematodes, but the nature of the symbiosis with *R. similis* is unknown.

5.2.2.3. Host reaction

Root damage by *Radopholus* is typical of that caused by *Pratylenchus* – reddish, brownish to black lesions caused by cell wall collapse as nematodes move inter- and intracellularly. The nematode usually penetrates the region of elongation but if terminals are penetrated, root tips can become swollen and stubby. Tissue rot occurs following secondary infections. Primary and secondary roots as well as corms can suffer severe damage in crops such as banana, whereas only non-lignified fibrous roots of woody plants are damaged. The quality of root crops such as turmeric is affected because rhizomes lose their bright yellow colour. Stunting, wilting and chlorosis characterize heavily infected plants. Tree damage includes smaller and sparser foliage and fruit. Branch ends become bare and eventually entire branches die. Disease progression is greatest during the dry season in affected tree crops.

5.2.2.4. Dispersal

The worldwide occurrence of burrowing nematodes is due to the spread of contaminated plant material over many centuries. Use of infected planting material and movement of soil for construction purposes or on agricultural machinery routinely spread the nematode within regions. The wide host range and virulent behaviour of the nematode results in efficient local dispersion at rates as high as 15 m year^{-1} . Reinfestation of disinfested banana fields in the French West Indies appears to be primarily the result of surface water run-off during heavy rainfall.

5.2.2.5. Ecology, host range and distribution

Radopholus similis is highly polyphagous, attacking more than 250 plants in 16 families. There are many reports of differential host preference among populations but two races of the nematode are commonly recognized. A citrus race exists in Florida that reproduces on most citrus species and varieties in addition to banana and the other known hosts of the genus. Populations that do not reproduce on citrus are collectively known as the banana race. The citrus race was given species status (*R. citrophilus*) in 1984, but subsequent research showed that *R. citrophilus* is a junior synonym of *R. similis*, based on karyotype identity, morphological and genetic identity and reproductive compatibility (see Chapter 12).

The pantropic distribution of *R. similis* makes it one of the major species of economic importance. Following black Sigatoka and perhaps the banana weevil, burrowing nematodes are the most serious banana malady in many countries. The nematodes cause ‘toppling disease’, so named because fruit-laden, mature plants become top heavy when the root system is damaged beyond its ability to anchor the plant (Fig. 5.5). During the stormy rainy season losses can be very high. In addition, *R. similis* damage stunts plant growth, delays maturity, reduces bunch weight and shortens the productive life of the mother and daughter plants. Burrowing nematode populations vary in their capacity to damage banana. An inverse relationship between nematode population development and banana plant growth suggests that the degree of damage results from differences in nematode numbers rather than differing virulence (Fig. 5.6). Populations with exceptionally high reproductive capacity may even reproduce well on some normally resistant cultivars.

During 20 years following the Second World War, 22 million black pepper vines in Indonesia were lost to ‘yellows disease’, eventually found to be the result of *R. similis*. The disease occurs throughout South-east Asia and India. Leaf yellowing is followed by drop and then branch dieback. Progression of decline during the dry season followed by recovery during the monsoons results in a gradual decline of the plants, called ‘slow wilt’ in India.

For several decades, ‘spreading decline’ was the most important disease of citrus growing on the deep sandy soil of central Florida. Eventually, the citrus race of *R. similis* was identified as the causal agent and aggressive management programmes were initiated. The race is known only in Florida and in addition to *P. coffeae* is the most virulent nematode pathogen of citrus. The name of the disease is descriptive of the very rapid spread of decline incidence from the location of the initial infestation. A prominent feature of the disease is that fibrous root density is relatively

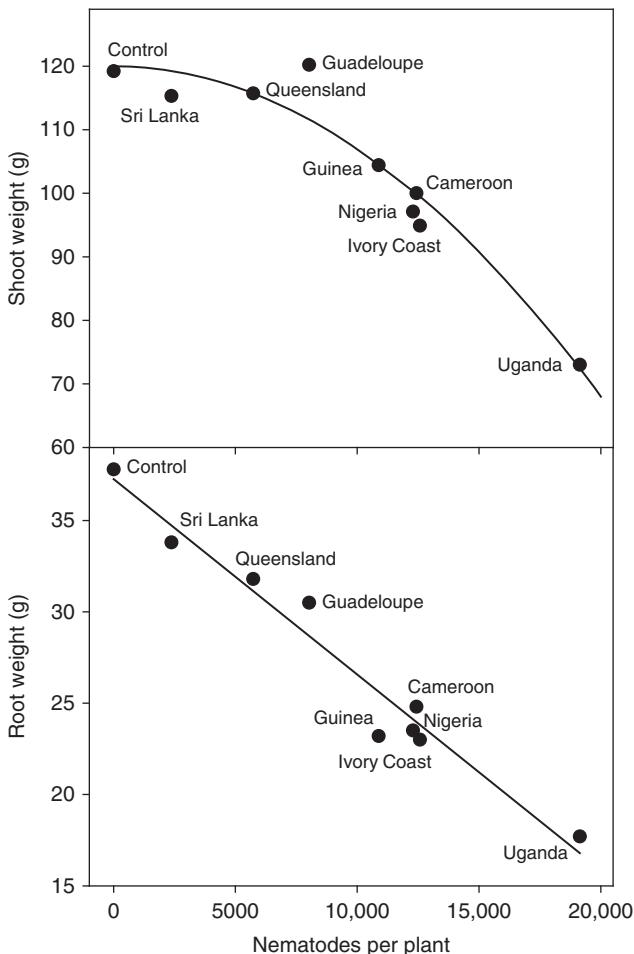


Fig. 5.6. Relationships between population growth of different populations of *Radopholus similis* and the growth of banana seedlings (*Musa AAA* cv. Poyo). These data support the idea that differences in virulence of burrowing nematode populations is caused by differences in the numbers of nematodes parasitizing the host. (Adapted from Fallas *et al.*, 1995.)

normal near the soil surface, but is severely reduced below depths of 25–50 cm. During the dry season, the trees lack the normally deep root system needed to avoid moisture stress. As with slow wilt of pepper, the tree condition alternates with the rainy and dry seasons, becoming progressively weaker over time. Above-ground symptoms are as those for yellows disease of black pepper. Without proper management, fruit yield can decline by more than half and orchards become economically non-viable.

Radopholus similis is increasingly a problem on tea in Sri Lanka where it tends to occur at lower elevations, whilst *P. loosi* is a problem at higher altitude. Other crops attacked by burrowing nematodes in their Indo-Malayan centre of origin and beyond include coconut palm, betel vine, ginger, turmeric, cardamom and nutmeg.

5.2.2.6. Molecular diagnosis

Populations of *R. similis* from many countries and host plants, as well as several of the recently described species in the genus, have been characterized by rDNA sequences (ITS and D2/D3 regions). PCR primers (ITS) specific to the genus and to just *R. similis* have been optimized for use in several assays to detect the nematode, and in some cases other pathogens, in soil and plant samples. Populations of *R. similis* appear to be remarkably homogeneous at these phylogenetically important gene regions, perhaps due to their relatively recent dissemination worldwide via major crops such as banana (Marin *et al.*, 1998). A 2.4 kb sequence tag site was used as a marker to demonstrate that hybrid progeny are produced when citrus and banana races of *R. similis* are mated. Significant variability of total DNA among *R. similis* populations (detected by RAPD analysis) has thus far failed to discriminate populations based on host range, pathogenicity or geographic origin.

5.2.2.7. Interaction with other pathogens

Burrowing nematodes can predispose plants to attack by other pathogens (e.g. black pepper to infection by *Fusarium solani*); however, unlike some lesion nematodes, *R. similis* is not known for its involvement in synergistic disease complexes. Nevertheless, the incitement of secondary infections by burrowing nematodes greatly exacerbates the root system damage. In addition to cortical rot, fungi may colonize the stele of plants such as banana, which is not affected directly by the nematode. Thus, the presence of the nematode exacerbates Panama wilt on banana caused by *F. oxysporum* f. sp. *cubense*.

5.2.2.8. Management and control

As with *Pratylenchus* spp., sanitation is one of the most important ways to manage burrowing nematode. Citrus nursery certification in Florida results in added crop value of more than US\$17 million year per year by slowing the spread of *R. similis*. The use of tissue culture-derived banana plants ensures clean planting material and infected planting material such as banana corms can be treated profitably with hot water or by paring the lesions (Fig. 5.5). Ditching between banana fields is employed to prevent the dissemination of *R. similis* from infested to disinfested fields in surface run-off of rainwater.

Some cultural practices have proved useful in helping plants tolerate the nematode. *Radopholus similis* damage is restricted to the deeper citrus root system, so growers protect the shallow roots by mowing or using herbicides rather than cultivation for weed control. Use of frequent irrigation and fertilization maintains nutrients in the shallow horizon, allowing trees to grow reasonably well even during the dry season. Mulching of infested black pepper vines provides similar benefits during the dry season to help the vines tolerate nematode damage to the root system. Mulching also reduced soil temperatures, which was associated with lower population growth and damage to cooking bananas by *R. similis*. *Radopholus similis* does not survive for long periods in the absence of host roots, so crop rotation is an option.

In Martinique, treatment of banana plants with herbicide to kill roots in soil before uprooting, in combination with non-host rotations and use of clean planting stock, allows banana to be grown without nematicides for several years and the programme has reduced the use of chemical nematicides by 60%. However, many of the crops commonly grown in heavily infested areas of South-east Asia are perennial (less amenable to rotation) and are hosts of the nematode.

Resistant germplasm is known in banana, citrus, coconut and betel nut, among other crops. Resistance-breaking biotypes of the citrus race of *R. similis* can develop on the commercially available resistant rootstocks. Two confirmed sources of resistance in banana are 'Pisang Jari Buaya' and 'Yangambi km5' and several potential sources have been reported, including lines resistant to nematodes in addition to *R. similis*. Breeding programmes to develop acceptable cultivars are ongoing. They are complicated by the variable reactions among nematode populations. Recent high-throughput molecular characterization of *R. similis* has identified several putative parasitism genes (primarily cellulases) that may be good targets for developing transgenic sources of resistant germplasm. Banana and plantain have been transformed to express both a cystatin and a peptide that can provide a dual mode of action against *R. similis* by inhibiting digestion and chemoreception, respectively (see Chapter 15).

Soil fumigants were formerly used heavily in citrus and banana orchards and resulted in serious environmental and health problems. Systemic nematicides have the potential to control profitably migratory endoparasitic nematodes, but their usage is becoming more restricted because of safety concerns and awareness of problems such as accelerated microbial degradation.

5.2.3. Rice root nematodes, *Hirschmanniella* spp.

This genus is aptly described by its common name because more than half of the 24 recognized species are parasites of rice and nearly 60% of the world's rice fields are infested by species of *Hirschmanniella* (Bridge *et al.*, 2005). These nematodes predominate in moist and even aquatic habitats on hosts such as blackberry, cabbage palm (*Sabal palmetto*), bulrush (*Scirpus robustus*), sagittaria (*Sagittaria subulata*) and hydrilla (*Hydrilla verticillata*). A half dozen species occupy temperate zones but the majority occur in the tropics and subtropics. The female is long (1–4 mm) and slender. The head region is not offset and may be hemispherical (e.g. *H. spinicaudata*) or low and flattened (e.g. *H. oryzae*). Other major diagnostic characters include stylet length, presence or absence of sperm in the spermatheca (and males in the population), and presence or absence of a mucro or a ventral, subterminal notch on the long pointed tails.

In rice paddies, *Hirschmanniella* spp. are efficiently dispersed in water, so that almost all of the plants may be infected. Symptoms are non-specific and include reduced early growth rate, occasional chlorosis and decreased tillering. Infected roots are yellow initially, then they rot. The rot is caused in part by transcriptional reprogramming by the nematode that induces programmed cell death, oxidative stress and obstruction of normal root metabolic activity. By comparison, root-knot nematode infection of rice roots affects transcription in ways that suppress the local defence pathways while stimulating metabolic pathways and nutrient transport towards the induced root gall. Rice root nematode eggs are deposited within the

roots and the life history is like that of other pratylenchids. Depending on the region and nematode species, from one to three generations per year have been reported. It is common to recover both *H. oryzae* and *H. spinicaudata* from a sample. These two species infect predominantly monocotyledons, but *H. oryzae* also infects some broadleaf plants such as cotton. The nematodes overwinter in roots of rice stubble or many species of native plants. In the absence of hosts they persist in soil, and low soil moisture induces a quiescence that extends longevity for up to a year. Crop rotation is effective in reducing populations of rice root nematodes. The use of trap crops such as *Sesbania rostrata* and *Aeschynomene afraspera*, that permit infection but not egression from the roots, are very effective and are good sources of nitrogen for the following crop, but may be uneconomical if used in place of a cash crop in the rotation.

5.3. Anguinids and the Stem and Bulb Nematode, *Ditylenchus dipsaci*

The family Anguinidae contains mycophagous nematodes and nematodes that attack plant aerial parts, bulbs and tubers (Boxes 5.2 and 5.3). Of several economically important genera, *Ditylenchus* spp. have the widest impact on agriculture. The genus has over 60 described species but only a few parasites of higher plants. The numerous races of *D. dipsaci* probably represent a species complex. From this complex the ‘giant race’ multiplying on Fabaceae was singled out and described as *D. gigas* (Vovlas *et al.*, 2011). *Ditylenchus destructor* and *D. africanus* are important pests of potato tubers in Europe and North America and groundnuts in South Africa, respectively. These two species are also mycophagous, as is *D. myceliophagus*, which is a pest of the cultivated mushroom, *Agaricus bisporus*. ‘Ufra’ is a serious disease of deep-water and lowland rice in India and South-east Asia, caused by *D. angustus*, which reside in water films, feeding ectoparasitically on the growing rice stems and leaves, or accumulate in the leaf sheaths and developing inflorescences. *Ditylenchus dipsaci*, known commonly as the ‘stem’ or ‘stem and bulb’ nematode, is equally as virulent as the former species, but is economically more important by virtue of its wide host range.

Seed gall nematodes, *Anguina* spp., inhabit the aerial parts of cereals and forage grasses. Eleven species are recognized and it is thought that host specialization has

Box 5.2. First report of a plant-parasitic nematode.

The Reverend John Needham made the first known report of a plant-parasitic nematode in a 1743 communication to The Royal Society of London. When he added a drop of water to improve resolution of the microscopic bundles of dry, white fibres he discovered in ‘small black grains of smutty wheat’, he was greatly surprised to find that the fibres ‘took life, moved irregularly, not with a progressive, but twisting motion; and continued to do so for the space of nine or ten hours, when I threw them away’. At the time, Needham’s observation generated interest because of its bearing on the question of spontaneous generation. The nematode he observed, *Anguina tritici*, was described by Steinbuch in 1799.

Box 5.3. Classification of the subfamily Anguininae.

Phylum Nematoda Potts, 1932
Class Chromadorea Inglis, 1983
Subclass Chromadoria Pearse, 1942
Order Rhabditida Chitwood, 1933
Suborder Tylenchina Thorne, 1949
Infraorder Tylenchomorpha De Ley & Blaxter, 2002
Superfamily Sphaerularioidea Lubbock, 1861
Family Anguinidae Nicoll, 1935
Subfamily Anguininae Nicoll, 1935 (1926)
Genus *Anguina* Scopoli, 1777
Ditylenchus Filipjev, 1936

acted as an isolating mechanism in the evolution of this genus. Restriction sites in the internal transcribed spacer 1 (ITS1) region of rDNA have been used to discriminate eight anguinid species of regulatory importance. *Anguina tritici* invade ovules where they induce galls, mate, lay eggs and reside as second-stage juveniles (J2). The J2 can remain as anhydrobiotes within dried seed galls for many years (see Chapter 7). When the infective J2 exit the moistened galls they infect the stem growing point, which elongates carrying the nematodes upward with the developing ear. Up to 60% seed reduction can result from sowing infected seed, but modern seed cleaning methods have largely eliminated the problem, except in regions where farmers save the seeds they sow. *Anguina funesta* and *A. agrostis* infect seeds of different forage grasses. Both species can introduce the bacterium *Rathayibacter toxicus*, which can be fatal to grazing livestock. As with *A. tritici*, rotation and use of clean seed effectively control these pests.

5.3.1. Morphology and identification

The species of *Ditylenchus* can be categorized as members of the ‘*D. dipsaci* group’ or the ‘*D. triformis* group’. Members of the former (including *D. angustus*) have sharply pointed tails and four incisures in the lateral field. They feed on plants almost to the exclusion of the more primitive trait of mycophagy (*D. dipsaci* is an obligate phytoparasite). The *D. triformis* group (including *D. destructor*, *D. myceliophagous* and *D. africanus*) have rounded tail tips, six lateral incisures and are mainly fungal feeders.

Female *D. dipsaci* are slender, less than 1.5 mm long and not curved when relaxed. Stylets are short and delicate, less than 15 µm long. The median bulb is muscular with distinct valve plates. A basal bulb may extend over the intestine. The ovary is outstretched with one or two rows of oocytes and post-uterine sac. The tail is elongate, conoid and acute. The male testes are outstretched and the bursa is adanal to subterminal, enveloping one-quarter to three-quarters of the tail. *Ditylenchus gigas* can be distinguished from all other *Ditylenchus* spp. by several morphological characteristics (body size, lateral field, stylet and post-vulval uterine sac). *Ditylenchus gigas* can be separated from *D. dipsaci* by its longer body length and longer vulva–anus distance.

5.3.2. Life cycle and behaviour

As a parasite of above-ground parts of plants, *D. dipsaci* is not buffered from changes in ambient weather conditions as are root parasites. Consequently, the nematode is highly resistant to desiccation and can survive in a state of anhydrobiosis for many years (see Chapter 7). Species adapted to more humid habitats such as *D. angustus* do not have this ability. Masses of anhydrobiotic *D. dipsaci* ('eelworm wool') frequently overwinter in dried plant debris in the field. The fourth-stage juvenile (J4) is the primary survival and infective stage. When conditions permit, the nematodes migrate to germinating host plants and invade hypocotyls or petioles, entering through stomata or penetrating the epidermis, where they moult to the adult stage. The nematode feeds on parenchymatous tissue where all life stages occur. Reproduction is by amphimixis and population growth can be very rapid; females lay 500 eggs from which the J2 hatch within 2 days and develop into females within 4–5 days. Females live more than 10 weeks and the life cycle requires 19–23 days at 15°C. The rapid population growth of this nematode can result in severe crop damage even when the initial population density is low.

5.3.3. Host reaction

Early infection of plants in the field causes high rates of seedling mortality. Surviving plants are stunted and deformed. The host response to *D. dipsaci* is due partly to enzymatic action that dissolves the middle lamellae and separates host cells. Plant hormone imbalance also occurs. Cell hypertrophy and formation of intercellular cavities causes local swelling. Affected leaves and petioles are often stunted and distorted and internodes are shortened. Stems of onion or garlic also become distorted with pimple-like spots called spikkles. Eventually stems become soft and puffy and often collapse. In plants grown from bulbs the nematodes migrate downward to infect the outer scales, eventually migrating throughout the bulb. Symptoms in infected bulbs progress downwards over time to reveal discoloured rings internally (Fig. 11.1B). Onion bulbs rot readily in storage whereas garlic bulbs tend to desiccate. Damage caused by *D. gigas* differs from that caused by *D. dipsaci* in a heavier distribution of symptoms through the main stem, leaves and pods, and in the greater percentage of infected seeds.

5.3.4. Dispersal

Active dispersal of *D. dipsaci* is limited; however, its capacity for desiccation survival makes it readily disseminated as a contaminant of planting material (seeds and bulbs), plant debris or contaminated equipment. The nematode is also frequently transmitted in irrigation water, which can result in greater crop loss than when infestation is from a point source.

5.3.5. Ecology, host range and distribution

The host range of *D. dipsaci* includes about 500 plant species. More than 30 physiological races of the nematode are known, some being host-specific and others widely polyphagous. Races of the nematode are named after the crop from which they were

identified or after a major host. *Ditylenchus dipsaci* is a serious pest of clover, pea, broad bean, celery, garlic, onion, potato, strawberry, oats and rye, and is the most important nematode pest of several crops, such as lucerne and the bulb ornamentals narcissus and tulip. In addition to broad bean, *D. gigas* parasitizes a number of *Lamium* spp., other dicotyledonous weeds and *Avena sterilis*.

Ditylenchus dipsaci is adapted to temperate conditions and occurs at higher elevation in some areas of the subtropics and tropics, providing humidity is adequate. The use of irrigation facilitates the nematode's survival in agriculture in hot, arid climates. The nematode's requirement for free moisture on plant surfaces makes it a seasonal pest in some regions. Winter crops in Italy are more susceptible to *D. dipsaci* damage due to the greater incidence of dew, fog and precipitation. Dense canopy formation in crops such as bean create humid conditions that permit heavy population development even during drier parts of the year. Fine-textured soils are most favourable for population growth and damage by *D. dipsaci*, perhaps due to their greater water-holding capacity.

Survival of *D. dipsaci* in soil is enhanced by low temperature and moisture deficit. The nematode is freeze-tolerant and will survive for many years in a frozen or desiccated state. In the absence of a host, the nematode survives for a relatively short time in warm moist soil.

5.3.6. Molecular diagnosis

The mainly morphologically indistinguishable races of *D. dipsaci* greatly complicate management based on crop rotation or use of resistance. Attempts to distinguish races of the nematode with esterase and catalase profiles and polyclonal antibodies were unsuccessful. Use of monoclonal antibodies proved to be specific to only one of several populations of the oat race. *Ditylenchus gigas* differs from related *Ditylenchus* spp. in the ITS1–5.8S–ITS2 region, the D2–D3 fragment of the 28S gene of rDNA, the small subunit 18S rDNA sequence, mtCOI gene and hsp90 gene sequences and chromosome numbers.

5.3.7. Interaction with other pathogens

Although *D. dipsaci* has been reported to interact synergistically with other pathogens, such as *F. oxysporum* on lucerne, true disease interactions do not appear to be common. Nevertheless, secondary infections in many crops are an important cause of increased damage to above- and below-ground organs, particularly with ornamental and food bulb crops.

5.3.8. Management and control

Clean planting material is critical to avoid damage by stem and bulb nematodes. Some garlic and onion producers rely heavily on programmes to produce and distribute certified planting material. Fumigants and nematicides are uneconomical for management of *D. dipsaci* in most crops but can be used in nurseries to reduce the infection rate of planting

material. Seed disinfection by methyl bromide fumigation, hot water or formaldehyde treatment is also practised. Disinfection of equipment used between fields is important.

Short persistence of the nematode in warm moist soil makes it amenable to control by 2–3-year rotations and by soil solarization in suitable climates. The success of rotation depends on the host range of the race in question and availability of suitable non-host crops. Sources of resistance for many of the races are known and commercial cultivars are available in crops such as lucerne, clover, oat, garlic, strawberry and sweet potato. Use of resistance against this nematode can be highly profitable, more than doubling yields in many crops for which cultivars are available.

5.4. Plant-parasitic Aphelenchs

There are four trophic types within the Aphelenchoidea: (i) entomopathogenic; (ii) mycophagous; (iii) plant-parasitic; and (iv) predatory. Aphelenchs are predominantly free-living and mycophagous in habit. Only a small number of more than 400 species are known to damage plants. A few species of *Aphelenchoides* (Box 5.4) are plant parasites of economic importance: *A. fragariae* and *A. ritzemabosi* live in buds and leafs of higher plants, *A. besseyi* is an important parasite of rice worldwide and *A. composticola* can destroy mushroom beds. Among approximately 100 species within the genus *Bursaphelenchus* only two are considered plant parasitic; they are both vectored by insects. *Bursaphelenchus xylophilus*, vectored by *Monochamus* spp., is the cause of the pine wilt disease. *Bursaphelenchus cocophilus* is vectored by *Rhynchophorus palmarum* weevils and causes red ring disease in several palm species in the Caribbean and Latin America.

5.4.1. The bud and leaf nematodes, *Aphelenchoides fragariae* and *A. ritzemabosi*

These two species with similar life cycles attack hundreds of herbaceous and woody plant species. By feeding on leaf mesophyl, they cause typical leaf blotches and when feeding on buds, they may kill the growing point and prevent flowering.

Box 5.4. Classification of the superfamily Aphelenchoidea

Phylum Nematoda Potts, 1932
Class Chromadorea Inglis, 1983
Subclass Chromadoria Pearse, 1942
Order Rhabditida Chitwood, 1933
Suborder Tylenchina Thorne, 1949
Infraorder Tylenchomorpha De Ley & Blaxter, 2002
Superfamily Aphelenchoidea Fuchs, 1937
Family Aphelenchoididae Skarbilovich, 1947
Subfamily Aphelenchoidinae Skarbilovich, 1947
 Genus *Aphelenchoides* Fisher, 1894
Subfamily Parasitaphelenchinae Rühm, 1956
 Genus *Bursaphelenchus* Fuchs, 1973

5.4.1.1. Morphology

Aphelenchoides fragariae and *A. ritzemabosi* show the general characters of the genus *Aphelenchoides* (Hunt, 1993). They are small to long nematodes, usually between 0.4 and 1.2 mm long. Heat-relaxed females die straight to ventrally arcuate, whereas the males assume a ‘walking stick’ shape with the tail region curled ventrally. The stylet is often about 10–12 µm long; the ovoid or spherical median bulb with central valve plates is well developed. The vulva is post-median, usually between 60 and 75% of the body length, and the female genital tract is monoprodelphic. A post-uterine sac is usually present. The tail is conoid with a variable terminus. The male tail is strongly hooked ventrally to form the characteristic ‘walking stick’ form; spicules are thorn-shaped and a bursa is absent.

Aphelenchoides fragariae and *A. ritzemabosi* are separated from each other using morphological characteristics (Fig. 5.7) of both females and males (Table 5.2).

5.4.1.2. Life cycle and behaviour

Bud and leaf nematodes are facultative plant-parasitic nematodes and can readily reproduce on fungi. They are not true endoparasites, being essentially tissue-surface feeders. They are not attracted by host plants and their orientation is not affected by gravity or light, but is positively influenced by CO₂. The nematodes enter leaves through stomata when the surface is covered with a thin film of water or by penetrating the epidermis of the under-surface. Within the leaves the nematode destroys the spongy mesophyll cells and its movement seems to be delimited by leaf veins. On some plants the nematode lives ectoparasitically within the folded crowns and runners (strawberry) or flower buds (violets, azalea).

Aphelenchoides fragariae and *A. ritzemabosi* are bisexual species for which amphimixis ($n = 4$) seems to be obligatory. Fertilized females of *A. ritzemabosi* go on reproducing for 6 months, without refertilization. It is suggested that caryogamy (hybridization) between both species is possible. Their life cycle is simple and very short (10–14 days at 18°C). J2 hatch from eggs in 4 days and about 20–30 eggs are laid per female, the low fecundity being compensated for by the short life cycle. It survives in soil for as little as 4 weeks.

5.4.1.3. Host reaction

By feeding on leaf mesophyll, bud and leaf nematodes cause typical leaf blotches initially limited by the veins (Fig. 5.8). Breakdown products of damaged tissues are nematotoxic and the nematodes move progressively from the invasion site until barrier veins prevent access to fresh tissue. This discontinuous process produces discrete blotches with different stages of discoloration. Eventually, dead, shrivelled and hanging leaves extend from the tops of plants. Ferns infested with *A. fragariae* typically show narrow or linear patches perpendicular to the midrib of the frond. Sometimes blotches have an irregular shape and look water-soaked (*Begonia*). On strawberry, the nematode causes malformations such as twisting and puckering of leaves, discoloured areas with hard and rough

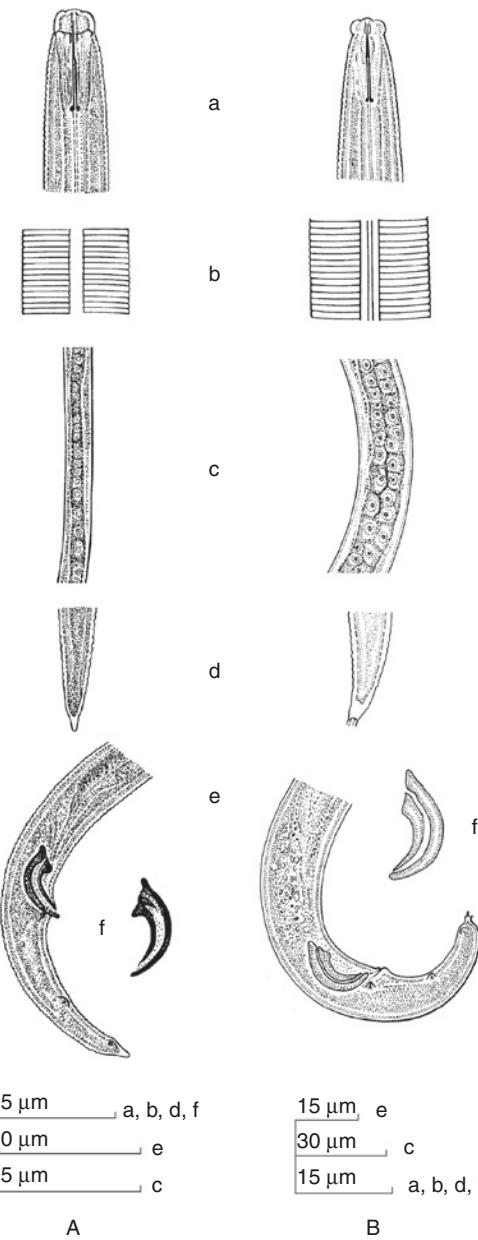


Fig. 5.7. Morphological comparison between male and female *Aphelenchoides fragariae* (A) and *A. ritzemabosi* (B); a: female head end; b: female lateral field; c: oocytes; d: female tail tip; e: male tail end; f: spicule. (From Siddiqi, 1974a, 1975.)

surfaces, undersized leaves with crinkled edges, rending of petioles, short internodes of runners, reduced flower trusses with only one or two flowers and death of the crown bud. Malformations also occur when *A. ritzemabosi* infects lucerne seedlings. Infestations of *Lilium* spp. by *A. fragariae* cause dieback of the leaves

Table 5.2. Differential diagnosis of *Aphelenchoides fragariae* and *A. ritzemabosi* based on female and male morphological characteristics. (From Siddiqi, 1974b, 1975.)

Characteristic	<i>A. fragariae</i>	<i>A. ritzemabosi</i>
Female		
Lateral field	Two incisures	Four incisures
Cephalic region	Smooth, elevated, anteriorly flattened with sides straight to rounded, almost continuous with body contour	Lip region spherical offset by a constriction, slightly wider with adjacent body
Excretory pore	With or close behind nerve ring	0.5–2 body widths posterior to nerve ring
Oocytes	In single file	In multiple rows
Tail end	Simple blunt spike devoid of any processes	Terminal peg with 2–4 minute processes pointing posteriorly, giving paintbrush-like appearance
Male		
Tail end	Arcuate through 45–90° not sharply curved like a hook	Usually curved through about 180° when relaxed
Spicules	Simple blunt terminal spine Moderately developed apex and rostrum; dorsal limb 14–17 µm long	2–4 processes, of variable shape Lacking a dorsal or ventral process at proximal end; dorsal limb 20–22 µm long

from the base of the stem upwards. When young shoots are attacked, stunting and distortion result from the nematodes feeding externally around the growing point.

5.4.1.4. Dispersal

Bud and leaf nematodes can spread from plant to plant when tissues are touching and the humidity is high enough to create a water film needed by nematodes to move on the plant surface. Rain splash also helps local spread of the bud and leaf nematodes. Over longer distances, the nematodes can be spread by infected propagating material.

5.4.1.5. Ecology, host range and distribution

Bud and leaf nematodes are found in temperate and tropical regions of all continents and both species may occur sympatrically. The nematodes overwinter in dormant buds and growing points. In dry leaves, adults of *A. ritzemabosi* coil and can survive in a desiccated state for several months, mostly as late-stage juveniles and adults. They do not survive exposures to -20°C, but most populations survive from -2 to 1°C.

The host range of both species is extremely large and composed of ferns and species of the Compositae, Liliaceae, Primulaceae and Ranunculaceae. *Aphelenchoides fragariae* is a major pest in strawberries and is found on more than 100 fern species. It occurs on common weeds in addition to cultivated plants.

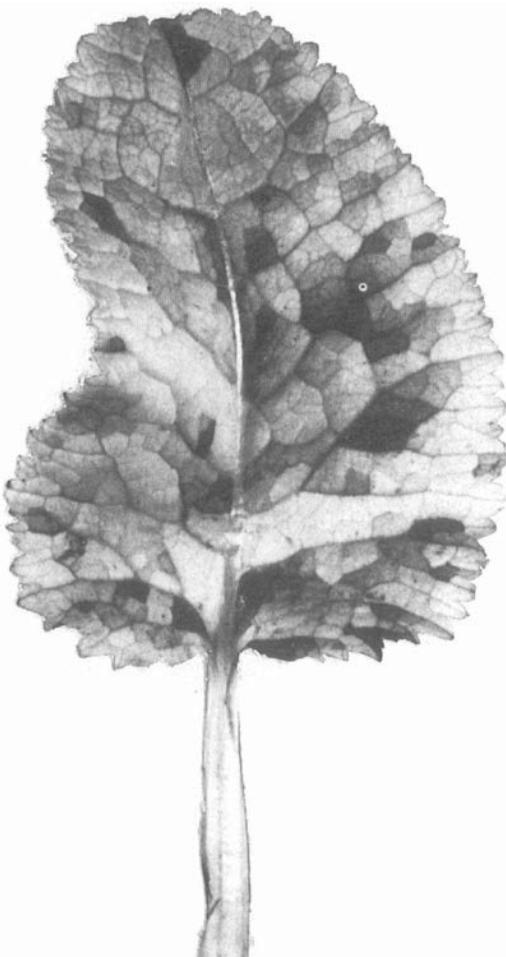


Fig. 5.8. Interveinal necrosis in a leaf of *Primula* sp. infested with *Aphelenchoides ritzemabosi*. (From Southey, 1993.)

5.4.1.6. Interaction with other pathogens

Aphelenchoides ritzemabosi was found in association with *Phytophthora cryptogea* on diseased gloxinia in Florida. In strawberry runners the ‘cauliflower disease’ was experimentally induced through co-inoculation of *A. ritzemabosi* and *Rhodococcus fascians*. In *Barleria cristata*, *A. fragariae* is associated with the bacterium *Pseudomonas choricii*. When associated with *Xanthomonas begoniae*, the nematode causes leaf spot symptoms to appear earlier and to be more severe.

5.4.1.7. Management and control

Selecting nematode-free propagation material is of paramount importance to reduce the impact of bud and leaf nematodes; whenever possible certified stocks should be

used. Regenerating plants from clean, dormant, excised axillary buds is effective for eliminating *A. fragariae* on strawberry. Planting stock can be disinfested by hot water treatment. Sometimes symptom-free stock may be infested and suspect material should be isolated from other plants to observe if symptoms develop.

Chemical control with insecticides (e.g. parathion) or non-fumigant nematicides used as foliar or soil applications is effective. Care should be taken when using these chemicals as phytotoxic thresholds are difficult to predict.

5.4.2. *Aphelenchoides besseyi*

Aphelenchoides besseyi is seed borne and causes the disease ‘white tip’ on rice. The species is widespread in rice-producing areas. The nematode lives mainly ectoparasitically on young tissue; at the end of the growing season it lives in a quiescent state under the hulls of the seed.

5.4.2.1. Morphology

Within its genus, *A. besseyi* is characterized by lateral fields about one-quarter as wide as the body, with four incisures (Franklin and Siddiqi, 1992). The posterior part of the stylet has slight basal swellings 1.75 µm across. The secretory-excretory pore is usually near the anterior edge of the nerve ring. The spermathecae are elongate oval, usually packed with sperm. The ovary is relatively short and not extending to pharyngeal glands, with oocytes in 2–4 rows. The post-uterine sac is narrow, inconspicuous and does not contain sperm; the sac is 2.5–3.5 times the anal body width long but less than one-third distance from vulva to anus. The female tail terminus bears a mucro of diverse shape with 3–4 pointed processes; the male tail has terminal mucro with 2–4 pointed processes; spicules lack an apex at the proximal end.

5.4.2.2. Life cycle and behaviour

When infested grain is sown, the quiescent nematodes become active and move to the growing points of stems and leaves of the seedlings. There they feed ectoparasitically and lay eggs in the leaf axils and flower panicles. A rapid increase in nematode number takes place at late tillering and is associated with the reproductive phase of plant growth. *Aphelenchoides besseyi* is able to enter the spikelets before anthesis and feed ectoparasitically on the ovary, stamens, lodicules and embryo. However, *A. besseyi* is more abundant on the outer surface of the glumes and enter when these separate at anthesis. *Aphelenchoides besseyi* reproduces by amphimixis but can also reproduce parthenogenetically. There are several generations in a season.

As grain filling and ripening proceed, reproduction of the nematodes ceases, and the development of third-stage juveniles (J3) to adults continues until the hard dough stage. At this phase of grain development, the nematodes coil and aggregate in the glume axis. The nematodes (mostly adult females) are found beneath the hulls of rice grains. They may remain viable for 2–3 years on dry grain but die in 4 months on

grain left in the field and do not survive in soil. Survival is enhanced by aggregation and a slow rate of drying (Chapter 7) but the number and infectivity of nematodes is reduced as seed age increases.

5.4.2.3. Host reaction

The typical symptom is the emergence of the chlorotic tips of new leaves. Later, these tips become necrotic, while the rest of the leaf may appear normal. *Aphelenchoides besseyi* also causes crinkling and distortion of the flag leaf enclosing the panicle. In severe infections, the latter may be hindered from emerging. The size of the panicle and the number and size of grains is reduced. Viability of infected seed is lowered, germination is delayed and diseased plants have reduced vigour and height.

5.4.2.4. Dispersal

Aphelenchoides besseyi is disseminated primarily by infected seed. On a local scale the nematode can be transmitted in flood water in lowland rice. High concentrations of seedlings in infested seedbeds also facilitate dispersal.

5.4.2.5. Ecology, host range and distribution

The optimum temperature for oviposition and hatch is 30°C; the optimum temperature for development is 21–25°C. At this temperature the life cycle is 10 ± 2 days. No development occurs below 13°C. Rice is the most important host of *A. besseyi*. The nematode is able to infect rice in most environments but infection and damage are generally greater in irrigated lowland and deep water than in upland rice. The nematode occurs in rice-growing regions of Africa, Asia, Caribbean, Europe and the USA. On strawberry in Australia and the USA, it is the causal agent of ‘summer dwarf’. The nematode has hosts in more than 35 genera of monocotyledons and dicotyledons.

5.4.2.6. Interaction with other pathogens

In Bangladesh, *A. besseyi* is reported to occur with *D. angustus* and *Meloidogyne graminicola* but little is known of their association. The nematode appears to influence symptoms caused by some fungal pathogens of rice. It is reported that *A. besseyi* reduces the severity of *Sclerotium oryzae* symptoms, whereas the deterioration of *Pyricularia oryzae*-infected leaves is accelerated by the nematode reproducing in the blast lesions. In both cases, the concomitant infection of the fungus and *A. besseyi* reduced yield more than either organism separately.

5.4.2.7. Management and control

Prophylaxis is important for management of *A. besseyi*. Using nematode-free seed is of paramount importance; if unavailable, hot water seed treatment is probably the most cost-effective control measure.

Resistance to *A. besseyi* is reported from different countries and in the USA. *Aphelenchoïdes besseyi* is controlled principally through the use of resistant cultivars. Screening for resistance, based primarily on symptom expression, has commonly revealed symptomless but susceptible (tolerant) cultivars. Symptom expression in the field is particularly variable and variation occurs between plants of a cultivar.

Irrigating seedbeds or direct seeding into water reduces infection. Early planting in cooler conditions also reduces or eliminates *A. besseyi* infection. Various chemicals are used for seed treatments, soil applications or root dips; however, no economic assessment of the use of chemical control for *A. besseyi* has been made. Experiments studying the effect of *Pseudomonas fluorescens* on *A. besseyi* development and rice growth and yield demonstrated that biological control agents can be used to reduce the impact of the nematode.

5.4.3. The pinewood nematode, *Bursaphelenchus xylophilus*

Nearly all *Bursaphelenchus* species are associated with bark beetles. Many species have specialized third-stage juveniles, termed ‘dauer larvae’, which are usually ectophoretic and use the insect for transport. Several *Bursaphelenchus* species are associated with longhorn beetles. *Bursaphelenchus xylophilus*, the pinewood nematode (PWN), causes a serious disease of pines but also feeds on fungi (Mota and Vieira, 2008).

5.4.3.1. Morphology

Bursaphelenchus xylophilus shows the general characters of the genus *Bursaphelenchus* (Ryss *et al.*, 2005): the cephalic region relatively high and offset from the body by a constriction; a 10–20 µm long stylet with reduced basal swellings; the median bulb well developed, ovoid to elongate ovoid in shape. The dorsal pharyngeal gland opening is situated inside the median bulb; pharyngeal glands dorsally overlap the intestine. In the female, the post-uterine sac is long and the vulva is situated at 70–80% of body length. In the male, the tail is curved ventrally, conoid and has a pointed terminus. A small bursa-like cuticular structure is situated terminally; the spicules are well developed.

Within the genus *Bursaphelenchus* several groups of species are separated on the basis of their morphology (Braasch, 2004; Ryss *et al.*, 2005). The number of incisures in the lateral field, clearly visible by scanning electron microscope, is considered an important diagnostic feature, as are spicule shape, number and position of caudal papillae, presence and size of vulval flap and the shape of female tails. Within the *B. xylophilus* group, species can be distinguished by the shape of the female tail. *Bursaphelenchus xylophilus* is distinguished from other species in the genus (Fig. 5.9) by the simultaneous presence of: (i) in the male, spicules flattened into a disc-like structure (the cucullus) at their distal extremity; (ii) in the female, the anterior vulval lip is a distinct overlapping flap; and (iii) the posterior end of the body is rounded in nearly all individuals. This last character separates *B. xylophilus* from *B. mucronatus*, a non-pathogenic species in which the female has a mucronate posterior end. However, morphological differentiation between *B. mucronatus* and populations of *B. xylophilus* with mucronate tails in North America is difficult.

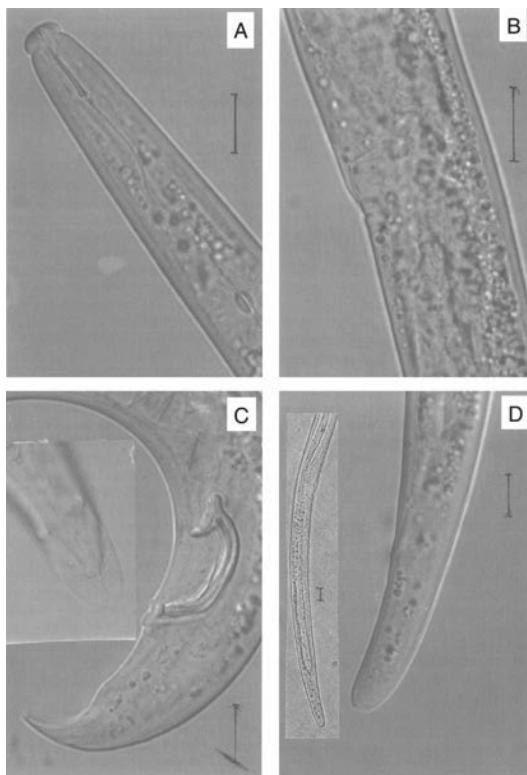


Fig. 5.9. Light microscope observations of *Bursaphelenchus xylophilus*. A: female head region with distinct labial region and stylet with reduced basal swellings. B: female vulva and vulval flap. C: male tail showing curved spicule with enlarged flattened cucillus (inset: ventral view showing bursa). D: female round tail terminus (inset: female tail with long post-uterine sac). Scale bars = 10 µm. (From Mota *et al.*, 1999.)

5.4.3.2. Life cycle and behaviour

Bursaphelenchus xylophilus has two different modes in its life cycle, a dispersal mode (primary transmission, phytophagous phase) and a propagative mode (secondary transmission, mycophagous phase) (Fig. 5.10). During primary transmission, the PWN, phoretically associated in the tracheae and elytra of young adults of their longhorn beetle hosts (*Monochamus* spp.), are transmitted to young twigs of a susceptible conifer host (usually *Pinus* spp.). This happens when the insects, loaded with fourth-stage juveniles (J4), feed on the bark of current or 1-year-old twigs. The initial feeding stage, lasting about 10 days to 3 weeks, is essential for the sexual maturation of the beetle. The nematodes enter the shoots through the feeding wounds.

In the young *Pinus* shoots, *B. xylophilus* spreads through the vascular system and resin canals, attacking their epithelial cells and living parenchyma. The nematode continues its life cycle, including four juvenile stages and an adult male and female stage. Under favourable conditions (20°C), the nematodes complete their life cycle in 6 days, each female laying between 80 and 150 eggs during a period of 28 days. As a result, nematodes block water transport in the xylem leading to plant death.

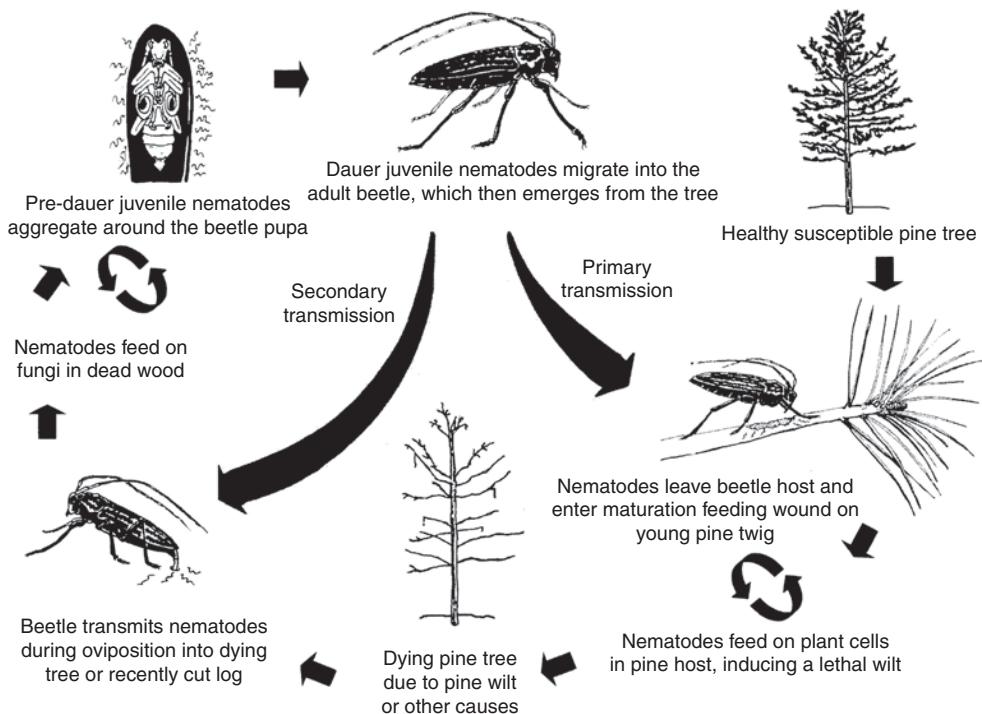


Fig. 5.10. Diagram representing the dispersal mode (primary transmission) and the propagative mode (secondary transmission) of the pinewood nematode, *Bursaphelenchus xylophilus* by its *Monochamus* vector on a susceptible conifer host. (From Giblin-Davis *et al.*, 2003.)

About 3 weeks later, the tree shows the first symptoms of 'drying out' or reduced oleoresin exudation. The nematodes now move freely throughout the dying tree. As a consequence of the reduction of its oleoresin defence mechanism, the tree becomes attractive to adult insects, which gather on the trunk to mate. At this stage, intensified wilting and yellowing of the needles is seen. In susceptible pines under the right conditions this can lead to classical pine wilt disease, resulting in the death of the tree within a year of infection (Giblin-Davis, 1993).

The secondary transmission pathway occurs when conifers wilt, die and become suitable breeding hosts for *Monochamus*. During oviposition by the insect vector in the bark of dead or dying trees or recently cut logs, the nematodes migrate out of the beetle into oviposition slits. This means of transmission, however, is much less significant than the one by maturation feeding. In the tree the PWN feed, reproduce and greatly increase their population densities on fungi transmitted to the wood by ovipositing beetles (usually *Ceratocystis* spp.). The biology of the PWN is synchronized with the development of the longhorn beetle progeny. A different type of third-stage juvenile, J_{III} (pre-dauer juvenile), develops as conditions in the host conifer become less suitable. This is usually correlated with the development of late instar larvae or pupae of its *Monochamus* vector and results in large aggregations of J_{III} around the pupal chambers where they overwinter with the beetle. A large proportion of the pre-dauer juveniles of *B. xylophilus* moult to non-feeding dauer juveniles

(fourth-stage, J_{IV}) and are attracted to CO₂ produced by newly enclosed adults. Nematodes also gather at the tips of perithecia formed by fungi growing on the sides of pupal chambers. When newly emerged beetles brush against the perithecial necks they acquire the nematodes, which settle below the elytra and in the tracheae. The immature adult beetle then flies from the wood carrying nematodes.

5.4.3.3. Host reaction

Two phases of symptom development occur after invasion of the wood by nematodes (Suzuki, 2002). In the early stage, cytological changes occur in the xylem parenchymatous cells, followed by cavitation and blockage within the tracheids. These internal symptoms are induced in both compatible and incompatible combinations of pine tree and PWN isolates. The first indication of the presence of nematodes in the tree is a reduction of oleoresin exudation rate, which marks the onset of advanced stage symptoms. Cambial death and cavitation in the outer xylem result in a water deficit that reduces transpiration and photosynthesis, causing the first obvious external symptoms, yellowing and wilting of the needles. The physiological water status of the tree and the nematode population density are both considered to be key factors in disease development. Experimental results suggest that pine seedlings do not wilt solely by virtue of the number of nematodes under favourable conditions such as a well-watered environment. Pine wilt disease seems to occur more frequently and to be more destructive in summers with little rainfall. The wilting may first appear on only one branch ('flag'), although the whole tree may later show symptoms. Infections with *B. xylophilus* eventually lead to death of the tree.

5.4.3.4. Movement and dispersal

The nematodes can move within the wood tissues and can leave one piece of wood to move into neighbouring pieces. However, without their vectors they are incapable of moving from one host tree to another. Adults of the vector beetles have a peak of flight activity about 5 days after emergence. Beetles are capable of flying up to 3.3 km, but, in most cases, dispersal is only for a few hundred metres. Infested wood is the most probable means of international transport of *B. xylophilus*, and the species has been intercepted on sawn wood, round wood and wood chips. The most serious pathway of introduction of *B. xylophilus* is when imported together with vector insects, which then carry the nematodes to coniferous trees.

Whether introduced with or without a vector insect, long-term establishment requires the nematode to find a means of coming into contact with a native vector, and this can probably be achieved only if the nematode first invades wood containing larvae or pupae of a potential vector.

5.4.3.5. Ecology, host range and distribution

Serious pine wilt disease is associated with higher temperature and occurs only where the mean summer temperatures exceed 20°C. In the laboratory, *B. xylophilus* can be

maintained on fungal cultures. It reproduces in 12 days at 15°C, 6 days at 20°C and 3 days at 30°C. Egg-laying starts on the fourth day after hatching and the juveniles hatch in 26–32 h at 25°C. The temperature threshold for development is 9.5°C.

Bursaphelenchus xylophilus is found mainly on *Pinus* spp. Apparently, the dead wood of all species of *Pinus* can act as a substrate for *B. xylophilus* development. However, only a limited number of species of *Pinus* are susceptible to attack as living trees. The Far Eastern species *P. bungeana*, *P. densiflora*, *P. luchuensis*, *P. massoniana* and *P. thunbergii* (in their native habitats), the European species *P. nigra* and *P. sylvestris* (planted in North America) and *P. pinaster* (planted in China) are the only species known to be killed by pine wilt disease as mature trees in the field. Many other species have been found to be damaged or killed by the nematode only under experimental conditions (mainly as seedlings in glasshouses). Other conifers can also act as hosts (primarily *Larix*, *Abies* and *Picea*) but reports of damage are rare. Isolated cases of death of *Picea* and *Pseudotsuga* due to this nematode have been reported in the USA.

It is presumed that *B. xylophilus* originated in North America and was transported from there to Japan in infested timber at some time around the beginning of the 20th century. The fact that native American conifers are mostly resistant, while Japanese species are susceptible, supports this view. From Japan, *B. xylophilus* spread to other Asian countries including China, Korea Republic and Taiwan. In North America the nematode is present in Canada, Mexico and the USA, probably wherever *Pinus* occurs except Hawaii. In Europe, *B. xylophilus* was detected for the first time in Portugal on *P. pinaster*. The nematode was later reported from Spain.

5.4.3.6. Molecular diagnosis

Bursaphelenchus xylophilus has a quarantine status in many countries (see Chapter 12) but is morphologically similar to *B. mucronatus*, so efficient diagnosis is critical. Both species (including the mucronate form of *B. xylophilus*) can be separated by DNA PCR amplification of the internal transcribed sequence (ITS) and intergenic spacer (IGS) regions in the rDNA, species-specific primers, RFLPs or satellite-DNA. The latter technique can discriminate a single nematode spotted on to a filter. Because the *MspI* satellite sequence is polymorphic within the species, this technique can also be used to characterize strains. Species-specific probes are also used with real-time PCR technology.

5.4.3.7. Interaction with other pathogens

The interaction of *B. xylophilus* with *Monochamus* spp. is of paramount importance because the insect is the natural vector for the nematode. *Monochamus alternatus* is the major vector in Asian countries; in Portugal the vector is *M. galloprovincialis*. *Monochamus* spp. are not only vectors but also pests of *Pinus* spp.

It is hypothesized that a third party, namely toxin-producing bacteria, is involved in pine wilt disease (Oku *et al.*, 1980). The disease would be a result of an integrative double vector system, where the nematode transmitted the pathogenic bacteria within the tree, and the cerambycid beetle transmitted the nematodes from tree to tree. Later, evidence of these bacteria in plant tissues infected with PWN as well as in the body surface of the nematode was also reported (Vicente *et al.*, 2012). However, this subject is still controversial and further studies are needed to understand the effective role of bacteria on this complex disease.

5.4.3.8. Management and control

Control measures against pine wilt disease aim at breaking the pine tree–nematode–insect disease triangle. Control must be concentrated on the activities that may represent risk of entry and dissemination. Wood trade between countries is nowadays highly monitored and all infested wood should be carefully treated before shipment or use in manufacturing.

In Japan, PWN control strategies have concentrated on a combination of removing dead or dying trees from the forest to prevent their use as a source of further infection, and control of vector beetles with insecticides. Research is ongoing to find alternative means of control, including natural and/or chemical nematicides/insecticides with low side effects, biological control of nematodes and vectors, use of insect attractants, and the more challenging and long-term approach, breeding programmes for resistance and tolerance of forest trees.

6

Ectoparasitic Nematodes*

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6.1. Introduction

Molecular research supports the hypothesis that plant parasitism in the phylum Nematoda evolved independently at least three times (see Fig. 2.9). Two of the three major plant-parasitic taxa, the tylenchs and the Longidoridae, show a similar adaptation to plant parasitism by the possession of a hollow protrusible stylet as tool for penetrating the plant cell wall, whilst in the Trichodoridae, the stylet is a modified styletiform mural dorsal tooth.

The tylenchs or tylenchids belonging to the suborder Tylenchina are the largest group of plant-parasitic nematodes. Within this group, the endoparasitic taxa, such as the family Meloidogynidae, which have developed a very close association with the plant cell, are economically important. The infraorder Tylenchomorpha De Ley & Blaxter, 2002 also includes the superfamily Aphelenchoidea Fuchs, 1937. Taxa of the Aphelenchoidea are mainly migratory endoparasites (see Chapter 5). The majority of the Tylenchomorpha, excluding the Aphelenchoidea, are ectoparasites that live in soil and feed on roots.

Ectoparasitic tylenchs *sensu stricto* can be grouped according to their parasitic strategies: (i) the migratory ectoparasites, which stay vermiform throughout their life cycle and feed for short periods along the root system (e.g. *Trichodorus*, *Belonolaimus*, *Dolichodorus*); and (ii) the sedentary ectoparasites, which are those species that may feed for several days on the same cell, either a cortical or an epidermal cell (Tytgat *et al.*, 2000). Other tylench taxa behave as semi- or facultative ecto-endoparasites (*Hoplolaimus*, *Helicotylenchus*) and may act as ectoparasites.

The Longidoridae and Trichodoridae are migratory root ectoparasites. They are responsible for substantial direct damage to a wide variety of plants, but their major pest status is as virus vectors, despite the rather low number of vector species in both families. Virus–vector association in Nematoda is only known from these two families; both families acquired the association with the non-related viruses independently, with Nepoviruses being associated with members of the family Longidoridae and Tobraviruses with Trichodoridae.

6.2. Definition of Ectoparasites/Ectoparasitism

The term ‘ectoparasite’ comes from the Greek: *ecto* (= outer), *para* (= with, at), *sito* (= feeding). An ectoparasite is a parasite that lives on the outer surface of a host; nematode ectoparasites do not enter the plant tissues with their body but use their stylet to puncture plant cells and feed upon the cytoplasm. The longer the stylet, the deeper the nematode ectoparasite can feed. In general, short stylet-bearing forms e.g. trichodorids and tylench taxa, such as *Tylenchorhynchus* and *Helicotylenchus*, usually feed on root hairs and/or epidermal cells; ectoparasites with long stylets, such as longidorids and the tylench genera *Belonolaimus* and *Dolichodorus*, exploit deeper tissues. All stages of ectoparasites may feed on the root. As ectoparasites *sensu stricto* do not enter the plant, the damage they cause is usually limited to necrosis of those cells penetrated by the stylet, and this may cause galling. However, cells are not always killed; cells can be punctured but not fed upon and cytoplasmic streaming

may be restored when the nematode moves away. According to Yeates (1971), the various migratory ecto- and endoparasites are not true parasites but a group of highly adapted plant browsers.

6.3. Classification

The classification (Boxes 6.1 and 6.2) follows the scheme proposed by De Ley and Blaxter (2002) as used throughout this book.

6.4. Tylenchina (Chromadorea, Chromadoria)

6.4.1. Selected genera of the Tylenchoidea and Criconematoidea

Morphological descriptions are based on females, unless otherwise stated.

Box 6.1. Classification of tylench ectoparasitic taxa.

Phylum Nematoda Potts, 1932
Class Chromadorea Inglis, 1983
Subclass Chromadoria Pearse, 1942
Order Rhabditida Chitwood, 1933
Suborder Tylenchina Thorne, 1949
Infraorder Tylenchomorpha De Ley & Blaxter, 2002
Superfamily Tylenchoidea Örley, 1880
Family Dolichodoridae Chitwood *in* Chitwood & Chitwood, 1950
 Subfamily Dolichodorinae Chitwood *in* Chitwood & Chitwood, 1950
 Genus *Dolichodorus* Cobb, 1914
 Subfamily Belonolaiminae Whitehead, 1960
 Genus *Belonolaimus* Steiner, 1949
 Subfamily Telotylenchinae Siddiqi, 1960
 Genus *Tylenchorhynchus* Cobb, 1913
 Subfamily Merliniinae Siddiqi, 1971
 Genus *Amplimerlinius* Siddiqi, 1976
Family Hoplolaimidae Filipjev, 1934
 Subfamily Hoplolaiminae Filipjev, 1934
 Genus *Hoplolaimus* Daday, 1905
 Subfamily Rotylenchoidinae Whitehead, 1958
 Genera *Helicotylenchus* Steiner, 1945
 Rotylenchus Filipjev, 1936
Superfamily Criconematoidea Taylor, 1936 (1914)
Family Criconematidae Taylor, 1936
 Subfamily Macroposthoniinae Skarbilovich, 1959
 Genus *Criconemooides* Taylor, 1936

Box 6.2. Classification of non-tylench ectoparasitic taxa.

Phylum Nematoda Potts, 1932
Class Enoplea Inglis, 1983
Subclass Dorylaimia Inglis, 1983
Order Dorylaimida Pearse, 1942
Suborder Dorylaimina Pearse, 1942
Superfamily Dorylaimoidea Thorne, 1935
Family Longidoridae Thorne, 1935
 Subfamily Longidorinae
 Genera *Longidorus* Micoletzky, 1922
 Longidoroides Khan, Chawla & Saha, 1978
 Paralongidorus Siddiqi, Hooper & Khan, 1963
 Subfamily Xiphidiorinae
 Genera *Australodorus* Coomans, Olmos, Casella & Chaves, 2004
 Paraxiphidorus Coomans & Chaves, 1995
 Xiphidorus Monteiro, 1976
 Subfamily Xiphinematinae
 Genus *Xiphinema* Cobb, 1913
Subclass Enoplia Pearse, 1942
Order Triplonchida Cobb, 1920
Suborder Diphtherophorina Coomans & Loof, 1970
Superfamily Diphtherophoroidea Micoletzky, 1922
Family Trichodoridae Thorne, 1935
 Genera *Allotrichodorus* Rodriguez-M., Sher & Siddiqi, 1978
 Ecuadorus Siddiqi, 2002
 Monotrichodorus Andrassy, 1976
 Nanidorus Siddiqi, 1974
 Paratrichodorus Siddiqi, 1974
 Trichodorus Cobb, 1913

6.4.1.1. *Dolichodorus Cobb, 1914 (Fig. 6.1 A–H)*

Dolichodoridae. Body long (1–3.5 mm) and slender. Lateral field with three incisures, areolated. Head offset, rounded, striated, roughly quadrangular to prominently four lobed in *en face* view; labial disc more often prominent; subdorsal and subventral lip sectors distinct; lateral lip sectors reduced or absent. Sclerotization of basal plate prominent. Stylet very long (50–160 µm), strong. Median and basal bulbs well developed, joined by a short, slender isthmus. Vagina variously sclerotized; no epiptygmata. Tail hemispherical-spiked, rarely conoid. Males with large, trilobed bursa; spicules most generally with prominent flanges; gubernaculum also robust, protrusible (Luc and Fortuner, 1987).

The genus *Dolichodorus* contains some 16 species, known as ‘awl’ nematodes (Geraert, 2011). The pathogenicity of *D. heterocephalus* Cobb, 1914 and the symptoms expressed by its host plants (see below) are well known. Those of some other species of *Dolichodorus* are less well known but from the information available appear to be very similar to those of *D. heterocephalus*. The genus has been

reported from widely separated states in the USA and from widely separated countries all over the world, predominantly in subtropical and tropical areas. The host range of *D. heterocephalus* is extensive and includes agronomic crops, vegetable crops, fruit trees, ornamental trees, shrubs, grasses and weeds. All stages of the nematode feed from the surface of roots by injecting its long stylet into cells mostly at root tips. If feeding occurs in the piliferous area for 1–4 h, the root becomes constricted in that area. Normal growth of the root tip is inhibited in the vicinity of a feeding site and the elongation of cells of the non-parasitized areas causes the root to curve. Root tips may appear enlarged because the tissues mature right to the tip, and occasionally galls appear on root tips. When an infested plant produces new root initials, the nematode feeds on them, stopping their growth as well. The result is a skeleton root system with almost no feeder roots and few, if any, secondary roots. This is often referred to as stubby root or coarse root or both. Root tissue

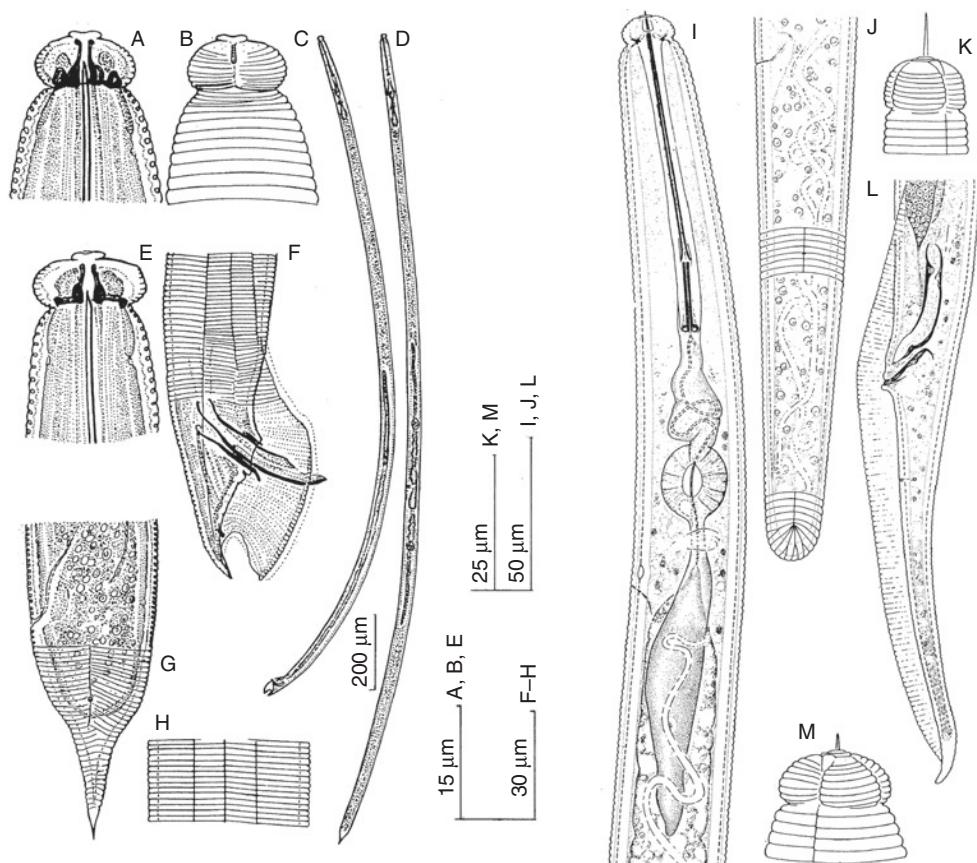


Fig. 6.1. A–H. *Dolichodorus heterocephalus* Cobb. I–M. *Belonolaimus longicaudatus* Rau. A, B and M, head ends of females; C, male; D, female; E and K, head ends of males; F and L, tail ends of males; G and J, tail ends of females; H, lateral field; I, pharyngeal region of female. (A–H after Siddiqi, 1976, courtesy *Nematologica*; I–M after Orton Williams, 1974, courtesy CABI.)

surrounding the feeding site is destroyed and discoloured. Above ground, the plants are stunted and chlorotic due to the lack of an adequate root system (Smart and Nguyen, 1991).

6.4.1.2. *Belonolaimus Steiner, 1949* (Fig. 6.1 I–M)

Belonolaimidae: Belonolaiminae. Large (2–3 mm) and slender ($a = 50\text{--}80$) tylenchs. Lateral field with a single, two or four incisures. Head large, rounded, mostly offset, divided by longitudinal grooves into four well-separated sectors; framework with basal plate moderately and arches lightly sclerotized. Scanning electron microscopy (SEM) face view shows a well-marked rounded labial disc, and first lip annulus divided into six sectors, lateral sectors almost completely regressed, seen only as small interruptions of the first one or two labial annuli. Stylet very long (90–160 μm), with conus more than twice the shaft length. Median bulb round, isthmus short; pharyngointestinal junction lies close to median bulb, anterior to main body of glands; pharyngeal glands overlapping intestine lateroventrally. Vulva equatorial, with epiptygmata; spermatheca axial, slightly offset; ovaries paired. Tail elongate, in female cylindroid, three to six anal body widths long; with post-anal extension of intestine and annulated terminus; in male elongate-conoid, enveloped by a low bursa. Spicules robust, slightly arcuate, flanged. Gubernaculum large, not protrusible (Fortuner and Luc, 1987).

The genus *Belonolaimus* contains about ten species of which *B. longicaudatus* Rau, 1958 is well known as an ectoparasite ('sting' nematode). Sting nematodes are predominantly found in sandy soils in the USA, with reports also from Middle and South America and even Australia. *Belonolaimus longicaudatus* has been recognized as an important pathogen since about 1950. A large number of plants, including most vegetable crops, agronomic crops, turf grasses and forage grasses, have been shown to be hosts. Symptoms caused by sting nematodes vary somewhat depending on such factors as inoculum level, host plant and age of the plant but, in general, the root system is greatly reduced and exhibits a combination of stubby roots and coarse roots with dark lesions along the root and at root tips. Sometimes roots are girdled completely. Apparently, salivary secretions from the feeding nematodes affect cells beyond those fed on. Shoot symptoms consist of stunting, premature wilting and leaf chlorosis (Smart and Nguyen, 1991).

6.4.1.3. *Tylenchorhynchus Cobb, 1913* (Fig. 6.2 A–E)

Belonolaimidae: Telotylenchinae. Body about 1 mm or less. Lateral field with two, three, four or five lines, sometimes areolated. Longitudinal ridges sometimes present on body. Head offset from body or continuous, annulated or rarely smooth; head framework light to moderately sclerotized. SEM shows various end-on patterns, the most typical with labial disc fused with first lip annulus, and with lateral sectors regressed; the remaining sub-median sectors give a distinctive square appearance to the face view; papillae often present on sub-median sectors. Stylet 15–30 μm long, thin to slender, with cone about as long as shaft, sometimes needle-like; knobs prominent. Median bulb round to oval, with distinct refractive thickenings; basal bulb usually offset from intestine, sometimes dorsally overlapping. Vulva near middle of

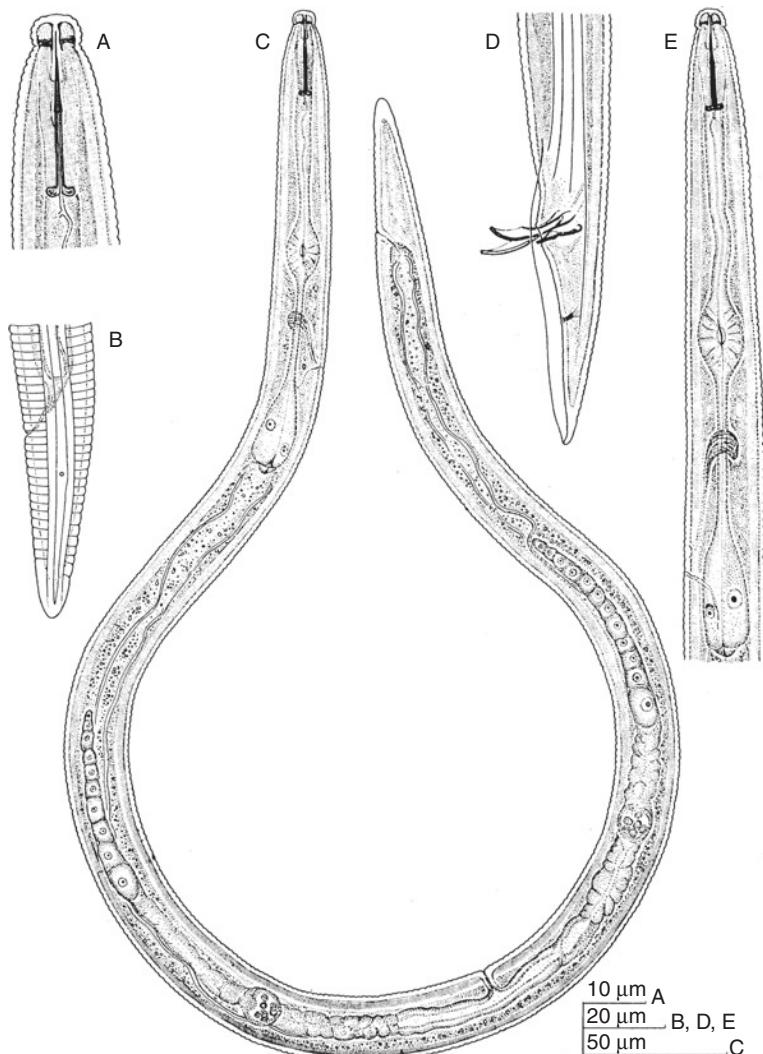


Fig. 6.2. *Tylenchorhynchus cylindricus* Cobb. A–C, neotype female; D and E, male. A, head end; B, tail end of female; C, entire female; D, tail end of male; E, pharyngeal region of female. (After Siddiqi, 1972c, courtesy CABI.)

body, generally at 50–54%, lips not modified; spermatheca round, axial; ovaries paired, outstretched. Tail conoid to sub-cylindroid (exceptionally clavate), about three times as long as wide ($c' = 2\text{--}4$), sometimes with thicker cuticle in the distal end. Male with caudal alae well developed, rarely lobed. Spicules flanged, gubernaculum about half as long as spicule (Fortuner and Luc, 1987; Geraert, 2006).

The genus *Tylenchorhynchus* contains more than 130 species. Several characters, such as the number of lines in the lateral field, presence of longitudinal ridges and thickening of tail cuticle, can be used to separate groups of species and they are useful for identification. Nomenclatorial status for such groups by naming them as genera is often proposed.

The species of *Tylenchorhynchus* constitute, with representatives of several related genera, a group of more than 250 species, generally known as ‘stunt nematodes’. Few have been proven as pathogens in the strict sense but about 8% are known ectoparasites. *Tylenchorhynchus claytoni* Steiner, 1937 is present in the north temperate zone, where various plants are attacked, in particular azaleas, holly and conifers, causing reduced growth. *Tylenchorhynchus annulatus* (Cassidy, 1930) Golden, 1971 is found in the subtropical and tropical regions of all continents, except Europe. It is a pathogenic ectoparasite of sugar cane, Bermuda grass and especially rice. *Tylenchorhynchus nudus* Allen, 1955 has been reported mainly from the Midwest USA, from the Orient and from India, and caused growth reduction in Kentucky bluegrass, spring wheat and sorghum, whilst sugar cane yield increased by 10–20% when nematodes were controlled by fumigation. *Tylenchorhynchus cylindricus* Cobb, 1913 (Fig. 6.2) is mainly found in North America. It causes moderate stunting in cotton and bean. *Tylenchorhynchus capitatus* Allen, 1955 is widespread on all continents and has a broad host range: hollyhock, apple, plum, strawberry, citrus, potato, wheat, tobacco and maize. *Tylenchorhynchus dubius* (Bütschli, 1873) Filipjev, 1936 is found all over the northern hemisphere and is very common in Europe; it is more a browsing ectoparasite. Many of the reports of detrimental *T. dubius* association with plants have indicated stunted growth and weight reduction as the main effects (Anderson and Potter, 1991).

6.4.1.4. *Amplimerlinius Siddiqi, 1976 (Fig. 6.3 A–L)*

Belonolaimidae: Telotylenchinae. Body medium to large (1–2 mm). Lateral field with six lines over most of the body, generally with areolations. Head continuous with body contour; labial framework robust. SEM face view broadly rounded, and laterally elongated, with labial disc partially or completely fused with first lip annulus, lip annulus sectors also partly or completely fused together. Stylet robust, 19–47 µm long; knobs large. Median bulb well developed, at or behind middle of pharynx; dorsal pharyngeal gland sometimes overlapping the beginning of the intestine for a short distance. Vulva with epiptygmata. Female tail cylindrical, occasionally sub-clavate, with hemispherical, annulated terminus; cuticle thickened at tip. Spicules robust, blunt and notched at tip; gubernaculum trough-shaped in lateral view (Fortuner and Luc, 1987).

The genus *Amplimerlinius* contains about 22 species (Geraert, 2011); two species are parasitic: *Amplimerlinius icarus* (Wallace & Greet, 1964) Siddiqi, 1976 and *A. macrurus* (Goodey, 1932) Siddiqi, 1976. Both species are morphologically very similar; they differ mainly in measurements, *A. icarus* being much larger. Both species are known mainly from Europe. They are ectoparasites and semi-endoparasites, feeding on oat and ryegrass roots, with the anterior third of the body embedded. They feed as fairly sedentary ectoparasites but also penetrate roots and feed on internal tissues. However, they cause little observable damage, except for darkening of the cells surrounding the embedded head; root growth appeared normal (Anderson and Potter, 1991).

6.4.1.5. *Helicotylenchus Steiner, 1945 (Fig. 6.4 A–I)*

Hoplolaimidae: Hoplolaiminae. Small to medium-sized (0.4–1.2 mm), spirally coiled or rarely arcuate. Lateral field with four lines. Head continuous to slightly offset,

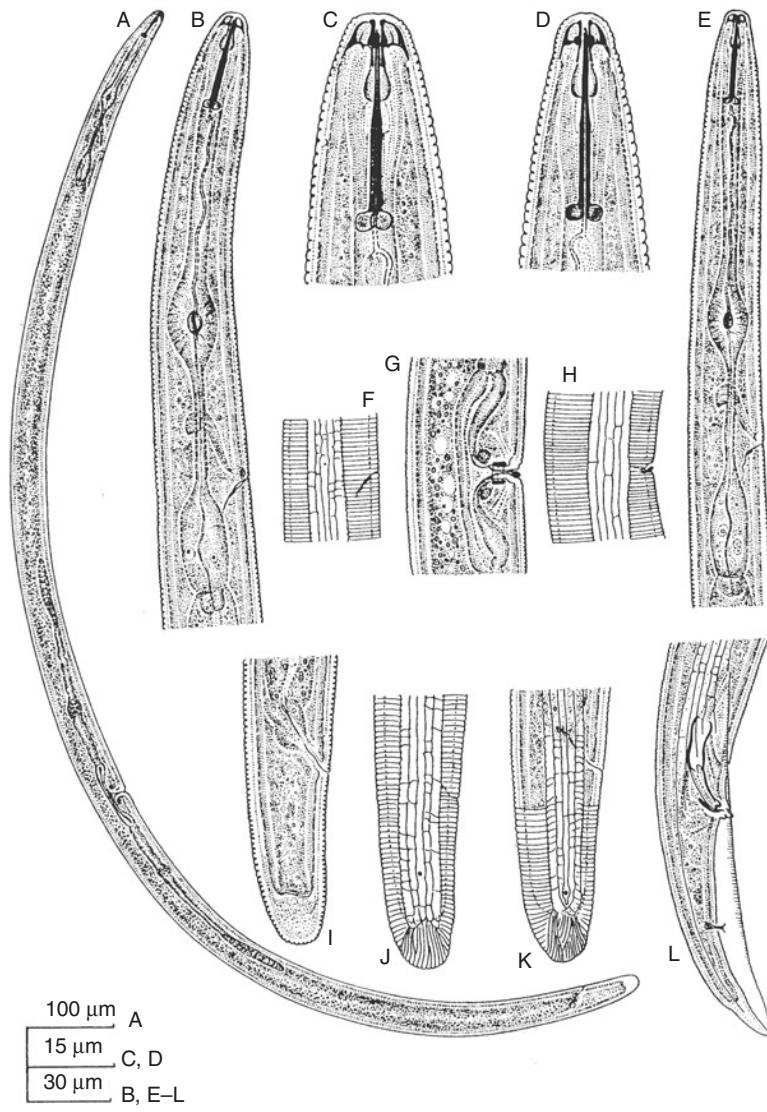


Fig. 6.3. *Amplimerlinius amplus* Siddiqi. A, female; B, pharyngeal region of male; C and D, head end of female and male, respectively; E, anterior region; F and H, lateral fields; G, vulval region; I–K, tail ends of females; L, tail end of male. (After Siddiqi, 1976, courtesy *Nematologica*.)

rounded or anteriorly flattened, generally annulated but almost never longitudinally striated; anterior lip annulus generally not divided into sectors. Cephalic framework well developed. Stylet robust, about three to four times maximum width of cephalic region. Orifice of dorsal pharyngeal gland 6–16 μm from stylet end. Median bulb rounded. Pharyngeal glands overlapping intestine on all sides with the position of the pharyngeal lumen situated between the dorsal gland and one of the sub-ventral glands, longest overlap being lateroventral. Two genital branches, the posterior one

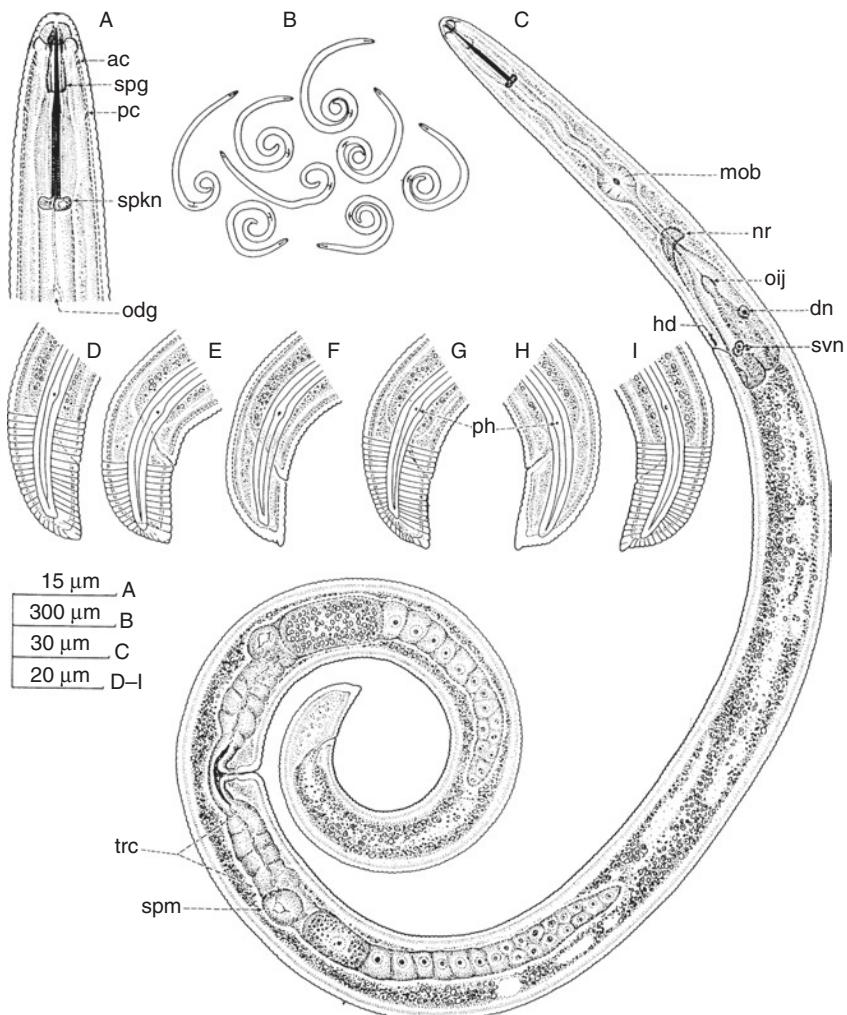


Fig. 6.4. *Helicotylenchus dihystera* (Cobb). A–I, topotype females. A, head end; B and C; females; D–I, tail ends; ac, anterior cephalid; dn, dorsal pharyngeal gland nucleus; hd, hemizonid; mob, median bulb; nr, nerve ring; odg, orifice of dorsal gland; oij, pharyngo-intestinal junction; pc, posterior cephalid; ph, phasmid; spg, stylet guide; spkn, stylet knobs; spm, spermatheca; svn, subventral gland nuclei; trc, tricolumnella (After Siddiqi, 1972a, courtesy CABI.)

sometimes degenerated or reduced to a post-vulval uterine sac. Epiptygmata present but folded inwards, into the vagina. Tail 1–2.5 anal body diameters long, typically more curved dorsally, with or without a terminal ventral process, sometimes rounded. Males sometimes with slight secondary sexual dimorphism in smaller anterior end. Caudal alae enveloping tail end. Male tail short, conical. Gubernaculum trough- or rod-shaped, fixed (Fortuner, 1987).

The genus *Helicotylenchus* is one of the largest genera in the tylenchs, probably containing more than 160 species. Several of them are known endoparasites.

Helicotylenchus dihystera (Cobb, 1893) Sher, 1961 is a cosmopolitan species with a very large host list. It is an ectoparasite or semi-endoparasite on the roots of many plants. The nematodes are partially or fully embedded in the root where they feed from a single cell over several days. Feeding resulted in cortical lesions of the roots. *Helicotylenchus multicinctus* (Cobb, 1893) Golden, 1956 is an important parasite of banana in the entire banana-growing areas of the world. It is an endoparasite in the cortex of the roots where it feeds and produces small superficial lesions. *Helicotylenchus brevis* (Whitehead, 1958) Fortuner, 1984 was found on cultivated plants (banana, mango) and uncultivated bush, ferns and bulbous plants in Southern Africa (Fortuner, 1991).

6.4.1.6. *Rotylenchus Filipjev, 1936* (Fig. 6.5 A–H)

Hoplolaimidae: Hoplolaiminae. Usually rather large (1–2 mm) ectoparasites, body spiral to C-shaped. Lateral field with four lines, with or without scattered transverse striae. Head offset or continuous with body contours, anteriorly rounded or flattened, generally annulated, with or without longitudinal striae on basal lip annulus. Labial framework well sclerotized, stylet and stylet knobs well developed; stylet knobs with rounded to indented anterior surface. Opening of dorsal pharyngeal gland often close to stylet end (6 µm) but sometimes more posteriorly (up to 16 µm). Median bulb well developed; pharyngeal glands overlap intestine dorsally and laterally; dorsal gland more developed than sub-ventral glands. Two genital branches outstretched, equally

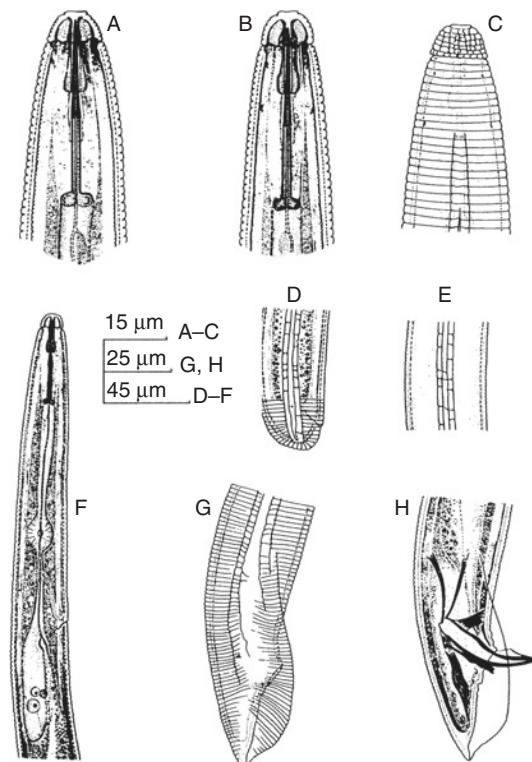


Fig. 6.5. *Rotylenchus robustus* (de Man). A and C, female head ends; B, male head end; D, female tail end; E, female lateral field at mid-body; F, female pharyngeal region; G and H, male tail ends. (After Siddiqi, 1972b, courtesy CABI.)

developed; posterior branch rarely degenerated. One or two epiptygmata present. Tail short, hemispherical, rarely with small ventral projection. Phasmids pore-like, small, near anus level. Males with caudal alae enveloping tail, not lobed. Spicules robust, flanged. Gubernaculum titillate (Fortuner, 1987).

The genus *Rotylenchus* in its broadest sense contains some 97 species occurring in every continent (Castillo and Vovlas, 2005; Cantalapiedra-Navarrete *et al.*, 2012). Several species are of economic importance in agriculture. The most widely distributed and most common species is *Rotylenchus robustus* (de Man, 1876) Filipjev, 1936, which has been reported from 25 countries and islands. This species is the causal agent of a destructive disease of carrots and lettuce, characterized by a severe reduction in the roots and stunting of top growth. *Rotylenchus buxophilus* Golden, 1956 caused stunted weak plants of young English boxwood, with reduced root systems showing numerous tiny brown necrotic lesions, which involved one or two cells on the surface or, more often, several cell layers deep in the cortex. *Rotylenchus laurientinus* Scognamiglio & Talamé, 1973 was found on carrots in Italy feeding as a semi-endoparasite and causing lesions and cavities in epidermal and cortical tissues. Other *Rotylenchus* species have been recorded in association with sugar cane by several authors in several countries (Fortuner, 1991).

6.4.1.7. *Hoplolaimus Von Daday, 1905 (Fig. 6.6 A-K)*

Hoplolaimidae: Hoplolaiminae. Body straight, large (1–2 mm). Lateral field with four lines or less, generally areolated at level of phasmids and anteriorly, sometimes with striae irregularly scattered over entire field, not areolated. Lip region offset from body, wide, anteriorly flattened, with clearly marked annuli and with longitudinal striae. Labial framework and stylet massive; stylet knobs anchor- or tulip-shaped. Dorsal pharyngeal gland opening 3–10 µm from stylet base. Pharyngeal glands overlap intestine dorsally and laterally; sometimes gland nuclei duplicated to a total of six nuclei. Two genital branches outstretched, equally developed. Tail short, rounded. Phasmids enlarged to scutella erratically situated on body, anterior to anus level and sometimes anterior to vulva level, not opposite each other. Male with caudal alae enveloping tail, regular. Secondary sexual dimorphism visible in labial region; pharyngeal structures smaller in males. Spicules massive with distal flanges; gubernaculum large, protrusible, titillate (Fortuner, 1987).

The genus *Hoplolaimus* contains more than 30 species, of which several are known endoparasites. *Hoplolaimus galeatus* (Cobb, 1913) Thorne, 1935 is widely distributed in the USA and has been reported from Canada, Central and South America and India. It has a large variety of hosts, in particular cotton, trees (pine, oak, sycamore, etc.), turf grasses and other graminaceous plants. It lives as an endoparasite on cotton, causing considerable damage to cortex and vascular tissue. On pine, most of the cortex of infested roots is destroyed. In sycamore it causes extensive root necrosis but it is unable to penetrate completely within the roots and its body partly protrudes out of the root. *Hoplolaimus pararobustus* (Schuurmans-Stekhoven & Teunissen, 1938) Coomans, 1963 is found in Africa, mostly within the roots of banana, but it can also parasitize other plants, including coffee, tea, sugar cane, palm trees, various tropical fruit trees, rice and yam. It has been described from grass in India and from various plants in Pakistan. On banana, it feeds mostly endoparasitically

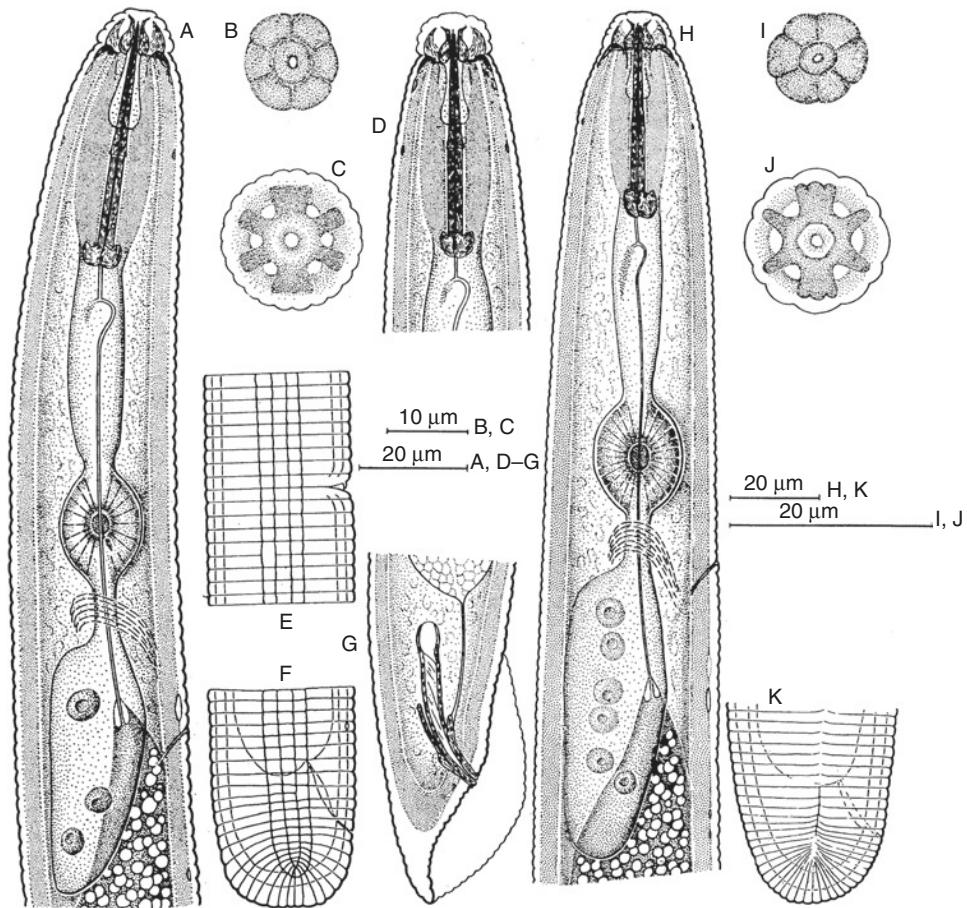


Fig. 6.6. A–G, *Hoplolaimus tylenchiformis* Daday. H–K, *H. seinhorsti* Luc. A and H, pharyngeal regions of females; B and I, end-on views of females; C and J, cross-sections through basal annulus of head of females; D and G, head and tail end of male, respectively; E, surface view at vulval region; F and K, tail ends of female showing lateral field. (After Sher, 1963, courtesy *Nematologica*.)

but it has occasionally been observed only partially embedded within the roots. On coffee, it feeds semi-endoparasitically. Cortex penetration results in cavities and ruptured cells. Numerous irregular brown necrotic lesions develop on the roots of infested coffee plants. *Hoplolaimus columbus* Sher, 1963 is an important parasite of soybean and cotton in the USA. It is an endoparasite of soybean roots, penetrating endodermis, pericycle and phloem. On cotton, it is a semi-endoparasite and it penetrates the cortex but not the endodermis (Fortuner, 1991).

6.4.1.8. *Criconemoides* Taylor, 1936 (Fig. 6.7 A–L)

Criconematidae: Macroposthoniinae. Sexual dimorphism. Females with a body of variable length (0.2–1 mm). Annuli large, distinct; mostly retrorse; 36–219; posterior

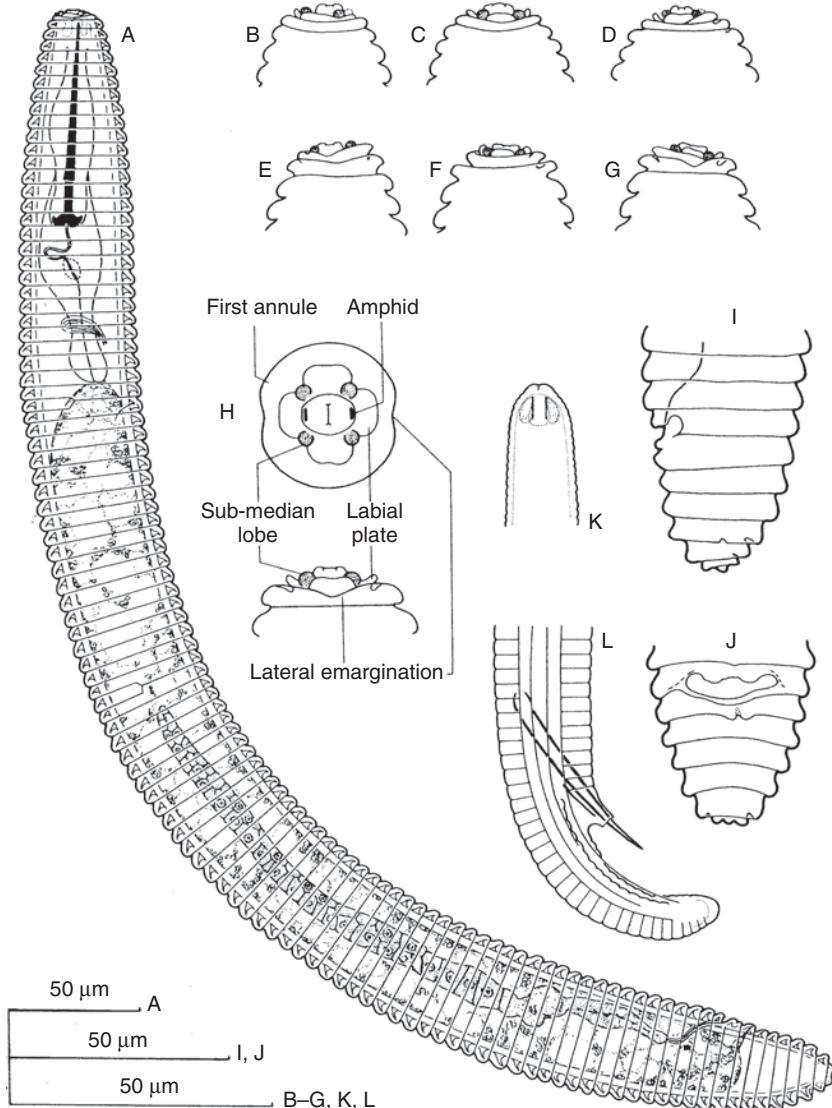


Fig. 6.7. *Criconemoides xenoplax* (Raski). A, entire body; B–H, head ends of females; I and J, female; K and L, male, head and tail, respectively. (After Orton Williams, 1972, courtesy CABI.)

edge smooth to finely crenate. Sub-median lobes generally well developed, but may be poorly developed or even absent in some species; separated or connected in different ways; first annulus may be reduced or even divided into plates. Vulva closed to open; anterior lip may be ornamented. Spear strong, stylet knobs mostly anchor-shaped; stylet rarely thin and flexible or exceptionally short with rounded basal knobs. Male with head end rounded to conoid; generally four lateral lines (rarely three or two); caudal alae distinct, exceptionally absent (Raski and Luc, 1987).

The genus *Criconemoides* (including *Mesocriconema*; some authors regard *Mesocriconema* as a valid genus based on characters of the labial region and whether the vagina is open or closed) contain more than 140 species. Several characters, such as head structure, vulva structure and structure of the lateral field in males, separate groups of species and they are useful for identification. Nomenclatorial status for such groups by naming them as genera is often proposed (Geraert, 2010). Three species are reported as important ectoparasites: *Criconemoides onoense* Luc, 1959, *C. ornatus* Raski, 1958 and *C. xenoplax* Raski, 1962. *Criconemoides onoense* causes root decay on rice in tropical regions, secondary roots being stunted with lesions near the club-shaped root tips. *Criconemoides ornatus* affects groundnut and fruit and nut crops, with death of lateral root primordia and extensive lesions and pits on roots; it occurs in subtropical and tropical regions worldwide. *Criconemoides xenoplax* attacks fruit and nut crops in the USA (Manzanilla-Lopez *et al.*, 2004). These three species were considered as representatives of *Mesocriconema* in Geraert (2010).

6.5. Enoplea

6.5.1. The family Longidoridae

Dorylaimida. Dorylaimoidea. Adults long to very long, slender, usually ventrally arcuate to C-shaped or spiral upon relaxation by gentle heat, rarely straight; body length (L) ranging between 1.5 and 13 mm. Tail usually short, hemispheroid or conoid, with or without peg, but may be elongate conoid to filiform. Body cuticle smooth by light microscopy, finely transversely striated by SEM; body pores present along the lateral, dorsal and ventral sides with the dorsal pores restricted to the anterior end, the lateral pores arranged in one (neck region) to two or three longitudinal rows (posteriorly) and ventral pores arranged along the body, but may be absent in vulva region or completely as in *Xiphidorus*. Lip region more or less rounded, continuous with body contour or offset to a variable extent (depression or constriction), with or without expansion of the lip region. Six lips largely or completely amalgamated. Anterior sensilla arranged in two circlets respectively of six inner labial sensilla and six outer labial sensilla + four cephalic sensilla; all papilliform or as small pores surrounded or not by a cuticular rim. Amphidial fovea, post-labial, with variable shape and size ranging from wide stirrup-shaped, funnel-shaped, goblet-like to pouch-like; amphidial opening either a pore or ranging from a minute to a large transverse slit. Cheilostome with posterior end marked by the guide ring and varying in length according to the groups, i.e. relatively short in the Longidorini, while long in the Xiphidorini and Xiphinematinae. Stylet greatly elongated, 95–350 µm long, consisting of a 50–220 µm long anterior odontostyle (forked or not) and a posterior supporting structure or odontophore, provided or not with flanges. Pharynx dorylaimoid, with an anterior slender flexible tube and a flask-shaped posterior bulb with enforced lumen wall, visible as three pairs of triangular platelets in transverse section, and three pharyngeal glands, one dorsal and two ventrosublateral glands. Intestine with pre-rectum. Female reproductive system typically didelphic–amphidelphic with vulva at mid-body but monodelphic and pseudomonodelphic conditions also occur with shift of vulva; uterus varying in length and in structure, from simple to complex, with or without various inclusions such as a Z-differentiation, granules, spines, crystalloids. Male reproductive system diorchic,

posterior testis reflexed. Spicules relatively large, dorylaimoid in shape; gubernaculum restricted to two lateral guiding pieces or crura. Pre-cloacal supplements consisting of an adanal pair and a medioventral series of 1–20 papillae, rarely staggered or arranged in a double row. Type genus: *Longidorus* Micoletzky, 1922 (Filipjev, 1934).

The family Longidoridae consists of two subfamilies, the Longidorinae Thorne, 1935 and Xiphinematinæ Dalmasso, 1969.

6.5.1.1. *Longidorinae* Thorne, 1935

Longidoridae. Guide ring single; dorsal pharyngeal gland nucleus (DN) smaller than ventrosublateral gland nuclei (VSN) and located at some distance posterior to its orifice.

The subfamily contains two tribes, the Longidorini and the Xiphidorini.

6.5.1.1.1. LONGIDORINI (FIG. 6.8). Longidorinae. Odontostyle non-furcate, base odontophore non-swollen to slightly swollen but without real flanges. Guide ring located within anterior third or two-thirds of odontostyle length from anterior end; compensation sacs present at level guide ring. The Longidorini contain two or three genera: *Longidorus*, *Paralongidorus* and *Longidoroides* depending on the acceptance or not of *Longidoroides* as a valid genus. *Longidoroides* was considered a junior synonym of *Paralongidorus* by Siddiqi *et al.* (1993) but was accepted as valid by Hunt (1993), Coomans (1996), Decraemer and Coomans (2007) and the present authors until additional morphological and molecular data become available. Currently, molecular data are only available for a single *Longidoroides*

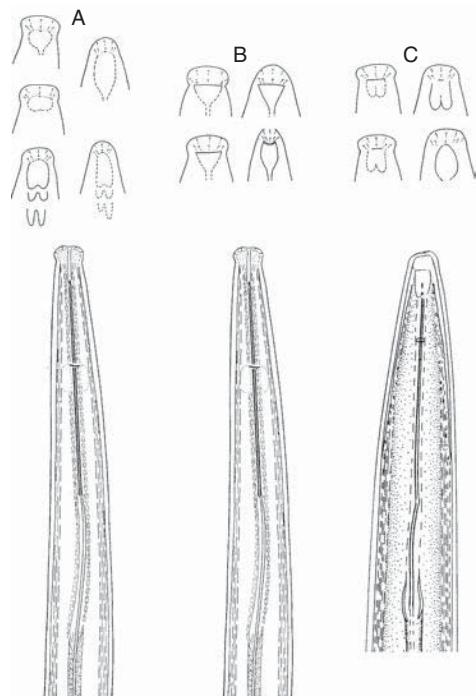


Fig. 6.8. Generic comparison of amphid and odontostyle region of the Longidorini.
A, *Longidorus*; B, *Paralongidorus*;
C, *Longidoroides*. Not to scale. (Based upon several authors.)

species (*L. bikanarensis*; Genbank, May 2012) and relationships of *Longidoroides* and *Paralongidorus* with the other genera within the family are not resolved yet (Gutiérrez-Gutiérrez *et al.*, 2011).

Longidorus Micoletzky, 1922

Longidorini. Lip region rounded or more or less flattened, continuous or marked by depression or constriction. Odontostyle non-forked; base odontophore without flanges. Cheilstome relatively long; guide ring single, anteriorly situated (at most at 40% of odontostyle length from anterior end); compensation sacs present. Amphidial fovea a pouch, its aperture a pore. Male with an adanal pair of pre-cloacal supplements (some species have two or three adanal pairs) plus a ventromedian series of up to 20 pre-cloacal supplements without hiatus between the adanal pair and the series; in some species ventromedian row forms a double staggered row. Female reproductive system didelphic–amphidelphic, dorylaimoid; uterus without sclerotizations. Tail short, dorsally convex-conical with a finely or broadly rounded terminus. Mostly four juvenile stages present, but some species with three. The latter are mainly restricted to Asia and occur less frequently in Africa and Europe. Type species: *Longidorus elongatus* (de Man, 1876) Thorne & Swanger, 1936.

Some 155 *Longidorus* species are known. Species identification is based upon the following features: (i) shape of pouch-like amphidial fovea; (ii) shape of lip region (rounded, flattened; offset by constriction, depression or continuous) and width of lip region; (iii) tail shape; (iv) length of odontostyle; (v) body length; (vi) tail length; (vii) ratios a and c'; (viii) distance of guide ring from anterior end; (ix) length of spicule; (x) number of ventromedian pre-cloacal supplements; and (xi) males known or unknown (Chen *et al.*, 1997). Seven *Longidorus* species are vectors of Nepoviruses, among them *L. elongatus* is an important plant parasite and widely distributed throughout temperate regions worldwide. There is usually one generation per year but on good hosts, such as strawberry, the life cycle may be half as long or less. Males are usually rare and reproduction is apparently parthenogenetic although bisexual reproduction is possible in populations where males are common. *Longidorus elongatus* is widespread in temperate regions worldwide and has been found in various types of soil but mainly has a preference for coarse, preferably stable, well-drained soils. It has a wide host range, among them many crops and weeds (De Waele and Coomans, 1991). As a root ectoparasite, *L. elongatus* aggregates around growing roots and feeds just behind the root tips, causing a characteristic swelling or galling and a general stunting of the root system. Severe attack of sugar beet results in a fangy, distorted root system. In Scotland, the species is found in the top 10 cm of soil in grass or cereal fields (Boag *et al.*, 1987). *Longidorus elongatus* can survive more than 2 years in a moist soil in a polythene bag in absence of host plants. The species is also known as a vector of the Scottish strains and English strain of raspberry ringspot virus (RRSV) and the Scottish strain of tomato black ring virus (TBRV). The virus particles are retained on the inner surface of the guiding sheath. Virus particles are not retained during the moulting process. Although the nematode does not retain the virus for much more than 2 months, it is able to reinfect itself from many weed hosts that carry the virus (Taylor and Brown, 1997).

Longidorus elongatus (de Man, 1876) Thorne & Swanger, 1936 (Fig. 6.9)

Adults several millimetres long (average 5.5 mm), body slightly coiled to an open ‘C’ upon heat killing or fixation. Lip region anteriorly flattened, continuous with the body

or slightly marked by a depression. Amphidial fovea, a large pouch, slightly bilobed. Odontostyle, 72–102 µm long, odontophore about half as long as odontostyle; guide ring far anterior, within one-third of odontostyle length from anterior end. Tail in female dorsally convex, ventrally flattened or slightly concave, about 1.3 anal body widths long, with a roundly conoid terminus and two or three lateral pores present on each side. Spicules about 60 µm long, lateral guiding pieces present; an adanal pair and a ventral series of 7–10 pre-cloacal supplements. Tail conoid to bluntly conoid, dorsally convex and ventrally concave with lateral body pores as in female. Four juvenile stages, the first emerges from the egg.

Paralongidorus Siddiqi, Hooper & Khan, 1963

Syn. *Paralongidorus (Siddiqia)* Khan, Chawla & Saha, 1978, *Siddiqia* Khan, Chawla & Saha, 1978 and *Inagreius* Khan, 1982.

Longidorini. Lip region continuous with body contour or expanded and offset; in some species with a second depression behind the groove. Odontostyle non-forked; base of odontophore slightly swollen or non-swollen but without flanges. Cheilstome relatively long; guide ring single, anteriorly situated (at most at 60% of odontostyle length from anterior end); compensation sacs present. Amphidial fovea variable: elongate funnel-shaped or stirrup-shaped, its aperture usually a large transverse slit. DN most generally some distance from its orifice; VSN most generally more developed than dorsal one. Male with an adanal pair of pre-cloacal supplements plus a ventromedian series of supplements without hiatus between the adanal pair and the series. Female reproductive system didelphic–amphidelphic; uterus without sclerotized structures. Tail short, rounded; may be conoid or hemispheroid. Four juvenile stages with appearance largely similar to females except for tail. First-stage juveniles with rather long conoid tail, becoming relatively shorter (higher c-value) in later developmental stages but not in absolute length. In one species, *P. duncani* Siddiqi, Baujard & Mounport (1993) only three juvenile stages recognizable by relative body length and length of functional and replacement odontostyles.

Type species: *Paralongidorus sali* Siddiqi, Hooper & Khan, 1963

The genus currently contains 72 species (14 *Longidoroides* species included). *Paralongidorus* can be distinguished from *Longidorus* mainly by the slit-like amphidial aperture and the stirrup-shaped fovea. Differentiation of *Paralongidorus* species is based on the following features: (i) shape of amphidial fovea; (ii) width of amphidial aperture; (iii) shape and width of lip region; (iv) tail shape in female; (v) length of odontostyle; (vi) body length; (vii) tail length; (viii) ratios a, c'; (ix) distance of guide ring from anterior end; (x) length of spicule; (xi) number of ventromedian pre-cloacal supplements; and (xii) males known or unknown (Escuer and Arias, 1997). A polytomous identification key to the genus was provided by Escuer and Arias (1997). One species, *P. maximus*, transmits a nepovirus, RRSV. *Paralongidorus maximus* shows a restricted distribution in Western and Eastern Europe but can be locally widespread (Germany) where it is often associated with vineyards; it was also recorded from South Africa in association with grapevine (Swart *et al.*, 1996). The species was also found in association with pine and river banks. *Paralongidorus maximus* has a preference for sandy and loamy-sand soils, showing highest densities between 20 and 40 cm; in Slovakia its occurrence was associated with soils derived from calcareous paternal rock (Liškova *et al.*, 2003). As a plant parasite, it is broadly polyphagous, attacking a wide range of woody and herbaceous plants.

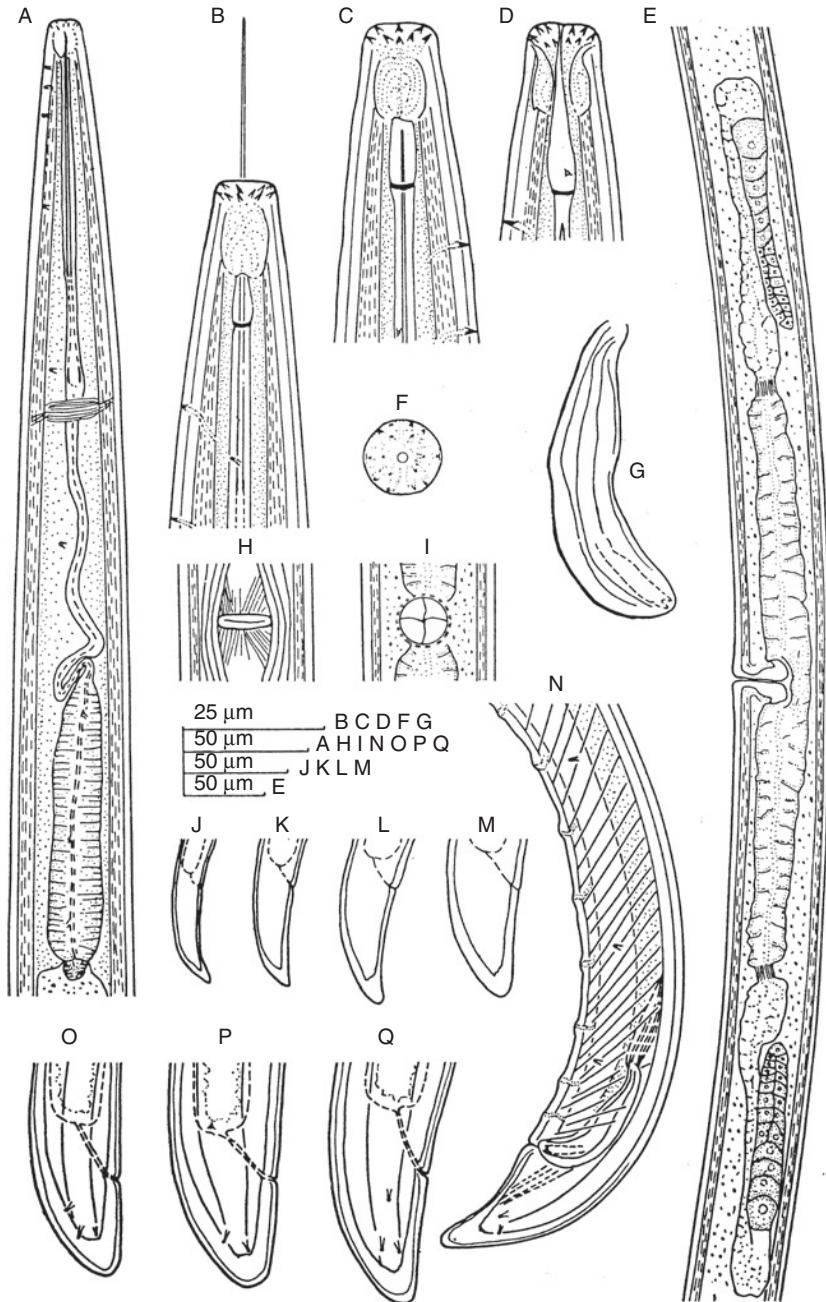


Fig. 6.9. *Longidorus elongatus*. A, pharyngeal region, dorsolateral; B–D, head region in surface view: lateral view of neotype (B), Scottish female, respectively in lateral (C) and ventral view (D); E, female reproductive system; F, lip region, *en face*; G, spicule and lateral guiding piece; H and I, ventral view of vulva and vagina, respectively; J–M, juvenile tails from first-stage juvenile (J) to fourth-stage juvenile (M); N, posterior body region male; O–Q, female tails, respectively of neotype, topotype and other specimen. (After Hooper, 1961, courtesy *Nematologica*.)

Paralongidorus maximus (Bütschli, 1874) Siddiqi, 1964 (Fig. 6.10)

Adults 7–12 mm long, heat-relaxed body ventrally curved in an open spiral. Lip region rounded, expanded and offset by deep constriction. Amphidial fovea stirrup-shaped, its wide slit-like opening two-thirds of lip region width. Odontostyle

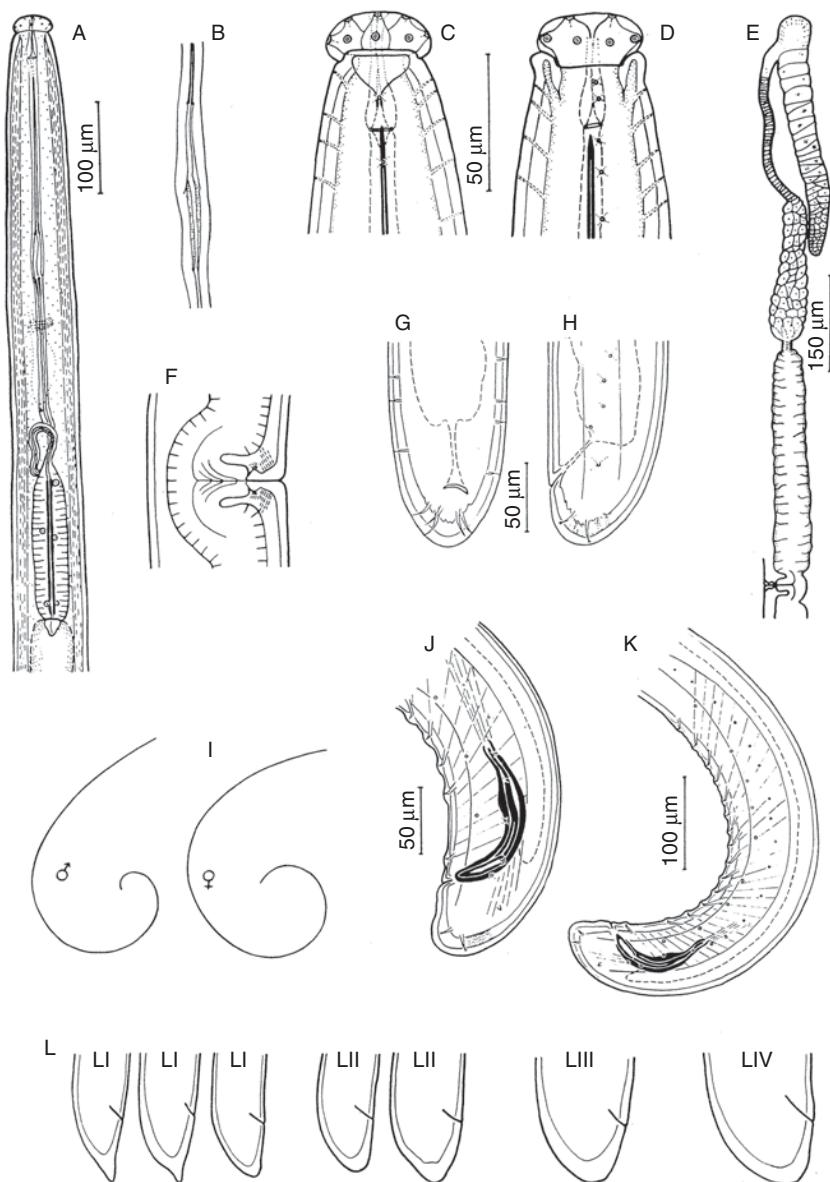


Fig. 6.10. *Paralongidorus maximus*. A, pharyngeal region; B, odontophore; C and D, head region in lateral view and dorsoventral view, respectively; E, anterior genital branch of female reproductive system; F, vagina region; G and H, female tail ventral view and view of left side, respectively; I, habitus of male and female upon thermal death; J and K, posterior body region of male; L, tails in lateral view of the four juvenile stages. (After Sturhan, 1963.)

152–187 µm long, odontophore 42–71 µm (female); guide ring far anteriorly (37–47 µm from anterior end). Tail in female short, bluntly rounded, about half the anal body width long. Males extremely rare. Spicules strong, 100–106 µm, lateral guiding pieces 32–35 µm; an adanal pair and a ventral series of 14–15 pre-cloacal supplements.

Longidoroides Khan, Chawla & Saha, 1978 (Fig. 6.8C)

Longidoroides Khan, Chawla & Saha, 1978, is characterized by: (i) the lip region usually continuous with the body, exceptionally expanded; (ii) a pouch-like usually bilobed amphidial fovea (the main difference from *Paralongidorus sensu stricto*); and (iii) its aperture a medium-sized to very small (1 µm) transverse slit. Fifteen species have been described, although no new species have been recorded since Escuer and Arias (1997) who accepted the synonymization with *Paralongidorus*. In the few data available, four juvenile stages occur as in *Paralongidorus*. Currently no *Longidoroides* species is known to transmit a nepovirus.

6.5.1.1.2. XIPHIDORINI (FIG. 6.11B–D). Longidoridae with a forked odontostyle, an odontophore with weakly developed flanges. Guide ring single, located at level of posterior third of odontostyle in resting position, compensation sacs lacking. Amphidial fovea variable in shape as well as the amphidial aperture.

The tribe contains three genera: *Xiphidorus* (eight species), *Paraxiphidorus* (three species) and *Australodorus* (one species). The geographic distribution is largely restricted to Central and South America. Currently, they do not appear to be of major economic importance and virus transmission has not been detected.

6.5.1.2. Xiphinematinae (Fig. 6.11A)

This subfamily has only one genus, *Xiphinema*. It is the oldest and most diversified genus of the family Longidoridae (Coomans, 1996; Coomans *et al.*, 2001). Within the genus 260 species are recognized as valid. They are divided into two species groups, the *Xiphinema americanum* group (54 spp.) and the non-*americanum* group. The genus includes in both species groups several economically important species, in particular vector species of nepoviruses: six virus vectors belong to the *X. americanum* group (*X. americanum*, *X. bricolense*, *X. californicum*, *X. rivesi*, *X. intermedium* and *X. tarjanense*) and three vector species to the *Xiphinema* non-*americanum* species group (*X. diversicaudatum*, *X. index* and *X. italiae*).

6.5.1.2.1. XIPHINEMA AMERICANUM GROUP (FIG. 6.12). *Xiphinema americanum* Cobb, 1913, the type species of the genus, was long considered to have a worldwide distribution. However, it is now regarded as a species complex of 51 putative species (Lamberti *et al.*, 2000), of which *X. americanum sensu stricto* is widespread in eastern USA, and also occurs in Arkansas, California and South Africa. Identification within the *X. americanum* group is of particular importance for phytosanitary regulation (see Chapter 12), as in North America these nematodes are natural vectors of four economically important nepoviruses that cause substantial damage to a wide range of crops. The identification of species within the *X. americanum* group is problematic as a result of general similarity of the morphology of the putative species.

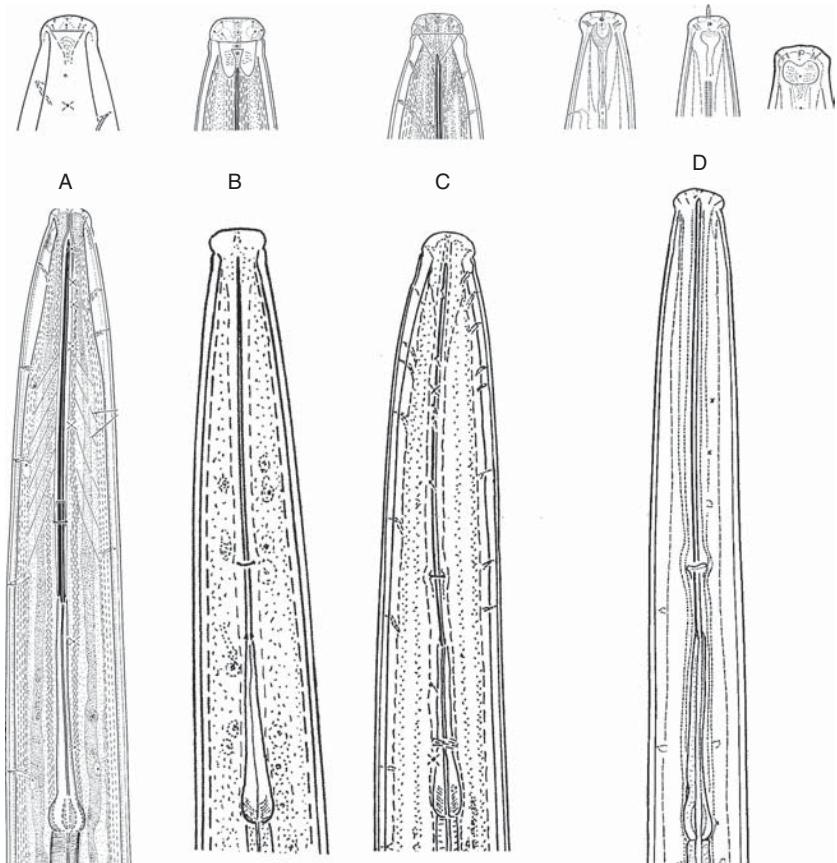


Fig. 6.11. Generic comparison of head and odontostyle region of Longidoridae (except the Longidorini). A, *Xiphinema*; B, *Australodorus*; C, *Paraxiphidorus*; D, *Xiphidorus*. Not to scale. (After various authors.)

The species of *Xiphinema americanum* *sensu lato* are characterized by: (i) adults with a more or less open C to spiral upon fixation or heat killing; (ii) body length small (<3 mm); (iii) lip region rounded, continuous with the body and marked by a depression, minor expansion or a constriction; (iv) odontostyle rarely longer than 150 µm; (v) female reproductive system didelphic, amphidelphic with rather short non-differentiated uteri, weakly developed sphincter muscles and oocytes usually with endosymbiotic bacteria, vulva between 42 and 65% from anterior end; (vi) tail short conoid with a more or less acute terminus, sometimes subdigitate; and (vii) male rare, female devoid of sperm. Four juvenile stages but species with three stages occur. The latter are widely distributed in the Americas but occur rarely in Africa, Europe and Asia (Lamberti *et al.*, 2000). Lamberti and Ciancio (1993) distinguished five species subgroups based on a hierarchical cluster analysis of morphometrics, among them a *Xiphinema pachtaicum* group; the latter also included *X. pachydermum*. There is no general agreement on the definition of the *Xiphinema americanum* species group morphologically as well as molecularly, especially with respect to the position

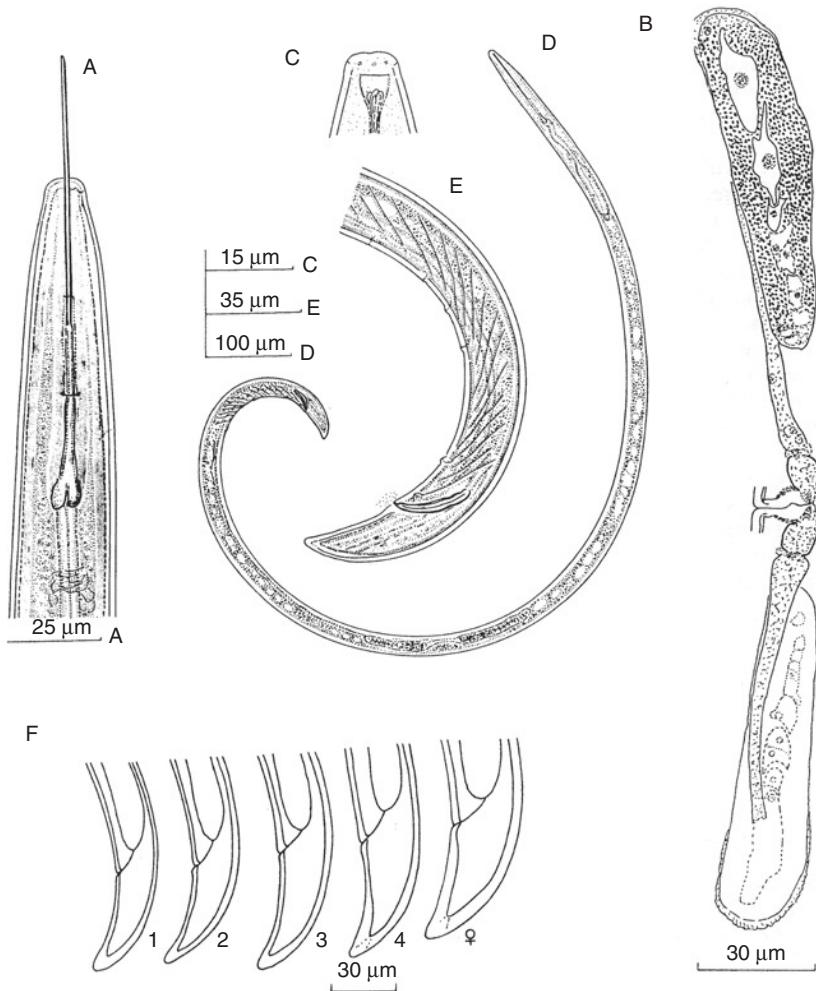


Fig. 6.12. *Xiphinema americanum*. Female: A, pharyngeal region; B, reproductive system. Male: C, head in surface view; D, total view; E, posterior body region. F, tail ends of the four juvenile stages (1–4) and female. (A, C–E, after Siddiqi, 1973; B, after Coomans and Claeys, 2001; F, after Coomans *et al.*, 2001.)

of *X. pachydermum* and related (mostly Portuguese) species. The latter differ from the typical *X. americanum* group in females which possess ovaries without associated symbiotic bacteria (except in *X. mesostilum*), a well-developed sphincter muscle vs weakly developed in the *X. americanum* species group, clearly longer uteri and males common (Luc *et al.*, 1998; Coomans *et al.*, 2001). Molecular analyses showed two groups within the *X. americanum* group but the *X. pachydermum* group does not cluster separately and includes some other species such as *X. pacataicum* (He *et al.*, 2005; Gutiérrez-Gutiérrez *et al.*, 2011).

In the polytomous identification key of the *X. americanum* species group (Lamberti *et al.*, 2004), the following characters are used: (i) odontostyle length; (ii) V%; (iii) value of ratio c' and c; (iv) body length; (v) tail length; (vi) shape of tail

terminus; and (vii) lip region continuous or offset from the body. Two additional characters could be added: (i) lip region marked by a depression; and (ii) the length of the genital branch/uterus in the female. Attention should be paid to the different use of characters and coding in the different polytomous identification keys (see also Lamberti *et al.*, 2000).

The *X. americanum* group includes the following vector species for four economically important nepoviruses with quarantine status IAI (EPPO, 1997) and naturally occurring in the USA: cherry rasp leaf (CRLV), peach rosette mosaic (PRMV), tobacco ringspot virus (TRSV) and tomato ringspot virus (ToRSV): *X. americanum* s.s. (CRLV, TRSV, ToRSV), *X. americanum* s.l. (CRLV, PRMV, TRSV, ToRSV), *X. bricolense* (ToRSV), *X. californicum* (CRLV, TRSV, ToRSV), *X. rivesi* (CRLV, TRSV, ToRSV), *X. intermedium* (TRSV, ToRSV), *X. tarjanense* (TRSV, ToRSV) (Lamberti *et al.*, 2000).

X. americanum has been recorded worldwide but often as *X. americanum sensu lato* and consequently these identifications need to be confirmed. *Xiphinema americanum* is widespread in North America but particularly in the eastern region. *Xiphinema americanum s. str.* has also been recorded from South Africa and the identification confirmed (see Lamberti *et al.*, 2000). The species prefers well-drained sandy soils but also occurs in clay soils; optimum soil pH ranged from 5.6 to 7.4. *Xiphinema americanum* can be found up to 60 cm depth with highest densities usually in the upper 15 cm. The highest population occurs in the autumn months or early winter months in California and high organic matter reduces the population. The species has a wide host range including woody and herbaceous plants, cereals, cherry, citrus, grapevine, strawberry, maize, tomato, tobacco, soybean and many others (Siddiqi, 1973). Marigold was found to support a 75-fold increase in population of *X. americanum* in 5 years, whilst millet, hairy indigo and cotton resulted in less than a 10-fold increase. By contrast, Sudan grass, crotalaria and beggar weed prevented multiplication (Brodie *et al.*, 1970).

6.5.1.2.2. XIPHINEMA NON-AMERICANUM GROUP: XIPHINEMA INDEX (FIG. 6.13). Xiphinematinae. Body length 1.2–7.5 mm, body straight to spiral. Lip region varying from well offset and knob-like to continuous with body contour, high to low. Amphids with stirrup- or funnel-shaped, rarely goblet-shaped, fovea with wide (40–90% of central body width) slit-like aperture, rarely narrower. Base odontostyle forked; base odontophore with sclerotized flanges. Cheilstome long, ending around posterior third of odontostyle; guide ring always double due to folding of guiding sheath anteriorly beyond guide ring. *Dilatores buccae* or cheilstome retractor muscles connecting cheilstome to dorsal and ventral body wall. True stylet retractor muscles degenerated, pharyngeal retractor muscles composed of three bands extending from near base of odontophore and splitting into four muscles attaching to body wall opposite anterior part of pharyngeal bulb. DN large, located at same level as its dorsal orifice (DO), more developed than the two nuclei of VSN. Pre-rectum well developed. Female reproductive system varying from typical didelphic–amphidelphic to various transitions (pseudomonodelphic) to complete mono-opisthodelphic; uterus varying from short and undifferentiated to long and differentiated into several parts and in many species provided with sclerotized structures (crystalloids, spines, Z- or pseudo Z-differentiation). Males diorchic, spicules dorylaimoid and gubernaculum reduced

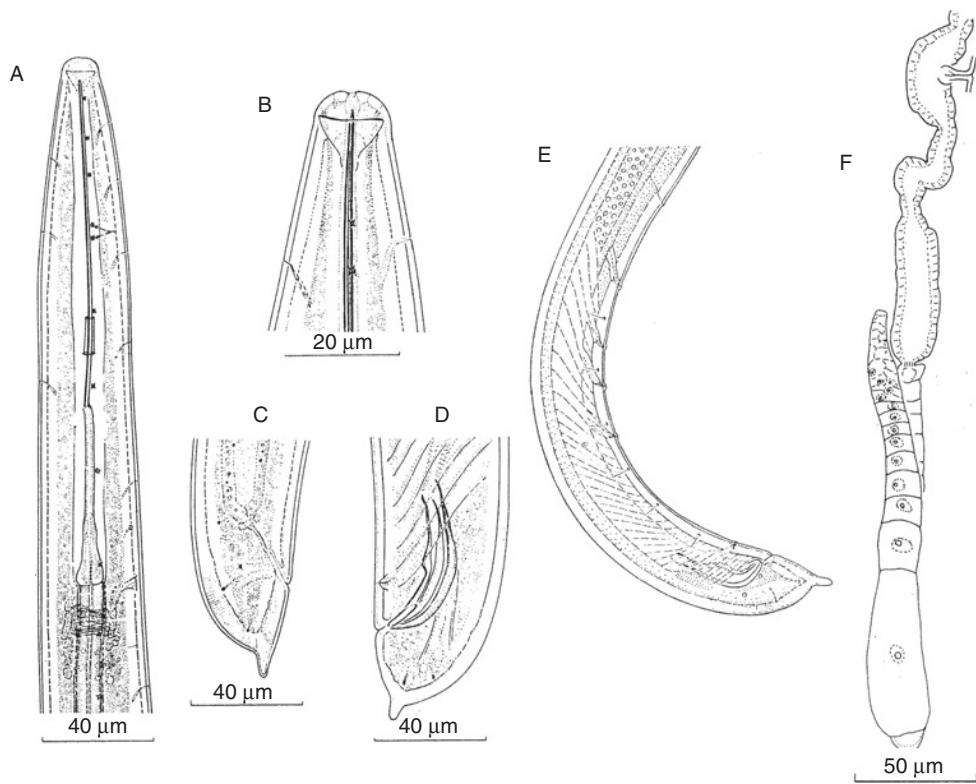


Fig. 6.13. *Xiphinema index*. A, pharyngeal region; B, head region, surface view; C, female tail; D, male tail region and copulatory apparatus; E, male posterior body region; F, female reproductive system, posterior branch. (From Siddiqi, 1974c.)

to sclerotized lateral guiding pieces or crura. Male genital pre-cloacal supplements consisting of an adanal pair followed by a hiatus and a ventromedian series varying from 1 to 11 (usually 2–5), mostly situated outside the range of retracted spicules, rarely absent. Tail shape varying from elongate filiform to short bluntly rounded; tails of both sexes similar or showing sexual dimorphism in some species. In about 40% of the species, males are abundant and reproduction is amphimictic. In 25% of *Xiphinema* species males are rare and in 35% of the species males are unknown; in both cases reproduction probably occurs through parthenogenesis. Four juvenile stages but species with three stages occur.

Species in the non-*americanum* group are subdivided into eight species groups based upon the following characters: (i) absence of anterior genital branch; (ii) absence of anterior ovary; (iii) anterior genital branch present but reduced; (iv) two equally developed genital branches with presence of a Z-organ; (v) as in (iv) but with a pseudo Z-organ with or without uterine spines; (vi) similar as in (iv) but without Z-organ or other uterine sclerotized structures; (vii) as in (iv) but uterus not differentiated and tail elongate to conical; and (viii) as in (vii) but tail short, rounded. Additional to these characters, species identification is also based on: (i) ratios c' and c; (ii) the presence or absence of a terminal canal in the cuticle at the tail tip; (iii) V%;

(iv) body length; (v) length of complete stylet; (vi) outline of lip region; (vii) habitus; (viii) presence of males; and (ix) tail shape in first- and fourth-stage juveniles (Coomans *et al.*, 2001).

The non-*americanum* species group includes several economically important species, in particular the three virus vector species, among them *Xiphinema index*. This species occurs worldwide and its distribution reflects the distribution of its major host, the grapevine, and includes almost every viticulture region in Africa, Australia, Europe, South America and the USA. *Xiphinema index* has been dispersed from the Middle East in soil adhered to grapevine propagation material and has become an invasive agricultural soil-borne pathogen. Recent studies showed that *X. index* is widespread in Spain and causes significant damage to grapevines; it occurs in a wide range of soil types (including heavy soils) but it has a preference for light- to medium-textured soils. Under controlled conditions, *X. index* had a reproduction factor higher than one in all Spanish commercial rootstocks. Difference in reproduction was influenced by the rootstock and decreased by about 12-fold with the increase in inoculum density from 100 to 1000 nematodes per plant (Gutiérrez-Gutiérrez *et al.*, 2011). Apart from grapevine, the species also attacks various woody plants, including citrus, fig, mulberry, *Prunus* spp. and *Pyrus* spp. By direct feeding, *X. index* causes necrosis, terminal galling and lack of lateral root development. The species transmits grapevine fanleaf virus (GFV). Males are extremely rare and reproduction is by meiotic parthenogenesis. The life cycle of *X. index* has been recorded as short as 22–27 days at 24°C in California and up to 7–9 months at 20–23°C in Israel.

Xiphinema index Thorne and Allen (1950) (Fig. 6.13)

Body about 3 mm long, forming an open spiral. Lip region hemispherical and except for a slight depression almost continuous with the body. Amphidial fovea stirrup-shaped with wide transverse slit-like aperture, about as long as the lip region width. Odontostyle 126 µm long (average), odontostyle 70 µm and provided with large flanges. Guide ring at 108 µm (average) from anterior end. Female reproductive system didelphic–amphidelphic, vulva at 38–40% of total body length from anterior end, uterus differentiated but without Z-organ. Tail 1–1.3 anal body widths long, convex-conoid with a digitate ventral or terminal peg, 9–13 µm long and inner protoplasmic core not extending into peg; peg may be absent. Males extremely rare, spicules 63 µm long; apart from adanal pair of pre-cloacal supplements, a ventromedian series of seven pairs of supplements outside the range of the retracted spicules.

6.5.2. The family Trichodoridae (Fig. 6.14)

Triplonchida. Diphtherophoroidea. Body straight cigar-shaped or J-shaped. Lip region rounded, continuous with body; lips amalgamated. Anterior sensilla papilliform and arranged in two circlets, an anterior circlet of six inner labial papillae and an outer circlet of ten papillae, the two subventral and two subdorsal outer labial papillae and four cephalic papillae form a kind of protruding double papillae. Amphidial fovea post-labial, cup-shaped and aperture a medium-sized transverse slit. Onchiostyle relatively long, ventrally curved stylettiform dorsal tooth; guide ring simple, at level of posterior end of onchiostyle. Pharynx with swollen posterior glandular bulb; five gland nuclei present: one posterior ventrosublateral pair, one smaller

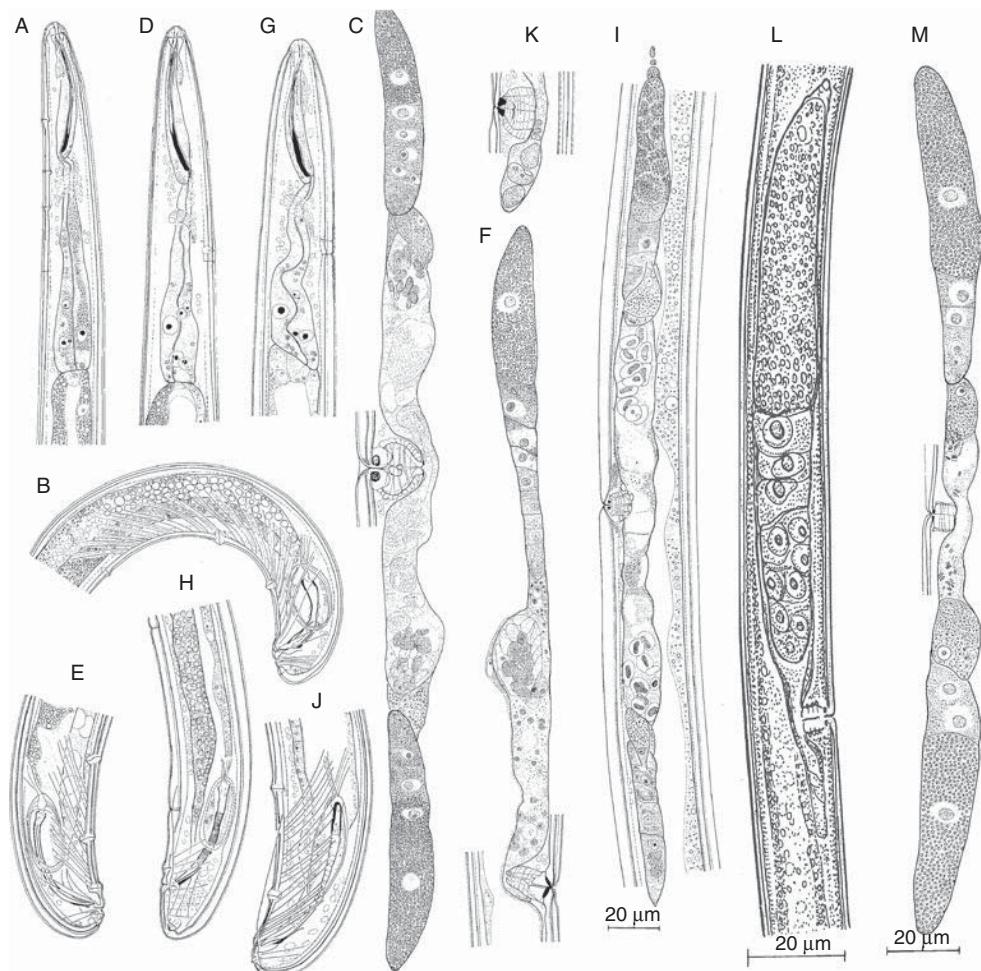


Fig. 6.14. The genera of the Trichodoridae. Anterior body region in male: A, *Trichodorus velatus*; D, *Monotrichodorus m. vangundyi*; G, *Paratrichodorus weischeri*. Male posterior body region: B, *T. velatus*; E, *M. m. vangundyi*; H, *P. pachydermus*; J, *Allotrichodorus campanulatus*. Female reproductive system: C, *T. taylori*; F, *M. m. vangundyi*; K, *A. campanulatus*, vulva region; I, *P. weischeri*; L, *Ecuadorus mexicanus*; M, *Nanidorus minor*. Scale bars = 20 µm. (A–K, after Decraemer, 1991, courtesy of Marcel Dekker Inc.; L, after Siddiqi, 2002; M, after Decraemer, 1995).

anterior ventrosublateral pair, and a single large dorsal nucleus. Intestine without pre-rectum, rectum almost parallel to longitudinal body axis; anus subterminal in female. Male with a single outstretched anterior testis, sperm cells variable in shape and size; 1–4 pre-cloacal supplements, one pair (rarely two) of post-cloacal papillae; weakly developed caudal alae present or absent; spicules straight to ventrally curved, variable in shape and ornamentation. Spicule protractor muscles transformed in a capsule of suspensor muscles surrounding the spicular pouch. Female reproductive system didelphic-amphidelphic or monodelphic-prodelphic, ovary (ovaries) reflexed; oviduct consisting of two finely granular cells; spermatheca(e) present or absent.

Tail short, maximum length one anal body width in male. Apparently four juvenile stages but first-stage juveniles have not been recovered from field samples and are known only from laboratory cultures. Type genus: *Trichodorus* Cobb, 1913.

The Trichodoridae includes 108 species and six genera: *Trichodorus* Cobb, 1913 (type genus), *Allotrichodorus* Rodriguez-M, Sher & Siddiqi, 1978, *Ecuadorus* Siddiqi, 2002, *Monotrichodorus* Andrassy, 1976, *Nanidorus* Siddiqi, 1974 and *Paratrichodorus* Siddiqi, 1974 (Fig. 6.14). The status of *Atlantadorus* introduced by Siddiqi (1974d) at subgenus level within *Paratrichodorus* and raised by him in 1980 to genus level is still under discussion (Decraemer, 1995; Duarte *et al.*, 2010).

The three largest genera, *Trichodorus* (62 species), *Paratrichodorus* (26 species) and *Nanidorus* (seven species), are didelphic and occur worldwide. The three remaining genera, *Monotrichodorus*, *Allotrichodorus* and *Ecuadorus*, have fewer species: *Monotrichodorus* with eight nominal species of which four are accepted species and one subspecies, *Allotrichodorus* with six species and *Ecuadorus* Siddiqi, 2002 with two species. These genera are monodelphic–prodelphic. They have only been recorded from Central America and the northern part of South America.

The main diagnostic characters for genus identification are: (i) female reproductive system (didelphic or monodelphic); (ii) length of vagina and size of vaginal sclerotized pieces; (iii) presence of advulvar lateral body pores; (iv) presence or absence of caudal alae in male; and (v) degree of development of copulatory muscles and related habitus and capsule of spicule suspensor muscles.

6.5.2.1. *Trichodorus Cobb, 1913: T. similis (Fig. 6.15)*

Trichodoridae. Cuticle usually not or slightly swollen after fixation, heat killing or fixatives. Pharynx usually with offset bulb, more rarely with short ventral overlap of pharyngeal glands or dorsal intestinal overlap. Secretory–excretory (S–E) pore usually at level of narrow part of pharynx or anterior part of pharyngeal bulb. Female reproductive system didelphic–amphidelphic; spermathecae present, rarely absent; vagina well developed, usually about half the corresponding body width long, variable in shape; vaginal constrictor muscle well developed; vaginal sclerotized pieces in lateral view conspicuous, variable in shape and size. Vulva a pore, a transverse slit, or rarely, a longitudinal slit. One to four lateral body pores present on each side, rarely absent; one post-advulvar pair (i.e. within one body width posterior to the vulva) present, rarely absent. Male common. One to three ventromedian cervical papillae present in neck region; rarely four or none. Lateral cervical pores usually present, rarely absent; usually one pair near onchiostyle base or just behind nerve ring. Sperm cells large, with large sausage-shaped or rounded nucleus. Spicules ventrally arcuate, rarely straight, usually with ornamentation (bristles, transverse striae or velum) or smooth. Capsule of spicule suspensor muscles well developed. Oblique copulatory muscles well developed, extending far anterior to spicular region, causing upon contraction the typical J-shaped ventral curvature of posterior end in male. Caudal alae absent, exceptionally present (*T. cylindricus* Hooper, 1962 and *T. paracedarus* Xu & Decraemer, 1995). Three pre-cloacal ventromedian supplements, rarely two, four or five supplements, more or less evenly spaced, usually one within range of retracted spicules. Tail with one pair of post-cloacal sub-ventral papillae and one pair of caudal pores. Type species: *T. obtusus* Cobb, 1913. Syn. *T. proximus* Allen, 1957.

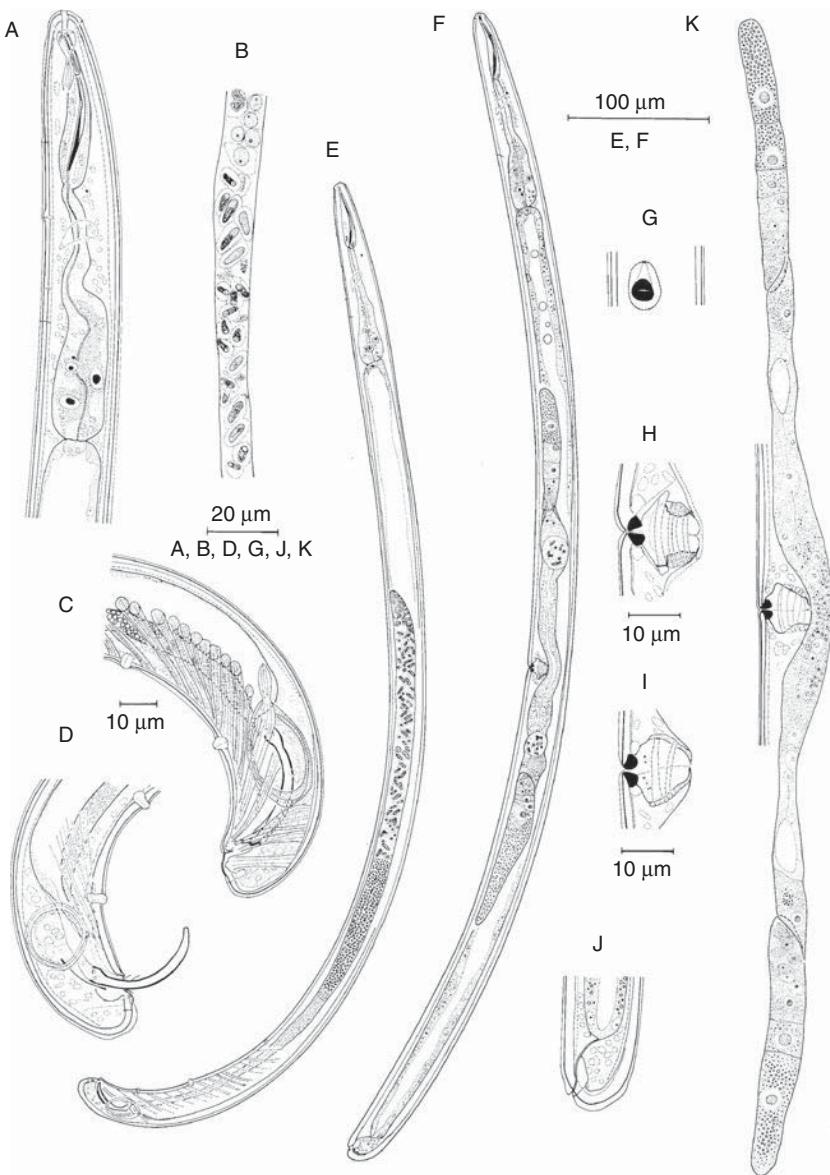


Fig. 6.15. *Trichodorus similis*. Male: A, pharyngeal region; B, sperm in testis; C and D, posterior body region; E, total view. Female: F, total view; G, ventral view; H and I, vaginal region; J, tail region; K, reproductive system. (After Decraemer, 1991, courtesy Marcel Dekker Inc.)

The main morphological features for species identification are, in the female, the shape and size of the vaginal sclerotized pieces and, in the male, the spicule shape; other characters are body length, onchiostyle and spicule length, number and position of pre-cloacal supplements and ventromedian cervical papillae in male.

Four *Trichodorus* species naturally transmit tobacco rattle virus (TVR) and/or pea early browning virus (PEBV) (see Section 6.7). *Trichodorus similis* is an important

plant pathogen that occurs in temperate and cooler regions of Europe (west, east and north); few records are known from the USA. The species is most widespread in sandy or sandy loam soils, with highest densities at between 20 and 30 cm depth. It shows a high tolerance to low acid soils (pH 5–6). In Belgium, for example, it is common under arable crops, pastures, grasses along roadsides and, to a lesser extent, in deciduous woodland, but is rare in coniferous woods (De Waele and Coomans, 1991). Reproduction is amphimictic and under optimum conditions the life cycle is completed in 45 days. *Trichodorus similis* causes direct damage to the root system of carrot, potato, onion, sugar beet, tobacco and trees such as *Prunus* (plums, cherry), *Juglans* (walnut) and *Picea* (spruce). It also acts as a vector of TRV to potatoes and *Gladiolus*.

Trichodorus similis Seinhorst, 1963 (Fig. 6.15)

Body J-shaped in male, about straight in female; 550–1060 µm long. Onchiostyle length, 35–52 µm. Males with three ventromedian cervical papillae anterior to the S-E pore, anteriormost papilla opposite the posterior onchiostyle region. Sperm cells with large sausage-shaped nucleus. Spicules, ventrally curved, with offset rounded manubrium and blade rather stout, tapered distally, without ornamentation except for a few bristles (only clearly visible when spicules protruded). Three ventromedian pre-cloacal supplements, the posterior one opposite or just anterior to the head of the retracted spicules. Females with rhomboid-shaped vagina, about half corresponding body width long; vaginal sclerotized pieces large rounded triangular with tip pointed to vulva; vulva a transverse slit in ventral view; spermathecae present.

6.5.2.2. *Paratrichodorus Siddiqi, 1974: P. pachydermus (Seinhorst, 1954)*

Syn. *Atlantadorus* Siddiqi, 1974.

Trichodoridae. Cuticle usually clearly swollen when fixed or heat-killed. Ventral pharyngeal and/or dorsal intestinal overlap usually present, bulb rarely offset. Female reproductive system didelphic–amphidelphic; spermathecae present, or absent with sperm throughout uterus. Lateral body pores present in about 50% of the species but rarely located advulvar; body pores exceptionally ventral or sub-ventral in position. Vagina short (about one-third of corresponding body diameter), constrictor muscles inconspicuous, vaginal sclerotization small to inconspicuous in lateral view. Vulva a pore or a longitudinal slit. Male rare or unknown. Ventromedian cervical papillae in male usually one papilla near the S-E pore, exceptionally with two. Lateral cervical pores absent or, if present, usually one pair near onchiostyle base or S-E pore. Male tail region straight; caudal alae present, obscure to distinct. Sperm cells variable, large with large sausage-shaped nucleus, medium-sized with rounded nucleus, small with small oval to rounded nucleus or thread-like (degenerated) with nucleus obscure, sperm exceptionally long, fusiform with elongated nucleus. Oblique copulatory muscles poorly developed and restricted to spicule region; capsule of spicule suspensor muscles inconspicuous. Spicules usually straight and blade about equally wide and often finely striated; no bristles or velum. Two or three ventromedian pre-cloacal supplements, exceptionally four; usually two supplements within spicule region. Usually one pair, rarely two, sub-ventral post-cloacal papillae. A pair of caudal pores

present. Type species: *Paratrichodorus tunisiensis* (Siddiqi, 1963) Siddiqi, 1974. Species identification is based upon the same characters as for *Trichodorus*. Additional features are the presence or absence of a spermatheca, the size and shape of sperm cells and nuclei and to a lesser extent the type of pharyngo-intestinal junction.

Within the genus *Paratrichodorus*, seven species are vectors of tobaviruses (see Section 6.7); among them, *P. pachydermus* acts as a vector of TRV and PEBV. *Paratrichodorus pachydermus* is common in sandy to light sandy loam soils but occurs occasionally in loam and peat soils, usually between 15 and 70 cm depth (De Pelsmaeker *et al.*, 1985); it can withstand both drought and waterlogged conditions. Reproduction is amphimictic. *Paratrichodorus pachydermus* is polyphagous, associated with a wide range of crops, trees and flowers.

Paratrichodorus pachydermus (Seinhorst, 1954) Siddiqi, 1974 (Fig. 6.16)

Body straight in male and female, 600–1020 µm long. Onchiostyle 44–60 µm. Pharyngo-intestinal junction with anteriorly directed dorsal overlap by intestine over pharyngeal bulb. Males with a single ventromedian cervical papilla anterior to the S–E pore and at level of isthmus. Sperm with large sausage-shaped nucleus. Spicules straight or posteriorly slightly ventrally curved, blade transversely striated, manubrium smooth, not offset. Three ventromedian pre-cloacal supplements, two well-separated supplements within region of retracted spicules. Tail long for the genus (about one anal diameter), a well-developed pair of post-cloacal supplements sub-terminal, near caudal pores and a pair of ventrosubmedian minute papillae or pores just post-anally. Bursa narrow, extending from level head of retracted spicule to sub-terminally on tail. Females with barrel-shaped vagina and small, rounded triangular vaginal sclerotized pieces slightly separated. Sperm distributed over uterus. Two to five lateral body pores on each side posterior to the vulva.

6.5.2.3. *Nanidorus* Siddiqi, 1974: N. minor

Based upon recent additional molecular support, *Nanidorus* is now generally accepted as a valid genus (Duarte *et al.*, 2010). Morphologically it is largely similar to *Paratrichodorus* but can be differentiated from the latter in the female by: (i) the vulva with more posterior position (60–64% vs <60% in *Paratrichodorus*); (ii) the transverse slit-like vulva opening vs a pore or longitudinal slit; and (iii) non-functional striated sperm, rarely small rounded, spread along the uteri instead of more diverse in shape and size and either in a spermatheca or spread along the uteri in *Paratrichodorus* and in male by the single pre-cloacal supplement vs 2–4 in *Paratrichodorus*. Males are rare in *Nanidorus* (only known in three out of seven species), lack ventromedian cervical papillae and caudal pores are absent in male and female vs present in *Paratrichodorus*.

The genus *Nanidorus* includes one of the most important trichodoridae plant pathogens, *N. minor*, which has been recorded mainly from tropical and subtropical regions worldwide, causing not only substantial damage by direct feeding on the root system but also as a vector for TRV in the USA and pepper ringspot virus (PRV) in South America; it has occasionally been found in southern Europe. *Nanidorus minor*

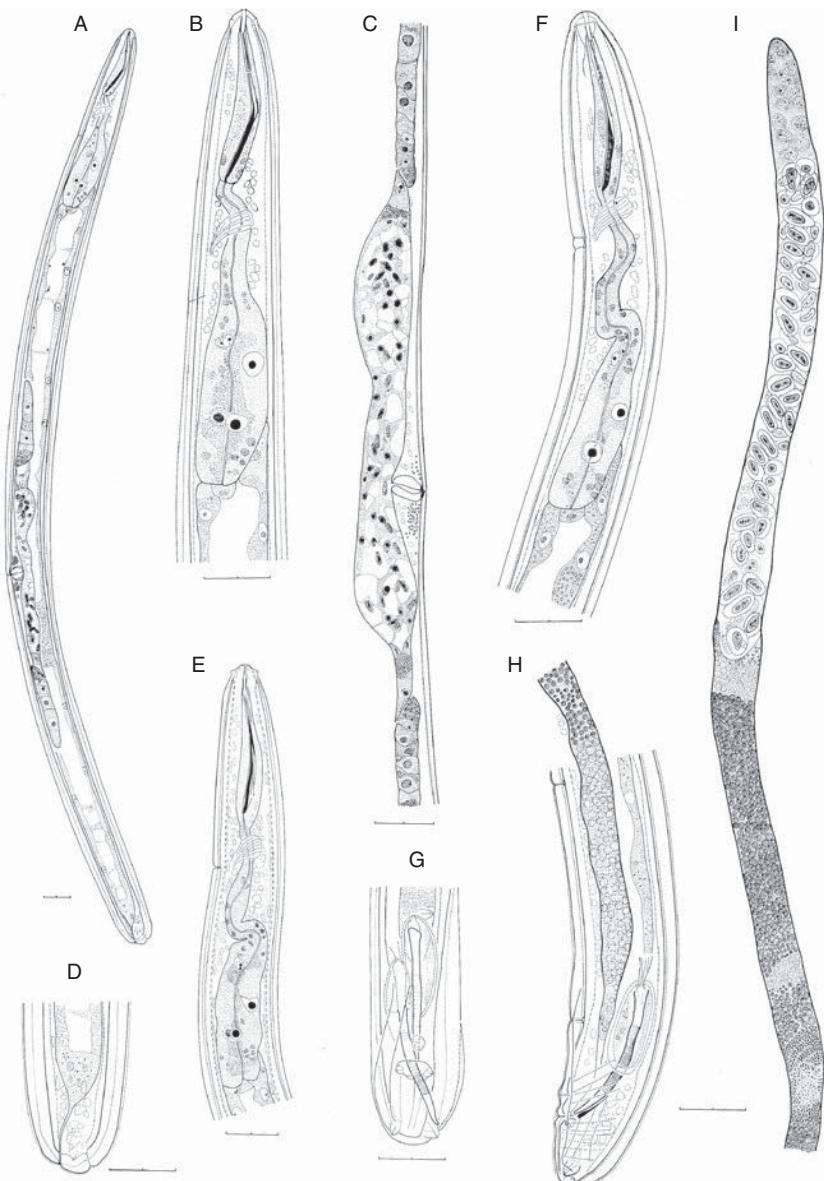


Fig. 6.16. *Paratrichodorus pachydermus*. Female: A, total view; B, anterior body region; C, reproductive system; D, tail. Fourth-stage juvenile: E, anterior body region. Male: F, anterior body region; G, ventral view of copulatory apparatus and tail; H and I, reproductive system and tail. Scale = 20 μm (After Decraemer, 1991, courtesy Marcel Dekker Inc.)

is common in sandy and sandy loam soils in warm climates and is also considered not to be endemic to South America. It has been associated with over 100 plant species including economically important crops such as sugar cane, walnut and a wide range of vegetables. On tomato its life cycle is complete in 21–22 days at 22°C and in 16–17 days at 30°C.

Nanidorus minor (Colbran, 1956) Siddiqi, 1974 (Fig. 6.17)

Syn. *Trichodorus minor* Colbran, 1956; *Paratrichodorus (Nanidorus) minor* (Colbran, 1956) Siddiqi, 1974; *Trichodorus christiei* Allen, 1957; *Paratrichodorus (Nanidorus) christiei* (Allen, 1957) Siddiqi, 1974; *Nanidorus christiei* (Allen, 1957)

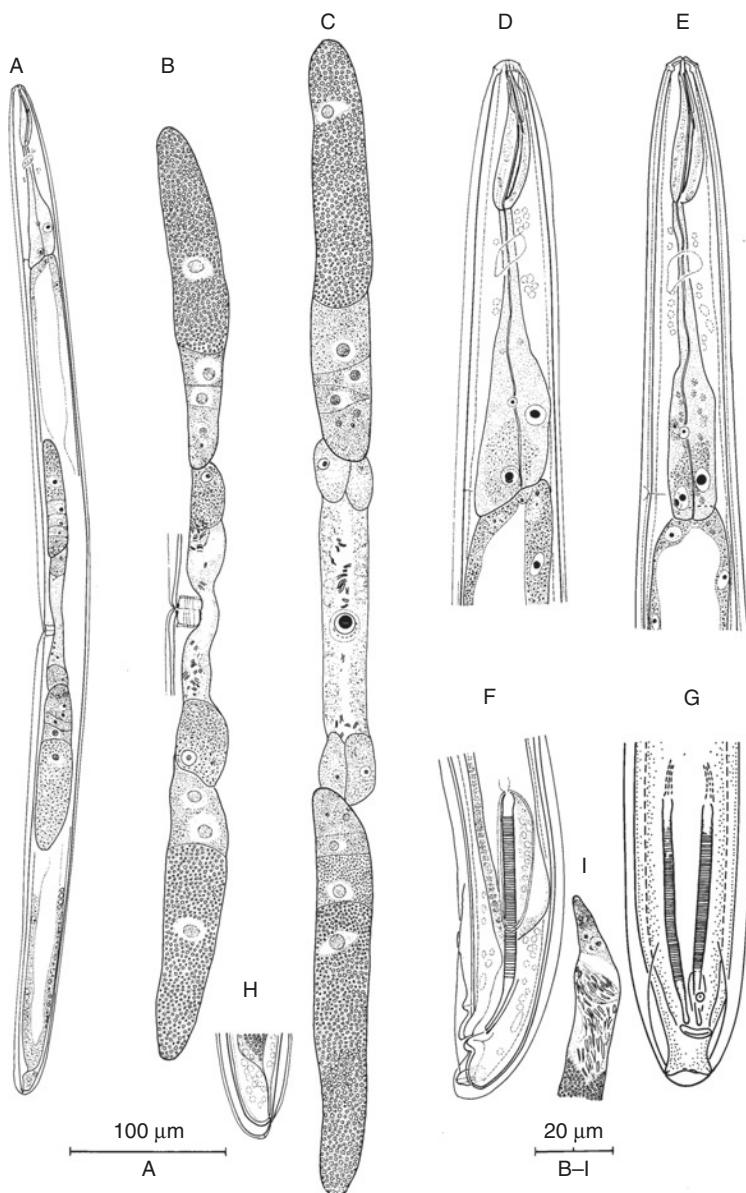


Fig. 6.17. *Nanidorus minor*. Female: A, total view (paratype *P. christiei*); B and C, reproductive system in lateral view and ventral view, respectively; D and E, anterior body region; F and G, male tail region and copulatory apparatus in lateral view and ventral view, respectively; H, female tail region; I, testis and vesicular seminalis. (After Decraemer, 1991, courtesy Marcel Dekker Inc.)

Siddiqi, 1974; *Trichodorus obesus* Razjivin and Penton, 1975; *Paratrichodorus (Nanidorus) obesus* (Razjivin and Penton, 1975) Rodriguez-M and Bell, 1978).

Female. Body straight, cigar-shaped, length 440–1530 µm (average between 490 and 700 µm). Onchiostyle length 26–47 µm, inner onchium (= replacement stylet) always present in adult. S–E pore near posterior end of pharyngeal bulb. Pharyngeal glands overlapping intestine ventrally and subventrally or bulb offset. Lateral body pores and caudal pores absent. Reproductive system without spermathecae; sperm cells appear degenerated; vagina small with typical quadrangular shape and small rod-shaped sclerotized pieces, parallel to longitudinal body axis and close together. Males extremely rare. Ventromedian cervical papillae and lateral cervical pores absent. Spicules long (48–73 µm), ventrally curved near distal tip. A single ventromedian pre-cloacal supplement near cloacal opening. Caudal alae extending from shortly anterior to pre-cloacal supplement to post-cloacal papillae. No caudal pores in male and female. Reproduction is parthenogenetic. Males are extremely rare except for a population in Argentina with 1:3 ratio male to female (Decraemer, 1995). This species has been recorded as a vector of TRV in the USA and PRV in South America.

6.6. Biochemical and Molecular Diagnostics

Molecular data are only available for a restricted number of species for tylench ectoparasites. The NCIB Genbank (May 2012) included molecular data for *Amplimerlinius* (two species), *Belonolaimus* (three species), *Dolichodorus* (one species), *Helicotylenchus* (11 species), *Hoplolaimus* (six species), *Rotylenchus* (18 species), *Tylenchorhynchus* (eight species) and *Criconemooides* (two species and additionally seven species under *Mesocriconema*). Sequence data have been obtained mostly from the partial 28S and the partial 18S rRNA gene and more limited from the complete 5.8 rRNA gene and internal transcribed spacers 1 (ITS1) and 2 (ITS2). Mitochondrial cytochrome c oxidase gene I (COI) was only available for *H. dihystera* and the partial cytochrome b (cytb) only for *M. curvatum* and *M. xenoplax* (now both in the genus *Criconemooides*). In addition, for *H. pseudorobustus* partial coding sequences, and sequence for heat shock protein 90 gene, are available in Genbank and for *Hoplolaimus stephanus* sequences of an actin-like gene are available.

Within Trichodoridae, molecular data in NCBI Genbank (May 2012) are only available for 25% of the species of the family (11 *Trichodorus*, two *Nanidorus* and ten *Paratrichodorus* species. Sequence data have been obtained mostly from partial 18S rRNA gene (16 species), the partial 28S rRNA gene and the complete 5.8 rRNA gene and ITS1 and/or ITS2. No information on the COI gene is available for trichodorids. Sequence data obtained for the 18S rDNA gene confirm the synonymy of *P. minor* (= *N. minor*) and *P. christiei* (Boutsika *et al.*, 2004). Duarte *et al.* (2010) developed a PCR–RFLP assay for the identification of species of the didelphic trichodoridae genera *Nanidorus*, *Paratrichodorus* and *Trichodorus* based on the variability of 18S SSU rDNA of Portuguese populations of 12 species and non-Portuguese populations of six of these species.

Within the Longidoridae, sequence data are available for 68 species of *Xiphinema* (27%), 48 species of *Longidorus* (31%), four species of *Paralongidorus* (5.5%) and four species of *Xiphidorus* (50%). As for the Trichodoridae, most sequence data are from the 18S rRNA gene (partial), the 28S rRNA and D1–D2 expansion segment

(partial) or the complete 5.8 rRNA gene and ITS1 and/or ITS2. Sequence for COI is available for 21 species of *Xiphinema*, the majority of the *X. americanum* group and for five species of *Longidorus*. For accurate identification of the quarantine nematode, *X. index*, sequence analyses of microsatellites that show high levels of polymorphism have been used as an effective diagnostic tool at species level (He *et al.*, 2003). The complex of cryptic species of the *X. pyrenaicum* group was resolved using nuclear ribosomal DNA genes (D2–D3 expansion segments of 28S, ITS1 and partial 18S) by Gutiérrez *et al.* (2011). Mitochondrial cytochrome c oxidase subunit 1 (cox1-mt DNA) served to check the taxonomic status of several populations of *X. brevicolle* in the Czech Republic (Kumari *et al.*, 2010). Gutiérrez-Gutiérrez *et al.* (2011) used this DNA region and the nuclear D2–D3 expansion segments of 28S rDNA to study the genetic structure of *X. pachtaicum* and *X. index* populations; both regions were considered suitable diagnostic markers for these species.

Phylogenetic relationships between *Xiphinema* and *Xiphidorus* species from Brazil, for example, have been inferred from 18S rDNA and appeared to match the taxonomic status of the taxa. Species of the *X. americanum* group formed a clade but within the group species separation was not always conclusive. The monophyly of the genus *Xiphidorus* was confirmed (Oliveira *et al.*, 2004). The phylogenetic relationship of *Longidorus* species based on the 28S gene has been studied extensively by He *et al.* (2005) and De Luca *et al.* (2004); the last study also included sequence data of ITS1–ITS2 + 5.8 – D1–D2 region. *Longidorus* appeared not to be exclusively monophyletic, as species of *Paralongidorus* were also included (He *et al.*, 2005).

6.7. Ectoparasitic Nematodes as Vectors of Plant Viruses

The most important pathogens associated with ectoparasitic nematodes are plant viruses. Longidoridae and Trichodoridae are the only families of plant-parasitic nematodes within the phylum Nematoda that can transmit plant viruses. Out of the 38 known nepoviruses, 13 have been shown to be naturally transmitted by longidorid nematodes, i.e. seven *Longidorus* species, one *Paralongidorus* and nine *Xiphinema* species, 5% of the total known species of the family Longidoridae. All three tobraviruses are transmitted by trichodorid nematodes. Within the Trichodoridae, four out of 57 *Trichodorus* species (7%), seven out of 26 *Paratrichodorus* species (26%) and two out of seven *Nanidorus* species (28.5%) are vectors of tobraviruses. For Europe, however, the percentages of virus vectors vs non-vector species are much greater for *Nanidorus* (43%), *Paratrichodorus* (73%) and *Trichodorus* (25%).

Nepoviruses and tobraviruses are positive-sense single-stranded RNA viruses and have two genomic RNAs, the larger one being referred to as the RNA1 and the smaller the RNA2. The nepoviruses have isometric particles while the particles of the tobraviruses are tubular-shaped and of two dominant lengths. In nepoviruses, the large RNA1 molecule carries the genetic determinants for host-range, seed transmissibility and some types of symptom expression, whilst the small RNA2 contains the coat protein (involved in serological specificity), nematode transmissibility and some symptom expression (Taylor and Brown, 1997). In tobraviruses, each molecule contains genetic determinants for symptom expression and the smaller RNA2 contains determinants for serological specificity (coat protein) and vector transmissibility. Nepoviruses and tobraviruses transmitted by nematodes are primarily pathogens of

wild plants, and most are seed transmitted. They have developed specific relationships with their vector species and became associated with cultivated plants. The viruses may have a wide host range, with TRV having the most extensive range of all plant viruses. Many of the nepoviruses and tobaviruses occur naturally as a range of serological and/or symptomatological variants. TRV disease symptoms in crops range from yellowing and necrosis in leaves to colour break in flowers and necrotic arcs in the tuber flesh of potatoes.

There is a marked specificity between virus and vector, and this appears most evident in Europe where a particular serological virus strain is transmitted by a single nematode species. For example, *X. diversicaudatum* only transmits arabis mosaic virus (ArMV), and the PRV serotype of TRV from Britain, Sweden and The Netherlands is transmitted only by *P. pachydermus* (Brown *et al.*, 2004). The acquisition and transmission of viruses is linked to their methods of feeding. In *Longidorus*, virus particles are adsorbed in a single layer onto the inner surface of the odontostyle, and in *L. elongatus* virus particles of RRSV may also be located between the odontostyle and the guiding sheath (Taylor and Brown, 1997). In *Xiphinema* vector species, virus particles are associated with the cuticular lining of the odontophore and the pharynx. In trichodoridae vectors, the tobaviruses particles are associated with the lining of the complete pharyngeal region but are not attached to the onchiostyle. However, Karanastasi and Brown (2004) observed that the sites of virus particle retention differed between the individual tobaviruses-trichodoridae combinations. In addition, virus particles of several strains of tobaviruses were acquired and retained by trichodoridae that are not natural vectors of the particular virus. In these instances, the particles were retained within the pharyngeal tract at locations from where, when released, they cannot be transferred anteriorly along the pharyngeal tract into plant cells but can move only posteriorly into the nematode intestine. Currently, tobaviruses are the most popular viruses for virus-induced gene silencing (ViGS), a useful system for investigating the role of different plant genes (Dubreuil *et al.*, 2009).

6.8. Management

Tylench ectoparasites are largely polyphagous and cause direct damage upon feeding. The agricultural importance of trichodoridae and longidoridae nematodes as plant pathogens is dual: (i) as agents of direct damage; and (ii) as vectors for tobaviruses and nepoviruses, respectively. It is common to find a mixture of several species occurring together within the rhizosphere of plant roots; within the Trichodoridae, a *Trichodorus* species is often found together with a *Paratrichodorus* species.

6.8.1. Direct damage

Among tylench ectoparasites, the sting nematode *Belonolaimus* can be controlled with nematicides, with resistant cover crops, by rotating susceptible crops with resistant varieties and by using organic soil amendments. Nematicides may be used to control the awl nematodes (*Dolichodorus*). In field studies, yields of marketable heads of lettuce were doubled when *R. robustus* was controlled with nematicides. Several crops such as wheat, maize, millet, lima bean, common bean, watermelon,

okra and numerous weeds are excellent hosts for *Helicotylenchus columbus*. This makes carefully planned crop rotations and good control of weeds important factors for successful control of this species.

Many species of the families Longidoridae and Trichodoridae cause direct damage to a wide variety of plants when population densities are high (Fig. 6.18), but some species, such as *N. minor*, are considered more economically important than others possessing some of the widest host ranges. The amount of damage to the root system varies with the crop attacked and also appears greatly influenced by season, soil type and fertility. For example, 'Docking disorder' of sugar beet in the UK resulting from feeding of *P. anemones* on seedlings was more severe after a prolonged period of rain in spring. In practical terms, management of these ectoparasites depends on suppression of their population densities to an acceptable level as determined by the costs of control measures in relation to the financial return of the resultant increased crop yield. Although most species are polyphagous, not all host species are good hosts, which makes crop rotation, for example, effective. Specific resistance against these ectoparasites is unknown.

6.8.2. Damage as virus vectors

The major pest status of Longidoridae and Trichodoridae results from the ability of some species to transmit plant viruses. The damage-level threshold (the minimum population density at which a particular nematode species is considered to cause

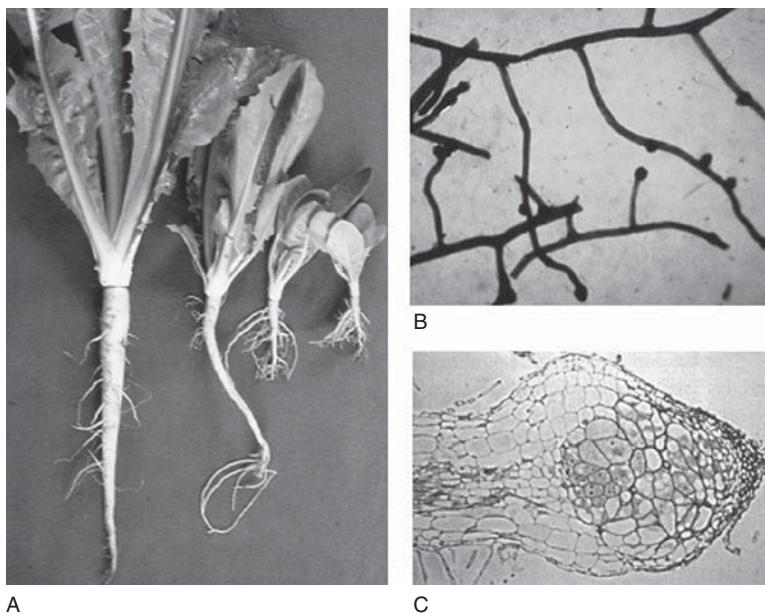


Fig. 6.18. Direct damage caused by Trichodoridae. A, stubby root symptoms in chicory caused by *Paratrichodorus teres*; B, galling on wheat caused by *Paratrichodorus* spp.; C, enlarged multinucleate cells in root gall caused by *Xiphinema index* on fig roots. (A, courtesy Plant Protection Service, The Netherlands; B and C, courtesy CABI.)

economically important damage to a particular crop host) is, in the case of virus vector species, equivalent to a single nematode. In most situations, nepovirus and tobavirus infections in the field are apparent only in crop plants in which symptoms are severe and which may induce plant death. By contrast, infection in wild plants is usually symptomless. Weeds provide a reservoir of infection and may provide overwintering sources of infection for some particular nepoviruses. The distribution and use of infected planting material appears to be the most important way by which nematode-transmitted viruses are introduced to areas where vector species may or may not have already been present.

Apart from measures such as the use of certified virus-free planting materials, taken to prevent viruses and vectors spreading to new areas, control measures are aimed at eliminating virus and vector from already infested land. The use of chemical nematicides (see Chapter 16) has, in general, proved to be satisfactory to prevent or, with perennial plants, to delay virus infection of newly planted crops. Crop rotation in general offers little prospect of practical prevention of damage because TRV and its polyphagous trichodoridae nematode vectors both have a wide host range. Knowledge of the pre-cropping history of a field is occasionally important as some crops, such as seed potatoes, are more sensitive to virus infection than others. Soil solarization using clear plastic mulch or a photoselective (black) polyethylene film may reduce populations of *N. minor* on tomato as effectively as fumigation with a mixture of methyl bromide and chloropicrin in warm climates. Increasing reliance is placed on plant resistance to virus infection but so far resistance to the vector nematode has not been achieved. Also transgenic resistance is primarily restricted to viruses and has not been applied to non-viral pathogens such as nematodes. Tissue culture micropropagation techniques may result in the production of virus-free plants. In general, crops should be kept free from weeds, especially before crops emerge. However, rigorous control of weeds increased TRV infection in potato, probably as a result of the virus-carrying vector being obliged to migrate and feed on potato instead of weeds.

Reducing nematode populations or freeing them from virus, through the culture of either non-susceptible crops or cultivars, may be an option to solve the complexity of plant virus infestation by nematodes in the long term. Also, new techniques such as the development of transgenic plants may offer strategies for suppressing crop diseases caused by these viruses.

Part II Nematode Biology and Plant–Nematode Interactions

Information on the functional biology of nematodes is central both to fundamental science and to practical aspects, such as targeting various management strategies effectively. This knowledge is also vital for identification and development of novel control targets. This is an exciting period for studies on nematode biology and host-parasite interactions because the availability of genome sequences, expressed sequence tag libraries and RNAi provides immense potential for functional genomic studies. The first two chapters in this section focus on nematode biology and lead into the third chapter, which reflects the advances in molecular information by focusing on molecular aspects of plant–nematode interactions.

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7

Reproduction, Physiology and Biochemistry*

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7.1. Reproduction and Development

Nematodes exhibit a variety of sexual and asexual reproductive methods and associated behaviours (Huettel, 2004; Denver *et al.*, 2011). Where there are two separate sexes, sexual reproduction, or **amphimixis**, can occur where male gametes (spermatoocytes) and female gametes (oocytes) fuse to form the zygote. Reproduction can occur asexually, termed parthenogenesis or **anmixis**, where males are not involved. A further type of reproductive method is **hermaphroditism**, where both egg and sperm are produced by the same individual. As will be evident in the following sections, there are several types of these basic reproductive forms and some genera can exhibit more than one type of reproduction (Table 7.1). Most information is available for *Meloidogyne* spp. (reviewed by Chitwood and Perry, 2009) and details are summarized in Table 3.1.

7.1.1. Amphimixis

Female nematodes are the homogametic sex (genetically XX) and males are heterogametic (sometimes XO, usually XY). This is found in *Anguina tritici* and *Ditylenchus dipsaci*, for example, and the sex ratio is determined genetically. In genera such as *Globodera*, *Heterodera* and *Meloidogyne*, sex chromosomes are absent and the sex ratio may be influenced by environmental factors such as food availability and temperature.

As with many animal groups, development of gametes includes meiotic division that results in the diploid ($2n$) complement of chromosomes being reduced by half (n). Fusion (fertilization) of the haploid gametes restores the diploid complement and ensures that the offspring receive genetic material from both parents. The following outline of oogenesis is based on observations of several species of nematodes, summarized by Triantaphyllou (1985). When the gonad is mature, oogonia are produced, which increase in size as they progress down the growth zone of the ovary. The young oocytes enter the spermatheca where development ceases unless they are entered by sperm. After copulation, the sperm is stored in the spermatheca and occasionally the uterine sac, if present. When young oocytes pass through the spermatheca they are individually fertilized with a sperm, thus stimulating further development. Soon after penetrating the oocyte, the sperm becomes temporarily inactive near the oocyte membrane it penetrated. Two meiotic divisions occur in the nuclei of the activated oocyte and the eggshell begins to form. One of the diploid nuclei from the first division is

Table 7.1. Reproduction method and chromosome numbers of some plant-parasitic nematodes. The haploid chromosome number is given first followed by the diploid number in brackets. (Modified from Evans, 1998.)

Nematode group	Chromosome number Haploid (diploid)	Type of reproduction
Class Enoplea		
Order Dorylaimida		
<i>Longidorus</i>	7	Parthenogenesis
<i>Xiphinema mediterraneum</i> (<i>X. americanum</i> group)	5	Meiotic parthenogenesis
<i>X. index</i>	10 (20)	Meiotic parthenogenesis
Class Chromadorea		
Order Rhabditida		
Family Anguinidae		
<i>Anguina tritici</i>	19 (38)	Amphimixis
<i>Ditylenchus dipsaci</i>	12 (24)	Amphimixis
<i>D. dipsaci</i>	(54)	Amphimixis
Family Dolichodoridae		
<i>Tylenchorhynchus claytoni</i>	8	Amphimixis
Family Pratylenchidae		
<i>Pratylenchus brachyurus</i>	(30–32)	Mitotic parthenogenesis
<i>P. coffeae</i>	7	Amphimixis
<i>P. neglectus</i>	(20)	Mitotic parthenogenesis
<i>P. penetrans</i>	5 (10)	Amphimixis
	6	Amphimixis
<i>P. scribneri</i>	6 (12) (25–26)	Meiotic parthenogenesis Mitotic parthenogenesis
<i>P. vulnus</i>	6	Mitotic parthenogenesis
<i>P. zeae</i>	(26) (21–26)	Parthenogenesis Parthenogenesis
Family Hoplolaimidae		
<i>Helicotylenchus dihystera</i>	6–8 (30, 34, 38)	
<i>H. erythrinae</i>	5 (10)	Amphimixis
<i>Hoplolaimus galateus</i>	10, 11 (20)	Amphimixis
<i>Rotylenchus buxophilus</i>	8	Parthenogenesis?
<i>Rotylenchulus reniformis</i>	9 (18)	Amphimixis
<i>Globodera mexicana</i>	9	Amphimixis
<i>G. pallida</i>	9 (18)	Amphimixis
<i>G. rostochiensis</i>	9–11 (18–24)	Amphimixis
<i>G. solanacearum</i>	9	Amphimixis
<i>G. tabacum</i>	9	Amphimixis
<i>G. virginiae</i>	9	Amphimixis
<i>Heterodera avenae</i>	9 (and rarely, 19)	Amphimixis
<i>H. betulae</i>	12, 13 (24)	Meiotic parthenogenesis
<i>H. carotae</i>	9	Amphimixis
<i>H. cruciferae</i>	9, 10	Amphimixis
<i>H. galeopsidis</i>	(32)	Mitotic parthenogenesis
<i>H. glycines</i>	9 (18)	Amphimixis

Continued

Table 7.1. Continued.

Nematode group	Chromosome number Haploid (diploid)	Type of reproduction
<i>H. goettingiana</i>	9	Amphimixis
<i>H. lespedezae</i>	(27) triploid	Mitotic parthenogenesis
<i>H. oryzae</i>	9 (27) triploid	Mitotic parthenogenesis
<i>H. sacchari</i>	(27) triploid	Mitotic parthenogenesis
<i>H. schachtii</i>	9 (18) 9 (19) 9	Amphimixis Amphimixis Amphimixis
<i>H. trifolii</i>	(24–27) triploid (26–34)	Mitotic parthenogenesis Mitotic parthenogenesis
<i>H. weissi</i>	9	Amphimixis
<i>H. rumicis</i>	(24) triploid	Mitotic parthenogenesis
Family Criconematidae		
<i>Hemicriconemoides</i> spp.	5	Amphimixis
Family Tylenchulidae		
<i>Tylenchulus</i> <i>semipenetrans</i>	5	Amphimixis, facultative meiotic parthenogenesis
<i>Cacopaurus pestis</i>	5, 6 (10, 11, 12)	Amphimixis
Family Aphelenchidae		
<i>Aphelenchus avenae</i>	8	Meiotic parthenogenesis, and amphimictic pseudogamy
	9	Meiotic parthenogenesis
Family Aphelenchoididae		
<i>Aphelenchoides composticola</i>	3	Amphimixis

reduced to a polar body and is extruded from the oocyte. The second division of the remaining oocyte nucleus results in a haploid pronucleus and a second polar body, which is also extruded. At about the time the egg is expelled from the uterus, the sperm pronucleus, also haploid, is formed and the gamete fusion occurs to produce the diploid zygote that commences development.

In many genera, including *Heterodera*, *Pratylenchus* and *Radopholus*, the female can be fertilized by several males, thus enhancing the genetic diversity of the offspring. In a few genera (e.g. *Trichodorus*), copulatory plugs deposited by a male nematode within the female vulva may interfere with subsequent mating (Decraemer, 2012). Amphimixis is often a secondary option in species where parthenogenesis is predominant.

7.1.2. Parthenogenesis

In species of nematodes that reproduce asexually, males are absent or occur only rarely. Among nematodes, parthenogenesis has been found to be particularly common in plant-parasitic forms. There are two main types of parthenogenesis, meiotic parthenogenesis (or automixis), which involves a reduction division, and mitotic parthenogenesis (or apomixis), where there is no reduction division.

7.1.2.1. Meiotic parthenogenesis

In this type of reproduction, there is a first meiotic division in the oocytes, although there are variations between species. This meiotic division allows the possibility of some genetic reorganization. *Meloidogyne hapla* race A exhibits facultative meiotic parthenogenesis, oogenesis and spermatogenesis proceeding as in amphimictic species to yield one haploid nucleus and two polar bodies per oocyte. If sperm are present, amphimixis occurs. However, if the egg is not fertilized by sperm, the diploid state is achieved by the egg pronucleus recombining with the second (haploid) polar body. In monosexual populations of *Aphelenchus avenae*, females produce only female progeny and reproduction is by obligate meiotic parthenogenesis. Meiosis produces only one polar body, with the egg nucleus having the $2n$ chromosome number that then develops into the zygote. Other species reproducing by meiotic parthenogenesis include *Xiphinema index* and *Pratylenchus scribneri* (Table 7.1).

7.1.2.2. Mitotic parthenogenesis

This is the most common method of asexual reproduction (Table 7.1). The only division that occurs is mitosis and the oocytes retain the diploid chromosome number. Mitotic parthenogenesis is always obligate. This prevents any genetic reorganization and, except for mutations, the ova produced should be clones. Frequently, mitotic parthenogenesis is associated with polyploidy (Table 7.1), which may increase the likelihood of mutation. There is no direct evidence for this, although variability in pathogenicity exists and can be selected for (Blok *et al.*, 1997). Mitotic parthenogenesis occurs in several species of *Meloidogyne* and *Pratylenchus*; in *Meloidogyne* it has evolved at least twice (Holterman *et al.*, 2009).

7.1.3. Hermaphroditism

In hermaphrodites, both egg and sperm are produced in the same individual. Usually the sperm is produced first and is stored in the spermatheca, then the gonad produces oocytes, which are fertilized by the sperm until the sperm supply is exhausted. Hermaphroditism is a common method of reproduction amongst rhabditids and other free-living nematodes but is relatively rare in plant-parasitic nematodes, being found in some members of the Criconematoidea and in species of *Paratrichodoros*. Pseudogamy is a type of hermaphroditism where sperm penetration activates oocyte development but the nuclei do not fuse. The sperm nucleus degenerates and the diploid state of the oocyte is restored after the first meiotic division. However, to our knowledge this form of reproduction has not been reported in plant-parasitic nematodes.

7.1.4. Development

Development includes embryogenesis and growth, about which there is a very large amount of information for *Caenorhabditis elegans* (see Priess and Seydoux, <http://www.wormbook.org>) but little for plant-parasitic nematodes. With the availability of genome

sequences, expressed sequence tag libraries and RNAi, there is now potential for functional genomic studies on development of plant-parasitic nematodes (Bird *et al.*, 2009; Rosso *et al.*, 2009). Embryogenesis has been described for many species of plant-parasitic nematodes (Hope, 2002) but most information derives from observations of fixed material. Most of the cell division occurs in the first half of embryogenesis. In the second half, the embryo elongates markedly, starts to move, and synthesizes the cuticle. The number of cells in most nematodes is presumed to be similar to that in *C. elegans* (959 somatic cells) despite differences in size between species and juvenile and, especially, adult stages.

Juveniles hatch from eggs that are laid by the adult female. Each egg is ovoid-shaped and contains a single juvenile. Despite the vast difference in size between adults of various species of nematodes, the majority of eggs are of similar size (50–100 µm long and 20–50 µm wide) and morphology. In most plant-parasitic nematodes the juvenile moults within the egg and the resulting second-stage juvenile (J2) hatches. The egg and the hatching responses are part of the survival attributes of many species of nematodes, and hatching is discussed in Section 7.6.5.

7.1.4.1. Moulting

The primary signal for moulting in nematodes is unlikely to be an increase in body size as growth takes place between moults. The cuticle is shed and replaced four times during the life cycle, although there is evidence that some species of *Longidorus* and *Xiphinema* have only three juvenile stages.

There are differences between species in the moulting pattern (Bird and Bird, 1991; Lee, 2002) but, in general, the process involves three phases: (i) the separation of the old cuticle from the epidermis (apolysis); (ii) the formation of a new cuticle from the epidermis; and (iii) the shedding of the old cuticle (ecdysis). Wright and Perry (1991) studied the moulting process in *Aphelenchus hamatus* from fourth-stage juvenile (J4) to adult. The process took 12–13 h to complete and showed features typical of most moulting patterns. Initially, *A. hamatus* did not move (a period termed lethargus) while the cuticle separated from the epidermis, which thickened and showed evidence of increased metabolic activity as the new cuticle was formed. There was also a loss of the knobs and shaft of the stylet. The water content of the nematode decreased, causing a reduction in body volume that enabled the adult to retract within and away from the old cuticle as the new stylet and head skeleton were formed. The entire body of the adult then moved within the old cuticle. The pharynx became active as the adult took up water and expanded to burst the old cuticle. It is possible that pharyngeal secretions and associated enzymic activity might aid in degrading the old cuticle before ecdysis. In some endoparasitic species, such as *Meloidogyne javanica* and *Heterodera glycines*, cuticles are partially or completely reabsorbed. During adverse conditions, the moulted cuticles of *Rotylenchulus reniformis* are retained as sheaths to aid survival (see Section 7.6.3).

7.2. Musculature and Neurobiology

Information on the musculature and nervous system of plant-parasitic nematodes is confined almost completely to morphological details (see Chapter 1). The information

here on physiology and biochemistry derives mainly from work on animal-parasitic and free-living nematodes, especially *C. elegans*, the assumption being that the basic features are likely to be the same for plant-parasitic forms.

7.2.1. Musculature

There are two types of muscle: somatic and specialized. Somatic, or body wall, muscles are obliquely striated (multiple sarcomere). The somatic muscle cell comprises three parts: the spindle that contains the contractile elements (called sarcomeres), a non-contractile muscle cell body that projects into the pseudocoelom, and the arm, which extends from the cell body to the longitudinal nerve chord or to the nerve ring. In many species of nematodes muscle cells have more than one arm. Before each arm reaches the nerve chord, it divides into several ‘fingers’, which subdivide into multiple fine processes that receive synaptic input from excitatory and inhibitory motor neurons. The fingers are interconnected by gap junctions, probably facilitating electronic spread of impulses between neighbouring muscle cells. Gap junctions occur between muscle cells and between neurons but are rare or absent between muscles and neurons. Specialized muscles are non-striated (single sarcomere) muscles and they include pharyngeal muscle cells, intestinal muscles and others associated with defecation, mating, fertilization and egg laying.

Each sarcomere consists of thick filaments flanked and interdigitating with thin filaments; the striated appearance of somatic muscle results from the alternation of regions containing thick and thin filaments. At the centre of each thick filament, a specialized region crosslinks the thick filaments to maintain their alignment. Thin filaments are anchored at one end to dense bodies. Thick filaments contain myosin and paramyosin, and thin filaments contain actins, tropomyosins and troponins. Actin forms the core component of thin filaments and binds and activates myosin. The process of muscle contraction depends on ATP and requires direct interaction of myosin head and actin. Cross-bridges, originating on the thick filaments, attach and detach cyclically to specific sites on the thin filaments, causing the filaments to be pulled past each other, resulting in shortening of sarcomeres and, thus, contraction of muscles.

Somatic muscles are arranged in longitudinal rows in a single layer divided by the epidermal chords into four nearly symmetrical quadrants; circular somatic muscles are absent in nematodes. Individual motor neurons of the ventral chord innervate either dorsal or ventral muscles, thus restricting body flexures to the dorsoventral plane only. Alternate contraction of the dorsal or ventral longitudinal somatic musculature results in sinusoidal locomotion. The more complex innervation of the anterior end permits the lateral and dorsoventral movements of the head that are components of oriented and unoriented behaviour (see Chapter 8).

7.2.2. Nervous system

The organization of the nervous system appears to be essentially conservative. The neurons have a simple, relatively unbranched morphology and a single gap junction is sufficient for functional coupling between neurons. The majority of nerve processes run longitudinally, as ventral and dorsal nerve chords, or circumferentially, as

commissures. The nervous system in *C. elegans* comprises two essentially independent parts, the pharyngeal system and the much larger extrapharyngeal (somatic) system, which are connected via a bilateral pair of gap junctions between a pair of pharyngeal interneurons and a pair of ring/pharynx interneurons. Most neuronal cell bodies of the extrapharyngeal nervous system occur around the pharynx, along the ventral mid-line and in the tail.

The circumpharyngeal nerve ring is the main mass of the central nervous system. Neuronal processes from the anterior sensilla (sense organs) run posteriorly as six cephalic nerve bundles, four of which have their cell bodies just anterior to the nerve ring in a region termed the ‘anterior ganglion’. The other cephalic nerve bundles, which contain processes from the lateral pair of amphid sensilla, run past the nerve ring to cell bodies in two lateral ganglia. Processes from the sensilla eventually synapse at the nerve ring. Processes from cells in the tail ganglia connect with the posterior (caudal) sensilla. Details of sensilla structure and function are given in Chapter 8.

The majority of the nerve processes leaving the nerve ring form the ventral nerve chord. The interneuron processes in this chord synapse either with the pre-anal ganglion or with excitatory motor neurons in the nerve chord. The motor neurons are arranged in a linear sequence and some have processes that run under the epidermis as commissures to the dorsal mid-line where they pass anteriorly or posteriorly forming the dorsal nerve chord. The latter is simpler in structure than the ventral nerve chord with few interneurons and no cell bodies.

7.2.3. Neurotransmission

Neurotransmission in nematodes has been reviewed by Wright and Perry (1998) and Holden-Dye and Walker (2011); for specific reviews on *C. elegans* see Jorgensen and Kaplan (2011). Classical synaptic transmission involves the arrival of an action potential at a pre-synaptic nerve ending, which leads to the opening of voltage-gated Ca^{2+} ion channels near the synapse and the influx of Ca^{2+} ions into the nerve cell. This results in the secretion, by exocytosis from the pre-synaptic nerve ending, of neurotransmitter molecules, which diffuse across the synaptic cleft and bind reversibly to specific receptor proteins on the post-synaptic membrane of a nerve or muscle cell. This causes a conformational change in the receptor proteins that are linked directly (ionotropic receptors) or via secondary messenger systems (metabotropic receptors) to ion channels. The release of the neurotransmitter and the threshold response of the receptors can be regulated by other chemicals ('neuromodulators') released in the area of the synapse. Whether the response is excitatory or inhibitory depends on the type of receptor and, thus, which ion channel is activated. Thus, the same neurotransmitter can be excitatory and inhibitory.

Classical transmitters include acetylcholine, probably the primary excitatory transmitter, several amino acids and various biogenic amines. Characterization of different molecular forms of acetylcholine has been conducted with several species of nematodes. There is extensive information on the amino acid transmitter, gamma-amino butyric acid (GABA), in *C. elegans*, including evidence for a novel excitatory GABA receptor as well a classic inhibitory GABA receptor (Schuske *et al.*, 2004). GABA has also been reported in J2 of *Globodera rostochiensis* and *Meloidogyne incognita* (Stewart *et al.*, 1994). Other putative neurotransmitters in nematodes include

the biogenic amines dopamine and 5-hydroxytryptamine, and various neuropeptides (see Section 7.3.1). Dopamine has been detected in a wide variety of nematodes, including *M. incognita* (Stewart *et al.*, 2001). An essential feature of all neurotransmitter systems is a mechanism for the rapid removal of neurotransmitter from the synaptic cleft. With acetylcholine, the mechanism is enzymatic and inhibition of acetylcholinesterase is the target site for the control of plant-parasitic nematodes by organophosphate and carbamate nematicides (see Chapter 16).

7.3. Biosynthesis

This subject was comprehensively reviewed for plant-parasitic and free-living nematodes by Chitwood (1998), who pointed out that much more is known about the chemical composition of nematodes than about their biosynthetic pathways. Increased knowledge of the latter may lead to the design of novel control strategies for plant-parasitic species by exploiting targets unique to nematodes.

7.3.1. Amino acids and related compounds

Work on *Caenorhabditis* spp. has shown a dietary requirement for arginine as well as nine typical mammalian essential (i.e. dietary) amino acids, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, although the requirement for methionine can be satisfied by a known precursor, homocysteine. Studies on various species of plant-parasitic nematodes (*D. triformis*, *Meloidogyne* spp., *Aphelenchoides* spp.) showed that they could all synthesize a number of typical mammalian non-essential amino acids. In addition, several amino acids that are essential in mammals could be synthesized, including tryptophan (in *Meloidogyne* spp.) and lysine, phenylalanine, leucine/isoleucine, threonine and valine (in *Aphelenchoides* spp.), although not necessarily in sufficient quantities to support reproduction.

Many putative neurotransmitters in nematodes, including biogenic amines and GABA, are related to amino acids and evidence for the pathways required for their synthesis has been obtained for *C. elegans* and to a more limited extent in some other nematodes (Wright and Perry, 1998; Perry and Maule, 2004). There has been little work on plant-parasitic nematodes, other than the localization of GABA and several biogenic amines within the nervous system (see Section 7.2.3). Various small polypeptides are also putative neurotransmitters or neuromodulators in nematodes. Typically, these are members of the FMRFamide-related peptides (FaRP) family and they have been localized in the nervous system of several species, including *H. glycines*, *G. pallida* and *M. incognita* (Johnston *et al.*, 2010a). Insect neuropeptides are derived from large parent polypeptides. Many genes encoding FaRPs and some other neuropeptides have been isolated from *C. elegans*.

The plant auxin indoleacetic acid (IAA), a tryptophan metabolite, and several related compounds, have been identified in species of *Meloidogyne* and *Ditylenchus* but there is no direct evidence of auxin biosynthesis by nematodes (Chitwood, 1998). Although most animals, including *C. elegans*, possess a nutritional requirement for B vitamins, *H. glycines* appears to have acquired the enzymatic machinery for biosynthesizing the amino acid derivatives biotin, thiamine and pantothenate via horizontal gene transfer (Craig *et al.*, 2009).

7.3.2. Nucleic acids and proteins

Like most organisms, nematodes are capable of synthesizing the purine and pyrimidine bases of nucleotides, although studies on the synthesis of nucleoside monophosphates are lacking. Work on *C. elegans* has provided evidence for the synthesis of cyclic nucleotides from their corresponding nucleoside triphosphates. The two enzymes at the start of the pentose pathway, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, have been demonstrated in several free-living and plant-parasitic nematodes (Barrett and Wright, 1998). However, during active nucleic acid synthesis in mammals the pentose pathway ends at the next step with the formation of D-ribose-5-phosphate, which is used for nucleotide synthesis. So the presence of the starting enzymes does not necessarily mean there is a complete cycle (see Section 7.3.4.).

Studies on nucleic acid polymerases in nematodes have focused on *C. elegans* and a large number of transcription factors have been identified in this species (Reinke *et al.* 2005). Translational mechanisms involved in protein synthesis in *C. elegans* have been reviewed by Rhoads *et al.* (2006).

7.3.3. Lipids

The total lipid content of the free-living and plant-parasitic nematodes examined ranges from 11 to 67% of dry weight and in general they have a greater lipid content than animal-parasitic nematodes, which rely more on glycogen energy reserves and, at least partially, anaerobic metabolism (Barrett and Wright, 1998). Neutral lipids are the primary lipid energy reserves and are predominantly triacylglycerols (containing three fatty acid molecules). These usually make up >70% of total lipid (up to 94%). Polar phospholipids generally comprise 6–16% of total lipid. Glycolipids have been reported to comprise 2.5% of total lipid in *M. javanica* females. Sterols occur in much smaller quantities, comprising 0.01–0.06% of dry weight in plant-parasitic species (Chitwood, 1998).

The fatty acid composition of nematodes is distinctive at the genus level; some qualitative differences have also been detected at the species level (Sekora *et al.*, 2010). Nematodes contain a much greater variety of fatty acids than their host. In general, the majority of fatty acids in most plant-parasitic nematodes contain 18 or 20 carbon atoms (see Box 7.1 for fatty acid nomenclature), and are mostly unsaturated (75–92%), although this can be influenced by the culture media and temperature. Between 19 and 24 C₁₂–C₂₀ fatty acids have been identified in *P. penetrans*, *Aphelenchoides ritzemabosi*, *Tylenchorhynchus claytoni*, *D. dipsaci*, *D. triformis*, *M. arenaria* and *M. incognita*; *D. triformis* also contained a 22:1 fatty acid. The major fatty acid group in each species was 18:1 and in species of *Ditylenchus* and *Meloidogyne* its major component was vaccenic acid, C18:1 (*n*-7). In *G. rostochiensis*, *G. pallida* and *G. tabacum solanacearum* the major fatty acid groups were 20:4 > 20:1 > 18:1. The differences in the fatty acids of host and nematodes indicate that *G. rostochiensis* is capable of fatty acid chain elongation and/or desaturation. In mycophagous *Aphelenchoides* spp., carboxyl-directed desaturation is indicated as the major pathway for the synthesis of long-chain polyunsaturated fatty acids (Ruess *et al.*, 2002). Some polyunsaturated fatty acids (20:4, 20:5) are known to be phytoalexin elicitors in potato and their presence in *G. rostochiensis* has been linked to pathogenesis.

Box 7.1. Fatty acid nomenclature.

Fatty acid nomenclature uses a numeric system. The general type of acid is designated as C_x with x as a number denoting the number of carbon atoms; for example, C_{18} (unsaturated acids) and C_{20} (polyunsaturated acids). They are further characterized as, for example, 18:1 or 20:4. The number after the colon represents the number of double bonds. When characterized with the double bond, the numbers are usually not written as subscripts and the C is omitted. In addition, the specific acid can be identified by including the location of the double bond, which is represented by, for example, $n\text{-}3$ (this is usually put in brackets). The number is the number of carbon atoms from the methyl end of the fatty acid. Thus 18:1($n\text{-}7$) is vaccenic acid and 18:9($n\text{-}9$) is oleic acid.

Phospholipids are the major components of cellular membranes and are typically derived from a molecule of glycerol, two fatty acids, phosphoric acid and another alcohol such as choline, ethanolamine or inositol. Another phospholipid, sphingomyelin, contains a long-chain nitrogenous base (sphingosine), esterified to a phosphoryl-choline molecule and amide-linked to a fatty acid. Ethanolamine and choline phosphoglycerides have been identified as the major phospholipids in *M. javanica*, *A. tritici* and *G. rostochiensis*. As in most organisms, phospholipids regulate membrane fluidity in nematodes. Electron spin resonance (ESR) spectroscopy of spin-labelled nematode polar lipid extracts reconstituted in water yielded plots that were linear between 0 and 30°C for cold-tolerant species (*M. hapla* and *A. tritici*) but had a sharp change in slope for *M. javanica* at 10°C, suggesting a physiologically important lateral phase separation or other localized event in the latter, more tropical, species. Phospholipids are also likely to be involved in signal transduction cascades in nematodes, including protein kinase C-mediated cascades. Incubation of *G. rostochiensis* eggs with tritiated inositol resulted in the formation of various inositol phosphates, which may reflect the involvement of inositol phosphoglyceride-mediated signal transduction in nematode hatching.

Glycolipids vary in structure from simple diacylglycerols attached to one sugar residue to glycosphingolipids, which consist of long chains of sugar residues attached to a lipophilic sphingosine base connected to a fatty acid moiety. Glycolipids often anchor proteins in cell membranes and also have roles in facilitating recognition events, and as precursors for lipoidal secondary messengers involved in signal transduction. The surface coat of nematodes contains glycolipids and can change in response to plant signals (Davies and Curtis, 2011); it is also implicated in the attachment of endospores of *Pasteuria penetrans* (see Chapter 13). There is evidence for the *de novo* synthesis of large amounts of a cerebroside, a glycosphingolipid in *C. elegans*. Similar glycolipids have also been identified in *M. incognita*. The sphingoid bases of these glycolipids have predominantly branched structures, unlike those of other organisms examined. The dauer stage of *C. elegans* contains unique glycolipids comprised of two fatty acids esterified to a trehalose molecule (Penkov *et al.*, 2010).

Sterols (tetracyclic polyisoprenoids) are biosynthesized in plants and higher animals via the hydroxymethylglutaryl Coenzyme A pathway. Nematodes possess a wide variety of sterols but cannot biosynthesize them *de novo*, unlike plants and higher animals. Sterols are one of the few essential nutritional dependencies that plant-parasitic nematodes

have upon their host plants, a dependency that could possibly be exploited for their control. Comparison of the sterol composition of plant-parasitic nematodes with their plant hosts has indicated that two major metabolic transformations can occur in these species: dealkylation of the C-24 side chain and the saturation of double bonds in the four-membered ring system to yield stanols (Chitwood, 1998). Sterols generally have two major functions. They act as modulators of cell membrane fluidity and as precursors of biologically active molecules, such as steroid hormones. In plant-parasitic nematodes, low membrane phase transition temperatures due to the predominance of unsaturated phospholipid fatty acids, plus the relatively small quantities of sterols present, suggest that the overall effects of sterols such as cholesterol on membrane fluidity are likely to be small, although effects upon specific domains cannot be ruled out (Chitwood, 1998). Studies on *C. elegans* suggest that nematode steroid hormones may be quite different from the ecdysteroid hormones of insects but information is lacking for plant-parasitic species. In *C. elegans*, entry into the dauer stage is regulated by 3-keto bile acid-like steroids termed dafachronic acids (Gerisch *et al.*, 2007).

7.3.4. Carbohydrates and related compounds

As in most other organisms, glycogen is the major storage carbohydrate in nematodes (3–20% of dry weight). Radiotracer studies have demonstrated its biosynthesis in at least two free-living species (Barrett and Wright, 1998; Chitwood, 1998). Specific sugars and related compounds, including the disaccharide trehalose, ribitol, inositol and glycerol, are synthesized by some nematodes as they undergo the process of anhydrobiosis (see Section 7.6.2.2). This synthesis is typically at the expense of glycogen or lipid depletion. The pathway for trehalose synthesis in nematodes involves, as in other organisms, the transfer of glucose by trehalose 6-phosphate synthetase from uridine diphosphate glucose to glucose 6-phosphate to form trehalose 6-phosphate and the conversion of the latter to trehalose by trehalose 6-phosphate phosphatase, the former process being the rate-limiting step (Behm, 1997; Chitwood, 1998). Hatching in cyst nematodes is associated with the release of trehalose, which is found in high concentrations in the perivitelline fluid (see Section 7.6.5).

An apparently unique feature of nematodes among the metazoa is the presence of the glyoxylate cycle, which synthesizes succinate from two acetyl-CoA molecules (see Section 7.4.4.). This pathway is used by many plants and bacteria to generate carbohydrates from acetate, and enables lipids to be converted into carbohydrates via β -oxidation of fatty acids.

Chitin, a polymer of *N*-acetylglucosamine, is a major component of nematode egg-shells (Bird and Bird, 1991) and in *C. elegans* it is involved in the prevention of fertilization by multiple sperm (Johnston *et al.*, 2010b). Evidence for chitin synthases has been reported in several nematode species, including *M. artiellia* (Veronico *et al.*, 2001).

7.4. Respiration and Intermediary Metabolism

There have been relatively few studies on the respiratory physiology of nematodes since the mid-1980s and the review by Wright (1998). Studies on intermediary metabolism show that free-living and plant-parasitic nematodes all appear to catabolize

energy reserves by glycolysis/β-oxidation and the tricarboxylic acid (TCA) cycle. Most biochemical studies on nematodes have been on animal-parasitic species and *C. elegans*. The relatively limited number of studies on plant-parasitic nematodes were reviewed by Barrett and Wright (1998). Absolute proof for the presence of a particular metabolic pathway is generally lacking in nematodes, although it is likely that nematodes possess the same pathways as those found in other metazoans. One unusual feature is the apparent presence of the glyoxylate cycle in many nematode species.

7.4.1. Respiratory physiology

The cylindrical body plan of nematodes, together with their lack of a circulatory system, places severe restrictions on their body radius for a given level of aerobic metabolism (Atkinson, 1980). However, almost all plant-parasitic nematodes are sufficiently small for diffusion to supply sufficient oxygen for full aerobic respiration at partial pressures of oxygen >15 mmHg. There are some habitats where microaerobic (<15 mmHg) conditions can occur. For example waterlogged soils, or organic soils with high levels of microbial activity, can have sufficiently low levels of oxygen to affect nematode activity. The roots of plants in low oxygen environments (e.g. mangroves, paddy and deep water rice) may also have low oxygen tensions.

Metabolically, nematodes are typical poikilotherms and show a marked change in their oxygen consumption with changes in ambient temperatures. Other factors that can influence their rate of oxygen consumption include ageing, osmotic and ionic regulation and locomotory activity, although the energy cost of movement appears low compared with metabolic maintenance and reproduction.

7.4.2. Amino acid catabolism and nitrogenous waste products

There is some evidence that starved nematodes, including J2 of *M. javanica* and *Tylenchulus semipenetrans*, can use endogenous amino acids as an energy source (Barrett and Wright, 1998). However, nitrogenous waste products in animals, usually in the form of ammonia, must either be excreted directly or converted to less toxic metabolites, such as urea. Nematodes, like most aquatic organisms, are ‘ammonotelic’, continually excreting ammonia (approx. 40–90% of non-protein nitrogen eliminated) into the environment as it is produced, thus avoiding the toxic storage problem. Urea is excreted by some nematodes (up to around 20% non-protein nitrogen) but not by others, although whether urea is produced via the ornithine–arginine (urea) cycle in nematodes is more problematic (Wright, 1998, 2004). The excretion of waste nitrogenous products into the soil environment may be a significant contribution to nitrogen availability to plants (Ferris *et al.*, 1998).

In amino acid catabolism, the amino group is removed first, followed by catabolism of the carbon skeleton. The two main routes for the removal of the amino group are transamination and oxidative deamination. There is also non-oxidative deamination of specific amino acids. All such enzyme systems have been identified in the free-living nematode, *Panagrellus redivivus*, the only nematode to be investigated in any detail and, in general, the ability of this species to transaminate and deaminate amino

acids is similar to other organisms. Following transamination or deamination, the carbon skeletons of amino acids are converted to glycolytic or TCA cycle intermediates and eventually to carbon dioxide.

7.4.3. Lipid catabolism

Neutral lipids are the major energy reserves in plant-parasitic nematodes and the lipids usually contain a high percentage of unsaturated fatty acids (see Section 7.3.3). The aerobic utilization of lipid stores has been demonstrated in a wide variety of nematode species and where investigated it is the triacylglycerol fraction that is catabolized. In some species of plant-parasitic nematodes, e.g. *G. rostochiensis*, a decline in infectivity has been correlated with a decline in lipid reserves. Organophosphate and carbamate nematicides cause reversible inhibition of nematode movement, and this reduces neutral lipid consumption in J2 of *G. rostochiensis*, enabling them to remain infective on recovery.

Under aerobic conditions, fatty acids are broken down by β -oxidation to give acetyl-CoA, NADH and reduced flavoprotein. The presence of β -oxidation enzymes has been reported in a number of plant-parasitic nematodes, and in several species radiotracer studies have demonstrated a functional pathway.

7.4.4. Carbohydrate catabolism

The main carbohydrate reserve in nematodes is glycogen (3–20% dry weight); significant amounts of trehalose (0.1–5%) have also been reported in some species but whether it has a role in energy metabolism as well as in anhydrobiosis has yet to be established. Glycogen utilization under aerobic and anaerobic conditions has been demonstrated in free-living and plant-parasitic nematodes as well as in animal-parasitic species (Barrett and Wright, 1998). In most nematodes, glycogen content is greatest in the non-contractile regions of the muscles, epidermis, intestinal cells and the epithelial cells of the reproductive system. Glycolytic enzymes have been demonstrated in nematodes together with a range of glycolytic intermediates. The regulatory properties of glycolytic enzymes in plant-parasitic nematodes have not been investigated. TCA cycle enzymes have also been demonstrated in a wide range of nematode species and radiolabel studies are consistent with a functional TCA cycle.

The glyoxylate cycle enables organisms to use acetyl-CoA from the β -oxidation of fatty acids for gluconeogenesis (see Section 7.3.4). This cycle has been found in bacteria, algae, fungi, plants, protozoans and nematodes. It consists of two enzymes, isocitrate lyase and malate synthase, which effectively ‘short circuit’ the TCA cycle by bypassing the two decarboxylation steps (isocitrate dehydrogenase and 2-oxoglutarate dehydrogenase) and catalyse the net conversion of isocitrate and acetyl-CoA to succinate and malate. Both glyoxylate cycle enzymes have been demonstrated in a range of plant-parasitic nematodes, including J2 of *M. incognita*; additional evidence for a functional glyoxylate cycle in nematodes has come from radiotracer studies and molecular genetic studies on *C. elegans* (Barrett and Wright, 1998; McCarter *et al.*, 2003; Jeong *et al.*, 2009).

Plant-parasitic nematodes produce ethanol as a major end product under anaerobic conditions. Other compounds that may be produced and excreted under anaerobic conditions include acetate, lactate, succinate and glycerol. In organisms that are periodically exposed to anaerobic conditions, resynthesis of carbohydrate from anaerobic end products is important in substrate conservation. For example, the presence of an active glyoxylate cycle would allow the resynthesis of carbohydrate from ethanol.

7.4.5. Cytochrome chains

NADH and reduced flavoprotein are oxidized by mitochondrial cytochrome chains, resulting in the reduction of oxygen to water and the formation of ATP. Cytochromes $a+a_3$ and c have been identified in *D. triformis* and $a+a_3$, b and c in *A. avenae* and there is some evidence for a non-mammalian type of terminal oxidase in *M. javanica*. Oxidation of iron (Fe^{2+}) centres in the cytochrome chain of nematodes has been suggested as one mode of action of halogenated aliphatic hydrocarbon fumigant nematicides (Wright, 1981). The cytochrome b gene has been used in molecular taxonomy (Plantard *et al.*, 2008; see Chapter 4) and mitochondrial cytochrome oxidase genes have been used for the same purpose for species of *Xiphinema* (Kumari *et al.*, 2010).

7.5. Osmotic and Ionic Regulation and Excretion

Nematodes only develop and reproduce in an environment where water is freely available and like other aquatic organisms they must deal with the opposing problems of osmosis and diffusion. Osmotic and ionic regulation in nematodes and techniques to study these aspects have been reviewed by Wharton and Perry (2011). In soil water, the infective stages of endoparasitic nematodes and all stages of ectoparasitic species are normally in a very dilute osmotic (hypo-osmotic) and ionic environment compared with their body fluids, although the osmotic potential of soil water may fluctuate considerably and become hyper-osmotic under some conditions. For nematodes living in soil the osmotic and ionic conditions will vary as the soil becomes saturated with water following rainfall. Animal- and plant-parasitic nematodes will face markedly different physiochemical conditions during their life cycle as they move from a parasitic to a free-living phase and back again.

Some nematodes, most notably free-living estuarine species, are regularly exposed to fluctuating osmotic conditions and in some cases appear to survive as osmoconformers. Species that live in hypo- or hyper-osmotic conditions for any appreciable period must regulate their water content and volume to retain normal locomotory activity (Wright, 1998, 2004). Whilst this would not apply to sedentary stages of endoparasitic species, such as root-knot and cyst nematodes, the maintenance of an osmotic and ionic balance is also important in other aspects of nematode biology, particularly feeding and reproduction.

7.5.1. Physiological ecology

Little is known about water and ion balance in plant-parasitic nematodes compared with animal-parasitic species or *C. elegans*, and the information available relates

almost entirely to free-living soil stages. As would be expected, the infective juveniles of plant-parasitic nematodes (e.g. *H. schachtii* and *G. rostochiensis*) that have been examined tend to be very good hyper-osmotic regulators but are less good hypo-osmotic regulators. For infective juveniles of plant-parasitic nematodes, the ability to maintain locomotory activity under a range of soil water solute concentrations is critical because almost all species actively infect their host. Marked increases in the solute concentration can occur in the upper layers of soils, where agricultural amendments and changes in the net evaporative rate have the greatest impact, and where irrigation can lead to ‘saline’ soils (Wright, 2004). However, the relative importance of the solute concentration compared with the soil matric potential on the water balance and movement of nematodes is uncertain. The matric potential of soil water can be tenfold greater than the solute component and increases at a much greater rate than the soil water osmotic pressure as the soil dries (Burr and Robinson, 2004). The matric potential is recognized as the more important factor for plant–water relations and as a predictor of nematode movement in soils (Robinson, 2004).

There have been no studies on osmotic and ionic regulation by plant-parasitic nematodes, although they would be expected to tolerate marked fluctuations in water potentials within plants, particularly at times of drought or nematode-induced stress. In the case of endoparasites, such as species of *Meloidogyne*, *Heterodera* and *Globodera*, osmotic and ionic balance could still be important for sedentary juvenile and adult female stages, particularly for feeding and reproduction.

J2 of *M. javanica* migrate away from areas of high salinity. *Meloidogyne incognita* and *R. reniformis* respond to the constitutive cations and anions of salts, respectively; the former species is strongly repelled by ammonium salts, whilst the latter species is repelled by chloride salts. How such differences in chemotactic responses relate to the behaviour and distribution of nematodes in soils of varying ionic content remains to be investigated. The amphids and phasmids of nematodes, which have chemosensory functions, are linked to osmotic avoidance behaviour in *C. elegans* and undoubtedly play a similar role in other species.

7.5.2. Mechanisms

Two complementary osmoregulatory mechanisms are found in animals: **isosmotic intracellular regulation**, where the osmolarity is adjusted to conform with the extra-cellular osmotic pressure, and **anisosmotic extracellular regulation**, where the extra-cellular fluid is maintained hypo- or hyper-osmotic to the external environment. The latter mechanism, which may involve a non-ionic component, has been demonstrated in several animal-parasitic nematode species (Wright, 2004). The pseudocoelom is the principal extracellular fluid compartment in nematodes and may act as a primitive circulatory system (Wright, 2004). In actively moving nematodes, sinusoidal waves of contraction and accompanying internal pressure changes will result in some mixing of the pseudocoelomic fluid, although the degree to which mixing is localized is unknown.

The body wall, including various epidermal glands, the intestine and the secretory-excretory system have been suggested as sites of urine production in nematodes capable of volume regulation in hypo-osmotic environments but again there is little direct evidence. Passive factors associated with osmotic and ionic regulation in hypo-osmotic

environments are thought to involve a relatively impermeable nematode cuticle or its underlying epidermal membrane (Wright, 1998). In *X. index*, invaginations of the outer epidermal membrane with continuity with the endoplasmic reticulum suggest a transport function. Most nematodes retain a functioning gut, and the regular removal of material by defecation in actively feeding nematodes suggests the intestine has an important role in fluid excretion.

The ionic composition of the pseudocoelomic fluid in several animal-parasitic species suggests that ionic regulation must occur. However, there is only limited, largely indirect, physiological and biochemical evidence for the ion channels and pumps that would be required to maintain electrochemical gradients across nematode epidermal and intestinal cells (Thompson and Geary, 2002). There is molecular evidence for KCl co-transporter proteins in *M. incognita*, which could be involved in the ionic and osmotic regulation (Neveu *et al.*, 2002).

7.5.3. Nitrogen excretion

In nematodes, the relative importance of the body wall, intestine and S-E system in the excretion of waste nitrogen (see Section 7.4.2) will vary between species and between stages of a particular species and is likely to correspond closely with the relative importance of these structures for the removal of water (Wright, 1998, 2004). All plant-parasitic nematodes have a small body radius (<20 µm) and a large surface area to volume ratio, with most cells adjacent to either the external environment or the alimentary canal. This suggests that the body wall and, where functional, the intestine are major routes for the removal of freely diffusible nitrogenous waste products, such as ammonia and urea. At physiological pH, most ammonia is charged (NH_4^+) but it is likely that most ammonia is excreted by nematodes in its non-ionised form (NH_3), which, unlike NH_4^+ , is freely diffusible across membranes. The significance of urea excretion in nematodes is uncertain but some vertebrate aquaporins and at least one nematode homologue can transport urea as well as water (Thompson and Geary, 2002).

7.6. Survival Strategies

7.6.1. Terminology

To survive unfavourable conditions, some nematodes are able to suspend development and survive in a dormant state until favourable conditions return. Dormancy is usually subdivided into **quiescence** and **diapause**. Quiescence is a spontaneous reversible response to unpredictable unfavourable environmental conditions and release from quiescence occurs when favourable conditions return. Quiescence can be facultative or obligate. Obligate quiescence occurs when the environmental cue affects a specific receptive stage of the nematode life cycle. For example, quiescence of unhatched juveniles is a common survival strategy among soil nematodes, including species of cyst and root-knot nematodes, *R. reniformis* and *P. penetrans*. By contrast, facultative quiescence is not stage-specific. Adverse environmental conditions and the types of quiescence they induce include cooling (cryobiosis), high temperatures (thermobiosis), lack of oxygen (anoxibiosis), osmotic stress (osmobiliosis) and dehydration, or desiccation (anhydrobiosis).

Cryptobiosis is a further term that has been used in connection with a quiescent state. Cryptobiosis is defined as a state where no metabolism can be detected, whereas dormancy involves lowered metabolism. In practice, it is frequently difficult to separate states on the basis of metabolic activity. The cause of the arrest in development is a more relevant criterion for separating states, and on this basis cryptobiosis can be viewed as the same kind of phenomenon as quiescence. Evans (1987) further distinguished between dormancy affecting ontogenetic development and that affecting somatic development.

In contrast to quiescence, diapause is a state of arrested development whereby development does not continue until specific requirements have been satisfied, even if favourable conditions return. It is either programmed into the life cycle (obligatory diapause) or is triggered by environmental stimuli (facultative diapause), such as day length.

The term ‘dauer’ is rarely used in relation to plant-parasitic nematodes but with the availability of numerous genomes, its use is likely to be increasingly justified (Perry, 2011). Dauer describes an alternative developmental stage for surviving unfavourable conditions for long periods and is a central component of the survival strategy of many free-living and entomopathogenic nematodes.

7.6.2. Quiescence, particularly anhydrobiosis

Separating different types of quiescence is somewhat artificial as many of the environmental stresses involve removal or immobilization of water. For example, desiccation concentrates body solutes and increases internal osmotic stress, exposure to hyperosmotic conditions causes partial dehydration of a nematode, and freezing may involve dehydration through sublimation of water from the solid phase.

The ability of some species of plant-parasitic nematodes to withstand dehydration for periods considerably in excess of the duration of the normal life cycle is a feature often associated with a dispersal phase, such as the cysts of *Globodera* and *Heterodera* species. Most research on the physiological and biochemical aspects of dormancy has focused on anhydrobiosis (Perry, 1999). Adaptations associated with desiccation survival serve primarily to reduce the rate of drying, either to prolong the time taken for the nematode’s water content to reach lethal low levels or, in true anhydrobiotes, to enable the structural and biochemical changes required for long-term survival to take place. Nematode anhydrobiotes can be grouped into those that depend on environmental factors to control water loss and those that have intrinsic abilities to control water loss; Perry and Moens (2011) termed these two groups **external dehydration strategists** and **innate dehydration strategists**, respectively. Both groups require controlled drying in order to survive, the first group to prolong the time to lethal low water content and the second group to enable biochemical changes to take place to facilitate long-term survival. Control of the rate of drying is the first phase; successful entry into long-term anhydrobiosis by the innate dehydration strategists depends on subsequent biochemical and molecular adaptations.

7.6.2.1. Behavioural and morphological attributes that enhance anhydrobiotic survival

The gelatinous matrix of species of *Meloidogyne*, which consists of an irregular mesh-work of glycoprotein material, shrinks and hardens when dried, thus exerting

mechanical pressure on the eggs to inhibit hatching during drought conditions and ensuring that the infective J2 are retained within the protection of the eggs and matrix. When exposed to desiccation, the permeability characteristics of the surface layers of the cyst wall and of the eggshell of *G. rostochiensis* change. The resulting control of water loss is a major factor in the survival of this species, as the hatched J2 is susceptible to environmental extremes. This susceptibility is offset by a sophisticated host-parasite interaction whereby the J2 does not hatch unless stimulated by host root diffusates (see Section 7.6.3). The ability of cyst nematodes to survive severe desiccation varies considerably between species and long-term anhydrobiosis seems to be associated primarily with those species, such as *G. rostochiensis*, that have a very restricted host range (see Chapter 4). In addition to its role in desiccation survival of unhatched J2, the eggshell enables J2 of *G. rostochiensis* to supercool in the presence of ice as a freeze-avoidance strategy for cryobiotic survival (Wharton *et al.*, 1993).

Rotylenchulus reniformis retains its moulted cuticles to protect the infective stages. J2 hatch in the soil and the subsequent moults are completed without feeding, resulting in a decrease in body volume. The young adults are enclosed in the three cuticular sheaths from the previous stages and remain inactive in dry soil over the summer months until favourable moist conditions return, allowing movement and exsheathment. The sheaths aided survival in the dry soil by slowing the rate of drying of the enclosed adult. The reduced rate of water loss assisted individuals to survive only for periods over which water loss was controlled; control of water loss merely prolongs the time taken for the nematode's water content to reach lethal low levels. Thus, control of the rate of drying does not, of itself, guarantee long-term survival but with protection of the sheaths plus the soil environment, where even if the soil water potential falls below -1.0 MPa the relative humidity in soil pores is still above 99%, survival in the dry season between crops is enhanced. Knowledge of the survival biology of *R. reniformis* formed the basis of a control strategy using alternating wetting and drying of soil; wetting the soil activates the adults and causes exsheathment, and drying the soil is lethal to the exsheathed adults.

Coiling and clumping (or aggregation) are two behavioural responses by some species of nematodes to removal of water (see Chapter 8). Nematodes will also coil in response to osmotic stress and increase in temperature. Coiling and clumping reduce the surface area exposed to drying conditions. The galls induced by *Anguina amsinckiae* contain hundreds of desiccated adults and juveniles of all stages, many of which are coiled. However, not all nematodes need to coil to survive drying. The galls induced by *A. tritici* contain tightly packed aggregates of J2 only, each of which remains uncoiled when dry. The classic image of anhydrobiotic nematodes is clumps of coiled, desiccated J4 of *D. dipsaci*, termed 'eelworm wool', associated with infected bulbs or inside bean pods. In infected narcissus bulbs, set out to dry at the end of the growing season, development is arrested at the J4 stage and hundreds emerge from the basal part of the bulb and aggregate before drying. The death of the peripheral J4 apparently provides a protective coat that, in a manner similar to the cyst wall and eggshell, aids survival of the enclosed nematodes by slowing their rate of drying. The cuticle of J4 is also able to resist water loss; the cuticular permeability barrier is heat labile and is destroyed by brief extraction with diethyl ether, indicating that an outer lipid layer, possibly the epicuticle, is involved.

A slow rate of water loss appears to allow orderly packing and stabilization of structures to maintain functional integrity during desiccation. This aspect has been

examined in detail in J4 of *D. dipsaci*, where an initial rapid loss of water is followed by a period of very slow water loss before the third phase of rapid water loss to leave individuals with no detectable water content (Fig. 7.1). The first two phases are separated by a permeability slump during which the permeability of the cuticle, and hence the subsequent rate of water loss, is reduced. During the first phase, there is a rapid shrinkage of the cuticle, the lateral hypodermal chords and the muscle cells, followed by a slower rate of shrinkage during the second phase. The contractile region of the muscle cells appears to resist shrinkage until desiccation becomes severe during the third phase (Fig. 7.1). The mitochondria swell and then shrink during desiccation, which may indicate disruption of the permeability of the mitochondrial membrane. A decrease in thickness of the hyaline layer, caused by shrinkage of its constituent muscle cells and epidermis, results in a decrease in diameter that is of a much greater magnitude than the accompanying change in length. By contrast, the reduction in the rate of water loss of *Rotylenchus robustus* is achieved by controlled contraction of cuticular annuli resulting in decreased length, but not diameter, of the nematode. Desiccation of J4 of *D. dipsaci* did not result in any appreciable denaturation of metabolic enzymes and intestinal cells changed little, possibly because the large lipid droplets they contain resist shrinkage and may prevent structural damage. Lipid reserves are maintained at high levels in many nematodes and provide a food source for the nematode after termination of dormancy and before they are able to feed on a host.

The dynamics of rehydration are also important for survival. With *A. avenae*, successful revival depends on slow rehydration in saturated atmospheres (approx. 100% relative humidity). By contrast, J4 of *D. dipsaci* rehydrate very rapidly on

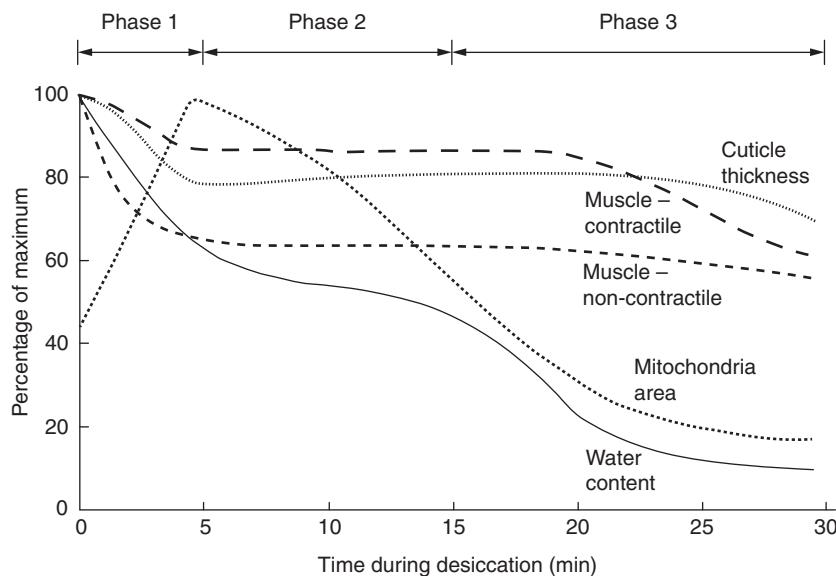


Fig. 7.1. Changes accompanying desiccation of J4 of *Ditylenchus dipsaci* following placement of hydrated individuals in 50% relative humidity at time zero. The three phases reflect differences in the rate of water loss. (From Perry, 1999.)

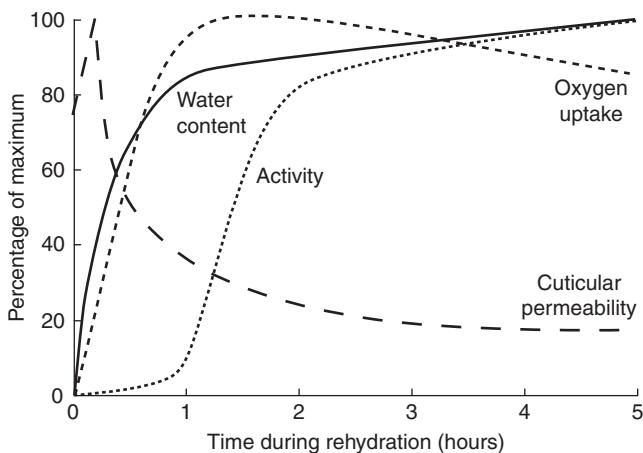


Fig. 7.2. Changes accompanying rehydration of J4 of *Ditylenchus dipsaci* following placement of desiccated individuals in water at time zero. Activity is defined as the percentage of J4s showing movement. The time difference between water uptake and activity is the 'lag phase'. (Redrawn from Barrett, 1991.)

direct transfer to water, although there is a delay, or 'lag phase', of several hours before the onset of locomotory activity (Fig. 7.2). The water permeability characteristics of the cuticles of *D. dipsaci* and *A. agrostis* are restored during the lag phase and this process can be prevented by inhibitors that block enzyme activity and post-transcriptional protein synthesis, indicating an active repair mechanism. Leakage of inorganic ions during rehydration of *A. tritici* ceases during the lag phase, indicating the repair of damaged membranes or the restoration of the permeability barrier.

Morphological changes occur gradually throughout the lag phase. Muscle cells of J4 of *D. dipsaci* increase in thickness, small lipid droplets coalesce within the intestine to form large droplets and there is a decrease in body length of *D. dipsaci*, which may indicate a contraction of the muscle cells as they recover. Metabolism of J4 of *D. dipsaci* begins immediately after hydration. The metabolite profiles recover quickly, with noticeable changes after 10 min rehydration and completion by 1 h. However, the ATP content does not recover as rapidly as those of the other metabolites; after 10 min there is little change and even after 1 h it is still low. The slow trehalose depletion (up to 48 h to return to pre-desiccation levels) may be associated with the slow recovery of ATP levels. Mitochondria swell during rehydration before adopting a normal morphology; immediately after hydration, the mitochondria are essentially uncoupled and there is no oxidative phosphorylation.

7.6.2.2. Biochemical adaptations of anhydrobiosis

Only a few species are able to survive beyond the period during which water loss is controlled. With these innate dehydration strategists, additional biochemical adaptations, reviewed by Barrett (1991, 2011), are required for long-term survival. At water

contents below about 20%, there is no free water in the cells. This 20%, usually referred to as ‘bound water’, is involved in the structural integrity of macromolecules and macromolecular structures, such as membranes. The water content of desiccated, anhydrobiotic nematodes is estimated to be about 1–5%, so it is probable that the bound water has been lost, although there is no experimental evidence that nematodes can survive the complete loss of structural water. Some molecules might replace bound water and preserve structural integrity but, in nematodes, there is only limited information on biochemical mechanisms of desiccation survival. The accumulation of the disaccharide trehalose, the only naturally occurring non-reducing disaccharide of glucose, during water loss of anhydrobiotic nematodes has been reported frequently. In *A. avenae*, glycogen and lipid reserves are converted to trehalose and glycerol, respectively. J4 of *D. dipsaci* and J2 of *A. tritici* also sequester trehalose, but not at the expense of lipid reserves; in these stages, other carbohydrates, such as myoinositol and ribitol, may be involved. There has been much speculation on the role of trehalose in desiccation protection. It may replace bound water by attaching to polar side groups on proteins and phospholipids, thus maintaining the balance between hydrophilic and hydrophobic forces acting on the molecules and preventing their collapse. Preventing crosslinkage of molecules and fusion of membranes as bulk water is removed also preserves membrane stability. Trehalose may prevent protein denaturation. Glucose reacts with the amino acid side chains of proteins to form brown pigments called melanoidins. By contrast, trehalose does not react with proteins in this way and also appears to suppress this adverse reaction of other sugars with proteins. Trehalose can act as a free radical scavenging agent to reduce random chemical damage.

Synthesizing trehalose during dehydration may indicate preliminary preparation for a period in the dry state, but it does not necessarily mean that survival during subsequent severe desiccation is assured. It appears that, following trehalose synthesis, other, at present unknown, adaptations are required at the cellular and subcellular levels for nematode survival, and rate of drying still has to be controlled.

7.6.3. Diapause

Diapause has been documented for cyst and root-knot nematodes as a strategy to overcome cyclic long-term conditions, such as seasonal conditions and/or the absence of the host, that are not conducive to hatch and infection (see Chapters 3 and 4). The incidence of diapause varies greatly between species and between populations of the same species. For example, among species of *Meloidogyne*, the percentage of unhatched J2 that enter diapause varies from less than 10% for the predominantly tropical *M. arenaria* to 94% for *M. naasi*, which has a temperate distribution.

Obligate diapause is initiated by endogenous factors and can be relieved by the J2 receiving exogenous stimuli for a required period of time. Nematodes can undergo obligate diapause only once in their life; in *G. rostochiensis* it is initiated by signals, such as photoperiod, via the host plant to the female nematode and thence to the developing juvenile. Temperature is the most important environmental cue for the termination of obligate diapause, with a fixed period of exposure to low temperatures relieving the arrested development.

Facultative diapause is initiated by exogenous, rather than endogenous, stimuli and terminated by endogenous factors after a critical period of time. This type of diapause is illustrated by the predictable periods (between autumn and spring from their second season onwards) of non-responsiveness to root diffusates of cyst nematode J2. Initiation of facultative diapause in *G. rostochiensis* is affected by both day length and low temperatures, whilst a range of environmental stresses on the female nematode initiate diapause in *H. avenae* and some *Meloidogyne* spp.

The different types of dormancy provide nematodes with a range of strategies with which to synchronise hatching with unpredictable and seasonal environmental changes. For example, the newly formed unhatched J2 of *G. rostochiensis* immediately enter obligate diapause, which is broken by the chilling stimulus of autumn and winter. In early spring, the unhatched J2 then enter obligate quiescence, which will be terminated by increasing soil temperature and potato root diffusate (PRD) to stimulate hatching of the J2. If no host is present, quiescence continues followed by facultative diapause as the unstimulated eggs enter their second winter. This combination of diapause and quiescence enables *G. rostochiensis* to persist in the soil for more than 20 years in the absence of the host plant.

7.6.4. Dauers

Dauers are a morphologically, behaviourally and physiologically adapted alternative juvenile stage for surviving unfavourable conditions (Grant and Viney, 2011). The dauer stage of *C. elegans* is enclosed by a dauer-specific cuticle and exhibits several characteristic resistance adaptations, including reduced metabolism, elevated levels of several heat shock proteins, an enhanced resistance to desiccation and a several-fold increase in trehalose levels (Erkut *et al.*, 2011). The dauer stage may be prevalent amongst nematodes as a group, including plant-parasitic nematodes, although some of the molecular information is inconclusive. In *D. dipsaci* under adverse conditions development stops at the J4 stage and, compared with J4 in a population feeding and developing under ideal conditions, the survival form of J4 has more lipid reserves and shows a propensity to aggregate (Perry and Moens, 2011), properties that reflect the dauer state. Dauers of *Bursaphelenchus xylophilus* survive on beetles of the genus *Monochamus*, using them for transport to susceptible hosts. Kikuchi *et al.* (2007) identified 31 homologues of 18 *C. elegans* genes, including nine homologues for *daf* (dauer formation) genes. *Meloidogyne hapla* has 14 orthologues of *C. elegans daf* genes (Abad *et al.*, 2008; Abad and Opperman, 2009) but it does not carry the *daf-28* orthologue, which is key in the signal transduction pathway. Abad and Opperman (2009) conclude that basic development mechanisms are conserved, although signalling is not. Comparison of expression profiles of dauer genes in *C. elegans* and in survival stages of parasitic nematodes (Elling *et al.*, 2007) reveals marked differences in expression patterns between *C. elegans* and other nematodes and there is insufficient information to be able to link individual *daf* genes to specific survival traits.

The decision of *C. elegans* to enter the dauer stage is influenced by pheromonal signals involving ascarosides, esters of short chain fatty acids to the dideoxyhexose ascarylose (Grant and Viney, 2011). Mixtures of ascarosides are produced by a wide range of nematode species, including *Pratylenchus penetrans* (Choe *et al.*, 2012); the discovery of pheromonal and other signalling roles for these molecules is likely.

7.6.5. Hatching

The eggshell affords protection to the enclosed J2. Once hatched the J2 is susceptible to environmental extremes and has to find a host and commence feeding. In some species this susceptibility is offset by a sophisticated host-parasite interaction whereby the J2 does not hatch unless stimulated by host root diffusates, effectively synchronizing hatch with the availability of nearby host plants. Although a few other species (e.g. *M. hapla*, *M. ottonsoni* and *R. reniformis*) hatch in response to host root diffusates, this phenomenon is most common among the cyst nematodes, although the reliance on host root diffusates to cause hatch varies considerably between cyst nematode species (Box 7.2). Species such as *G. rostochiensis* and *G. pallida* that have a very restricted host range are almost completely dependent on host diffusates for hatch. By contrast *H. schachtii*, for example, hatches well in water and its survival is correlated with a very wide host range (some 218 plant species, including many weeds). *Heterodera avenae* also has a large hatch in water but a relatively narrow host range; however, the hosts are very common. Most information on hatching mechanisms derives from research on *G. rostochiensis* and *H. glycines* (Jones *et al.*, 1998; Perry, 2002).

The production of the active chemicals in host root diffusate, the hatching factors (HFs), occurs over a relatively short period of plant growth in several species. *Heterodera goettingiana* hatched only in diffusates from 4- and 6-week-old pea plants, whilst hatch of *H. carotae* was largely restricted to diffusates of 5- and 7-week-old carrots. HFs are highly mobile in the soil, with hatch activity towards *Globodera* being detected up to 80 cm from the potato root zone. The first HF purified was the terpenoid, glycinoeclepin A, which induced hatch of *H. glycines*; subsequently two

Box 7.2. Grouping of some species of cyst nematodes into four broad categories, based on their hatching response to host root diffusates. (From Perry, 2002.)

Group 1	Very large numbers of juveniles hatching in response to host root diffusates; few hatching in water.	e.g. <i>Globodera rostochiensis</i> , <i>G. pallida</i> , <i>Heterodera cruciferae</i> , <i>H. carotae</i> , <i>H. goettingiana</i> , <i>H. humuli</i>
Group 2	Very large numbers of juveniles hatching in response to host root diffusates; moderate hatch in water.	e.g. <i>H. trifolii</i> , <i>H. galeopsidis</i> , <i>H. glycines</i>
Group 3	Very large numbers of juveniles hatching in response to host root diffusates; large hatch in water.	e.g. <i>H. schachtii</i> , <i>H. avenae</i>
Group 4	Hatching of juveniles induced by diffusates only in later generations produced during the host growing season; very large hatch in water for all generations.	e.g. <i>H. cajani</i> , <i>H. sorghi</i>

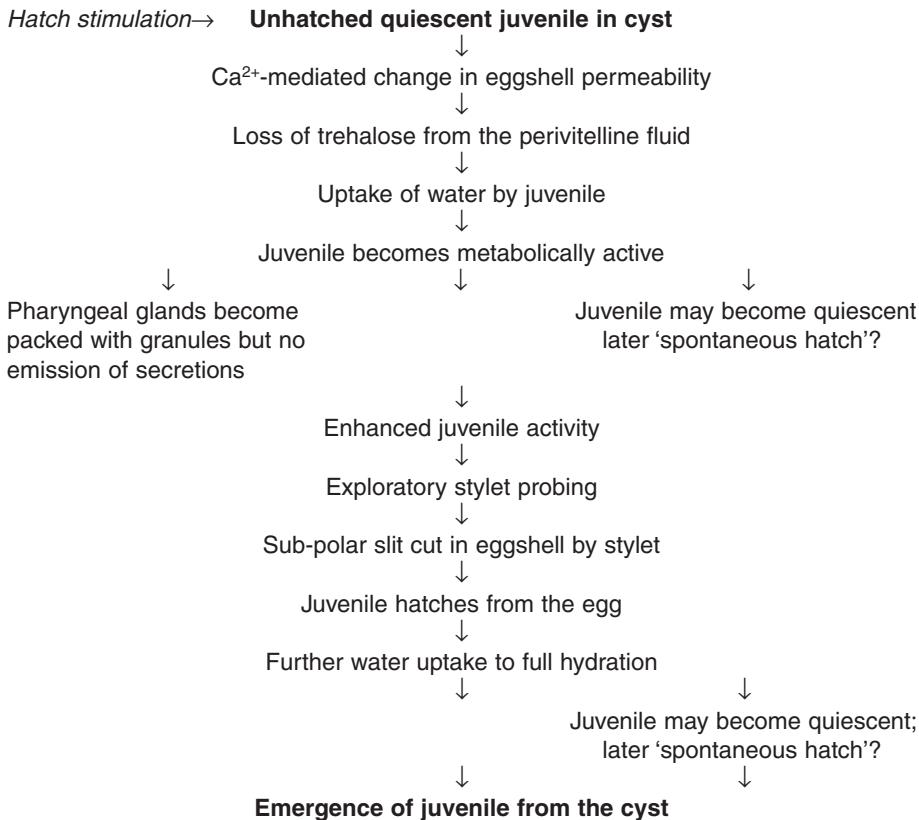
more HFs, glycinoeclepins B and C, were purified. Chemical synthesis of glycinoeclepin A has been achieved but would be prohibitively expensive for commercial use. The fractionation of potato root diffusate revealed the presence of more than ten HFs with activity towards PCN, and mass spectrometry has distinguished these into two classes of terpenoid molecules. HFs have very high specific activities, stimulating hatch of *H. glycines* and *G. rostochiensis* at concentrations as low as 10^{-10} g ml $^{-1}$. In addition, the potato steroid glycoalkaloids α -solanine and α -chaconine stimulate the hatch of *G. rostochiensis* and *G. pallida* (Jones *et al.*, 1998). *Globodera rostochiensis* hatches faster in response to these two glycoalkaloids and this may relate to the adaptation of *G. rostochiensis* to the bitter (i.e. high glycoalkaloid) potatoes of the Andes. Solanoeclepin A is a hatching stimulant for potato cyst nematodes and the total chemical synthesis of solanoeclepin A has been reported (Tanino *et al.*, 2011). As well as plant-derived HFs, there is increasing evidence for a role for microbial HFs in cyst nematodes hatching in soil.

Two other classes of hatching chemicals have been identified in potato root diffusate: hatch inhibitors (HIs) and hatching factor stimulants (HSs). HIs are produced earlier than the HFs, resulting in an initial net inhibition of hatch while the roots of the new plants become established. Subsequently, the J2 of *G. rostochiensis* hatched in response to a rise in the HF:HI ratio (Byrne *et al.*, 1998). HSs synergize the effect of HFs to increase the number and/or rate of hatching but are inactive by themselves.

There are considerable variations between species of nematodes in the sequence of events, the overlap of individual responses and their significance during the hatching process. Essentially, the hatching process can be divided into three phases: changes in the eggshell, activation of the juvenile, and eclosion (or hatch from the egg). In many species, such as *Meloidogyne* spp., activation of the juvenile appears to precede, and may even cause, changes in eggshell structure; in others, such as *G. rostochiensis*, alteration of eggshell permeability characteristics appears a necessary prerequisite for metabolic, and consequent locomotory, changes in the juvenile. Exposure of cyst nematodes to host root diffusates induces a cascade of interrelated events leading to eclosion. Most research has centred on cyst and root-knot nematodes, with the hatching mechanism of *G. rostochiensis* being best understood (Box 7.3).

Inside the egg, the J2 of *Globodera* and *Heterodera* spp. is surrounded by perivitelline fluid, which contains trehalose at a concentration of 0.34M in *G. rostochiensis* (Clarke *et al.*, 1978) and 0.5M in *H. goettingiana* (Perry *et al.*, 1980), for example. The osmotic pressure generated by the trehalose reduces the water content of the unhatched J2 and this partial dehydration inhibits J2 movement because the turgor pressure is insufficient to antagonize the longitudinal musculature. To ‘activate’ the unhatched J2, the pressure needs to be removed. In *G. rostochiensis* and some other species, this is achieved by the first event in the hatching sequence, a change in permeability of the inner lipoprotein membranes of the eggshell. This is caused by HF binding or displacing internal Ca $^{2+}$ (Box 7.4). In both *G. rostochiensis* and *G. pallida*, a 5-min exposure to PRD is sufficient to stimulate hatch, suggesting the involvement of a receptor–ligand interaction between the HF and the eggshell lipoprotein membrane. The change in eggshell permeability results in trehalose leaving the egg, permitting an influx of water and subsequent rehydration of the juvenile to a water content commensurate with movement. The presence of trehalose in eggs containing unhatched, viable J2 has been used as the basis for a viability assay for *G. rostochiensis* and *G. pallida* (van den Elsen *et al.*, 2012).

Box 7.3. Flow diagram showing events in the hatching process of J2 of *Globodera rostochiensis* after stimulation with potato root diffusate. The main components are in chronological order but there will be overlap in the sequence of some events. Secretions in the pharyngeal glands do not appear to be involved in hatching but their accumulation is a preparation for the subsequent invasion and feeding phases of the life cycle.



Box 7.4. Three classes of Ca²⁺-binding site have been distinguished in the *G. rostochiensis* eggshell (Clarke and Perry, 1985):

- (i) Sites in the outer layers that bind Ca²⁺ tightly; these sites are not involved in the hatching process.
- (ii) Sites in the lipoprotein layer, from which Ca²⁺ can be removed by hatching factors (HFs); these are proposed to be the sites associated with HF-stimulated hatch.
- (iii) Sites on the lipoprotein that bind additional Ca²⁺ ions in the presence of HF.

Atkinson and Taylor (1983) reported a sialoglycoprotein with high Ca²⁺ affinity that was involved in the hatching process, which may correspond to Ca²⁺-binding site class (ii).

The involvement of enzymes in eggshell permeability change has been postulated in several species. A Zn^{2+} -dependent enzyme mediates hatching of *H. glycines* and leucine aminopeptidase activity was found in the egg supernatant, although root diffusate does not increase its activity. Apparent softening of the eggshell before eclosion occurs in *X. diversicaudatum*, *A. avenae* and *M. incognita*, and in *M. incognita* lipase activity has been correlated with hatch. By contrast, the eggshell of *G. rostochiensis* remains rigid during the hatching process and there is no evidence of enzyme involvement. Devine *et al.* (1996) demonstrated that the potato steroidal glycoalkaloids, α -solanine and α -chaconine, induce hatch of *G. rostochiensis*; glycoalkaloids are known to destabilize lipid membranes, during which leakage of trehalose is possible.

Several other events accompany rehydration. Within 24 h of exposure of unhatched J2 of *G. rostochiensis* to root diffusate, O_2 consumption and utilization of lipid reserves commences, the adenylate energy charge falls while the content of cAMP (a possible secondary messenger in receptor-ligand interactions) rises. These effects on the rehydrated J2 are due in part to removal of osmotic pressure and hydration and in part to direct stimulation of the J2 by root diffusate. Changes in gene expression appear to occur during or immediately after the hatching process.

Vigorous movement of the J2 does not begin until at least 3 days after initial exposure to root diffusate, when the J2 starts local exploration of the inner surface of the egg, using its lips and stylet, and then begins thrusting movements with the stylet. This causes a regular pattern of perforations in the sub-polar region of the eggshell, which the J2 extends to a slit through which it hatches. Such use of the stylet to produce holes in the eggshell relies on the eggshell remaining rigid. J2 of *D. dipsaci* use a similar approach, except that the stylet thrusts are more random and the J2 uses its head to force open the slit in the eggshell. In *P. penetrans* and *H. avenae*, a single stylet thrust penetrates the eggshell and the head extends this into a tear. J2 of *H. iri* use the tail tip to make the initial penetration.

The integration between host and nematode to ensure survival and invasion has progressed furthest in cyst nematodes and the sophisticated hatching mechanism is one aspect of this integration. Hatching patterns of cyst nematodes with multiple generations during the host-growing season are further survival adaptations. In several species of tropical cyst nematodes, with many generations during a crop growing season, there are changes in the hatching biology (Perry and Gaur, 1996). For example, under favourable conditions, *H. glycines* produces most eggs in egg sacs and J2 in these eggs hatch readily in water to invade the host and result in a rapid population build-up. Under less favourable conditions, there is a shift towards production of encysted eggs, which have a greater requirement for hatch stimulation. In *H. cajani*, this shift occurs at the end of the host-growing season and is accompanied by increased energy reserves in the unhatched J2 to further enhance survival in the absence of a host.

8

Behaviour and Sensory Perception*

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* A revision of Robinson, A.F. and Perry, R.N. (2006) Behaviour and sensory perception. In: Perry, R.N. and Moens, M. (eds) *Plant Nematology*, 1st edn. CAB International, Wallingford, UK.

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8.1. Sense Organs or Sensilla

In this chapter behaviour is narrowly defined as neuromuscularly controlled movements of the nematode body or its parts that result in: (i) travel or rotation of the nematode through space (locomotion); (ii) events of physiological or developmental importance (hatching, feeding, copulation, egg laying, defecation, root penetration); and (iii) postures or integrated movements of groups of nematodes (coiling, clumping, swarming and nictating) enhancing survival and phoresis. In order to perform these behaviours, nematodes need to assimilate information from their external environment via sense organs or sensilla that are of two basic types: **internal** and **cuticular**. Internal sensilla are mechanoreceptors or, less frequently, photoreceptors, whereas the cuticular sensilla detect a much greater range of stimuli, including chemical, mechanical, temperature and osmotic pressure. The fundamental structure of a sensillum (Fig. 1.5F) comprises three basic cell types: a glandular sheath cell, which is deeply folded with a very large surface area, a supporting socket cell, surrounding the duct that encloses the distal regions of the receptors, and a number of bipolar neurons, or dendritic processes, which are bathed in secretions. The main concentration of cuticular sensilla is on the head of the nematode (Fig. 8.1), consisting of a hexaradiate pattern comprising six lips containing 12 labial sensilla, four cephalic sensilla and two amphids. These are arranged in three circles consisting of an outer circle of four cephalic sensilla, a middle circle of six outer labial sensilla and an inner circle of six inner labial sensilla (see Chapter 1).

8.1.1. Chemosensilla

Chemicals that cause interactions between organisms are called **semiochemicals**, which include **allelochemicals**, mediating interspecific responses such as nematodes responding

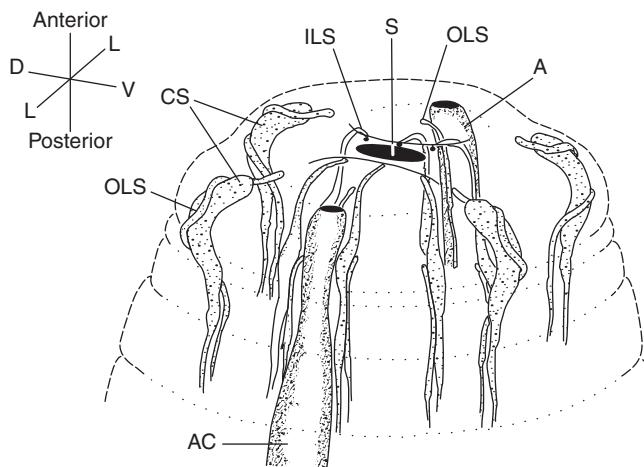


Fig. 8.1. Diagrammatic reconstruction summarizing the form and arrangement of the anterior sensilla of *Pratylenchus* spp. A, amphid; AC, amphidial canal; CS, cephalic sensillum; ILS, inner labial sensillum; OLS, outer labial sensillum; S, stoma. (From Perry and Aumann, 1998.)

to diffuse from host roots, and **pheromones**, mediating intraspecific responses such as male nematodes responding to sex pheromones from female nematodes. Semiochemicals may have volatile and non-volatile components. Sensilla that open to the environment have long been assumed to have a function in detecting semiochemicals.

The amphids are considered to be the primary chemosensilla but evidence from work on the free-living nematode, *Caenorhabditis elegans*, shows that they also have thermosensory and mechanosensory roles. Amphids are paired organs, positioned laterally with cephalic or cervical external openings, and contain several sensory receptors (the number varying among taxa). The socket cell surrounds the amphidial duct; often the socket cell secretes copious material into the duct. The ciliary region of the receptors is enclosed by the sheath cell and within the sheath cell, and near the base of the duct, gap junctions occur between adjacent receptors. Typically, the amphid sheath cell is deeply folded or otherwise modified, resulting in a very large surface area. In *Meloidogyne* males and to a lesser extent in second-stage juveniles (J2), for example, the sheath cell has many extracellular, fluid-filled caverns continuous with a larger pouch surrounding the receptors.

Phasmids are specialized pairs of sensory organs situated in the posterior lateral field (Fig. 1.3). Typically the structure is that of a simple chemoreceptor, including one or two neuron receptors, each with a typical ciliary region surrounded by a sheath cell; the receptors terminate in a duct that opens to the exterior. The duct is surrounded by one or two secretory socket cells. Although there is much information on the ultrastructure of the phasmids of many species of nematodes, there is only speculation about their function.

The socket and the sheath cells produce secretions, which may be specialized for parasitism. However, the secretions in the amphidial duct may derive only from the socket cell, where there is continuity between secretory vesicles and the secretions in the duct, rather than from the sheath cell, where there seems to be no direct contact with the amphidial duct as the receptor cavity of the sheath cell appears closed (Baldwin and Perry, 2004). The secretions may protect the dendritic ending of the nerve cells from desiccation or microbial attack, or may maintain electrical contact between the tips and the bases of the dendritic processes. Secretions from the posterior supplementary sensilla are likely to aid adhesion in copulation. The compounds present in the secretions are undoubtedly important in chemoreception and include N-acetylgalactosamine and fucose in J2 of *Meloidogyne incognita* and O-glycans in J2 of *Heterodera schachtii*, with mannose or glucose, N-acetylglucosamine and galactose and/or N-acetylgalactosamine forming the oligosaccharide chains. There is evidence that the composition of amphidial secretions differs between species. A glycoprotein associated with amphidial secretions of J2 of six species of *Meloidogyne* was not present in representatives from eight other genera that included *Globodera* and *Heterodera*, indicating a more specialized function for this protein in *Meloidogyne*. Secretions also may change at different stages of the life cycle of an individual species, perhaps reflecting different functional requirements.

8.1.2. Mechanosensilla

Mechanosensilla terminate beneath the cuticle surface and the ducts do not open to the exterior, although they may contain secretions that might aid in mechanical transmission.

Many mechanosensilla are expressed at the cuticle surface as raised areas, or bumps, termed papillae. In general, the inner, outer and cephalic papillae (Fig. 8.1) are the main concentration of anterior mechanoreceptors, although in Tylenchomorpha the inner labial papillae are chemoreceptors. Mechanosensory perception is involved in locomotion as well as responses to external stimuli, such as tactile responses during copulation mediated by papillae in the tail region, and internal processes, such as egg laying and pharyngeal pumping. The involvement of mechanosensory perception (as well as chemoreception) in hatching and selection by endoparasitic nematodes of root penetration sites has been inferred from observations on pressing and rubbing of the anterior end accompanied by stylet probing.

8.1.3. Other sensilla

Nematodes can exhibit extremely sensitive thermotaxis (see Section 8.4.2). It is not known which sensilla are thermoreceptors in plant-parasitic nematodes, although in *C. elegans* the amphids contain a neuron that is thought to detect thermal cues. Photoreception in certain marine and insect-parasitic nematodes is mediated by photoreceptive sensilla termed ocelli, which are associated with a pair of red/brownish pigmented spots on either side of the pharynx, or lie within the central pharyngeal region and are shaded by a hollow cylinder of haemoglobin pigment that provides directional resolution.

8.2. Undulatory Propulsion

With only few exceptions, nematodes move by dorsoventral undulations of the body that are propagated backwards from the anterior end. As a result, on flat surfaces they tend to follow sinusoidal paths, as do snakes, but while lying on their sides rather than their ventral surface. *In vitro*, vermiform stages of plant-parasitic nematodes in water and on agar typically exhibit rhythmic endogenously generated body waves that cease or reverse only when an obstacle or some other discontinuity in an otherwise homogeneous substrate is encountered. The typical response to an obstacle is to stop, withdraw one or two waves, probe in one or more directions with the anterior end, and then resume rhythmic forward movement in a new direction. In nature, plant-parasitic nematodes are usually on or within highly heterogeneous substrates, such as soil, plant tissue or foliar surfaces, where rhythmic propulsion is likely to be interrupted frequently.

The undulatory waveform varies with environment and species. Substrate resistance reduces wave amplitude, wave length and speed of wave propagation, and in water where resistance is minimal some species propagate waves ten or more times as fast as others. However, the rate of forward progression, ignoring substrate-dependent slippage, is strictly determined by the speed of wave propagation in almost all nematode species utilizing undulatory propulsion (Fig. 8.2). Moreover, in at least some species, observations show that the waveform can be differentially modulated along the length of the body in response to external forces or stimuli, thus allowing efficient passage over irregular surfaces; this may also occur in nematodes within soil, plant tissues or other habitats where they are rarely or never observed.

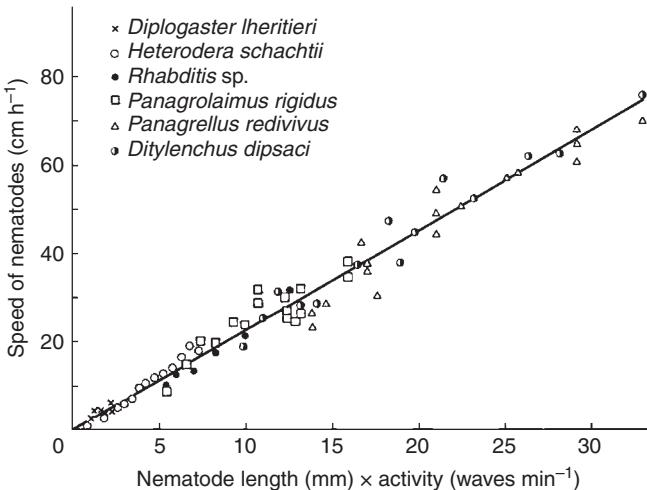


Fig. 8.2. Speed of propulsion by morphologically and ecologically diverse nematodes on a moist glass surface. (From Wallace, 1958b.)

8.2.1. Internal mechanics

Microscopic nematodes are not thought to be strong enough to dislodge most soil particles, thus restricting movement to channels within the soil. However, their bodies are clearly stiff and can transmit sufficient axial force to burrow through pluronic gel and dilute (0.5–1.5%) agar. This stiffness derives from a high internal pressure or turgor, which provides a hydrostatic skeleton. High internal pressure appears to be built up and maintained by pumping action and valves of the metacorus. Crossed fibres within the basal zone or layer of the cuticle offer a mechanical explanation for the observation that upon osmotically induced swelling, increases in circumference were restricted and most volume increase resulted primarily from increased length (Alexander, 2002). Anatomical evidence indicates additional factors contribute to body stiffness (Burr and Robinson, 2004). The sinusoidal waveform is produced by alternate contraction of the dorsal and ventral longitudinal muscle trunks of the body wall antagonized by the hydrostatic skeleton. Other antagonistic factors include stiffness of the muscle fibres themselves, elastic restoring force of the cuticle, and compartmentalization of turgor, especially between the so-called muscle bellies and the gut. The relative proportions of cuticle thickness, muscle belly diameter and body diameter vary more than tenfold across nematodes. Thus, it is reasonable to assume that the relative importance of these factors also varies.

Undulatory locomotion in nematodes presents six requirements related to neuromuscular control (Box 8.1). Although most is known about creation and propagation of the dorsal–ventral wave, understanding how all requirements might be met requires familiarity with nematode sensory structures (see Section 8.1) and elementary knowledge of nematode neuromuscular anatomy and function, much of which derives from work on *C. elegans* and the animal-parasitic nematode, *Ascaris suum* (Wright and Perry, 1998). Muscles consist of bundles of parallel elongated cells called muscle fibres. Each fibre in turn contains, in addition to essential cell organelles and a highly

Box 8.1. Requirements of neuromuscular control for nematode undulatory locomotion (Burr and Robinson, 2004).

1. Creation of a reciprocating pattern of tension and relaxation on dorsal and ventral sides of the body.
2. Propagation of this pattern along the body posteriorly for forward locomotion and anteriorly for backward locomotion.
3. Control of the switching from forward to backward locomotion based on sensory input.
4. A rhythmic pattern generator to generate the waves cyclically.
5. Regulation of wave frequency, amplitude, wavelength and rate of propagation according to environmental requirements.
6. Utilization of sensory feedback to adjust waveform according to the location of objects along the body.

specialized endoplasmic reticulum, a stack of contractile units (sarcomeres) positioned end to end along the fibre's length and separated by so-called z-lines, which serve as connective tissue within and between contractile units. Each sarcomere contains parallel and overlapping molecules of the linear contractile proteins myosin (also called thick filaments) and actin (called thin filaments) within each sarcomere so that each thick filament is positioned centrally and thin filaments are attached on one end to a z-line with the other end projecting into and partly overlapping the thick filaments. During muscle contraction, membrane depolarization events create a strong molecular affinity between thick and thin filaments, causing them to slide across each other, maximizing the overlap and shortening the sarcomere. Taken together, the alignment of z-lines and overlapping regions of thick and thin filaments gives rise to the striated appearance; somatic muscles in nematodes are obliquely striated.

Somatic muscles are oriented parallel to the body axis as two dorsal and two ventral muscle trunks. They are unusual in that each sarcomere is connected perpendicularly to the site of force application, the cuticle, by z-line functional equivalents consisting of a geometric arrangement of dense bodies and basal lamina that project into the fibre. The basal lamina, epidermis and cuticle are thought to comprise three tightly bound layers. Thus, the nematode body wall, consisting of somatic muscle, epidermis and cuticle, functions as a single differentially contractile organ (Burr and Robinson, 2004). As there are no circumferential muscles in nematodes, local constrictions in volume do not occur. Cuticular annuli allow for compression during bending of the body, but the magnitude of the longitudinal restoring force within the cuticle has not been measured, nor has its longitudinal elastic modulus during stretching in most species. However, it is clear that the nematode cuticle has little circumferential elasticity and longitudinal elasticity appears limited to a relatively narrow increase in length.

8.2.2. Sensory transduction

The organization of the nematode nervous system (see Chapter 1) is essentially conservative, and information from the two best-studied species, *C. elegans* and *A. suum*,

is likely to predict neuronal organization in other species (Martin *et al.*, 2002; Baldwin and Perry, 2004). The main mass of the central nervous system is the circumpharyngeal nerve ring, and the majority of nerve processes run longitudinally, as ventral and dorsal nerve chords, or circumferentially, as commissures. The neurons have a simple, relatively unbranched morphology and a single gap junction is sufficient for functional coupling between neurons.

Much of our understanding of neurotransmission comes from work on animal-parasitic nematodes and, especially, from *C. elegans* (Perry and Aumann, 1998; Perry and Maule, 2004). The initial pre-interactive events include passage of signals from the external environment through the sensilla secretions to the membrane-bound receptors of the nerve cells. Passage through the secretions is presumed to be facilitated by olfactory binding proteins (OBPs) that bind hydrophobic molecules, transport them through the secretions and deliver them to the receptors; however, convincing evidence of OBPs in plant-parasitic nematodes has yet to be obtained (Jones, 2002). On stimulation of a nerve ending, a neurotransmitter is released that triggers a downstream signalling event/cascade that results in a behavioural change. Further details of this process are given in Chapter 7.

Stimulation of a particular chemosensory neuron of *C. elegans* results in a distinct behavioural response (attraction or avoidance) demonstrating that the neurons are 'hard wired' and it is the neuron that determines the response not the nature of the receptor molecule being stimulated (Bargmann and Horvitz, 1991). Some receptors are expressed in a single sensory neuron whilst others are present in up to five sensory neurons.

8.2.3. Neuromuscular control

Sinusoidal waveforms are enabled by separate innervation of dorsal and ventral muscle trunks by their respective nerve chords along most of the body length. Innervation is achieved via somatic muscle arms that extend to and synapse only with their respective nerve chords (dorsal or ventral). Thus, contraction along most of the body is possible only in the dorsal–ventral plane and there is evidence that commissures between the dorsal and ventral nerve chords provide reciprocally inhibitory innervation. However, in the anterior end, additional innervation permits complex behaviours involving localized movement of the anterior in other planes, as observed during feeding, hatching, mating and tissue penetration. The most comprehensive information on sensory neurons and the circuitry by which forward and backward movements are triggered and effected derives from work on *C. elegans*, where numerous neurons and synapses have been carefully mapped and studied (see Section 8.6).

8.2.4. Transfer of forces and propulsion

The primary variables influencing nematode propulsion are body waveform geometry and forces arising from the nematode and its surroundings (Box 8.2).

Each sinusoidal body wave can be considered a locomotory unit (Fig. 8.3) exhibiting reciprocal inhibition of opposing dorsal and ventral sections of the longitudinal

Box 8.2. Variables influencing nematode undulatory propulsion.

(A) Geometric variables

1. Body wavelength.
2. Body wave amplitude.
3. Wavelength-to-amplitude ratio.
4. Body diameter-to-wavelength ratio.

(B) Forces

1. Net contractile force of body wall and speed of contraction (determines power).
2. Axially transmitted resistance (determines ratio of power to speed and energy lost to heat of friction):
 - (a) from friction along body:
 - (i) substrate resistance;
 - (ii) cuticular resistance; includes changes in coefficient of resistance of cuticle when compressed and expanded;
 - (b) from force against head penetrating substrate.
3. Substrate resistance locally perpendicular to body axis (determines slippage and energy lost to work done to surroundings).

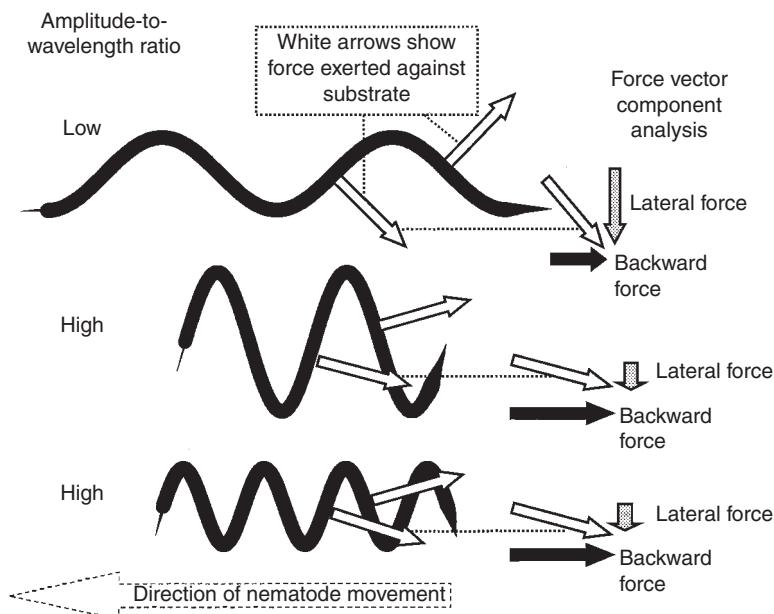


Fig. 8.3. Effect of amplitude-to-wavelength ratio on backward force exerted by nematodes when moving forward.

body muscles (Alexander, 2002; Burr and Robinson, 2004). Comparisons of cuticular folds show that dorsally convex regions of curvature along the body axis are dorsally extended and ventrally contracted, and conversely for ventrally convex regions. During forward movement, when waves are propagated backwards, the relatively straight regions of longitudinally oriented body trunk muscles just behind those that are maximally contracted ventrally are undergoing ventral contraction, while those immediately ahead are ventrally distending. Thus, although the precise way that wave propagation is controlled neurologically is uncertain, reciprocal anterior–posterior as well as dorsal–ventral motor inhibition is indicated, with different inhibitory relationships for forward and backward movement, and candidate neural processes have been identified.

Vector analysis can be applied to understand essential relationships between waveform, external resistance and internal contractions (Fig. 8.3). To move, a nematode must overcome surface tension and friction by pushing its angled body against the substrate laterally (more precisely dorsally and ventrally) relative to the sinusoidal body axis but obliquely backwards to either side of the direction of travel of the nematode's centre of mass. When a vector representing the obliquely backwards force applied perpendicular to the body axis is resolved into components perpendicular and parallel to the direction of travel, the relative magnitude of the backward component useful to overcome resistance clearly varies directly with the amplitude-to-wavelength ratio. Thus, it is not surprising that nematodes characteristically increase the amplitude-to-wavelength ratio upon encountering increased substrate resistance. The angle of leverage against obstacles or tracks in the substrate is thereby increased. However, the speed of propulsion is typically decreased so that overcoming greater substrate resistance with perhaps no additional strength (strictly, power) is achieved at the cost of speed, much like shifting a bicycle into a lower gear. Conversely, most nematodes that normally move on solid or semi-solid substrates exhibit body waves with greatly exaggerated wavelength and speed when suspended in water, which offers relatively little resistance to movement.

Microscopic nematodes are at the limit of size of organism at which the viscosity of water surrounding the body becomes a major force and they differ greatly in ability to negotiate free water and water-saturated porous substrates, as discussed in Section 8.3. The primary factors affecting efficiency of swimming by any animal are size, shape, speed, inertia and viscosity. The dimensionless index known as the Reynolds number predicts the relative importance of inertia and viscosity for a given shape, size and speed; for small nematodes in general the value has been estimated as 0.01. When the Reynolds number is this small, animals swimming in water are so strongly influenced by viscosity that they must use non-reciprocal motions, such as undulatory propulsion, or they will continually pull themselves back and forth with no net change in position (Dusenberry, 1996). When moving while held by surface tension within a film of water on a solid substrate, viscosity contributes to substrate resistance and purchase. Additional factors potentially influencing nematode propulsion but little investigated include the body diameter-to-wavelength ratio, wrinkling and smoothing of the outer layer of the cuticle during undulations and differences in general cuticular topology among species. The latter will be discussed briefly in relation to peristaltic movement in *Criconemoides* spp. (see Section 8.5).

8.3. Random Movement

As discussed in Section 8.2, the rate of progression of any organism depends on: (i) its intrinsic speed and strength; (ii) the resistance offered by its environment; and (iii) the efficiency with which it can gain purchase to overcome that resistance. Intrinsic speed and strength in turn can be influenced physiologically or via sensory input and the maximal rate of movement often appears attuned to ecological needs.

8.3.1. Physiological factors influencing activity

Probably the most important physiological factors influencing the rate of nematode movement are temperature, oxygen availability, toxins, water and ionic status, energy reserves and the nematode's state of activation as influenced by developmental triggers. As poikilotherms, nematodes tend to increase activity with increased temperature up to some limit; however, specific thermal optima for activity differ greatly among species and appear ecologically tuned (Fig. 8.4). In a study comparing nematodes from a common geographic region (southern Texas, USA), infective juveniles of two root parasites (*Rotylenchulus reniformis* and *Tylenchulus semipenetrans*) were found to have thermal optima for motility 10°C higher than those of two foliar parasites (*Ditylenchus dipsaci* and *D. phyllobius*), consistent with the difference between the warm median soil temperature in the summer, when most root growth occurs, and the need for foliar parasites to be maximally active during the cool rainy period of the summer, when the foliar moisture films required for infection of foliar buds are present. Ecological adaptation in speed of movement is afforded by the low energy cost of locomotion in microscopic nematodes, due to their small size

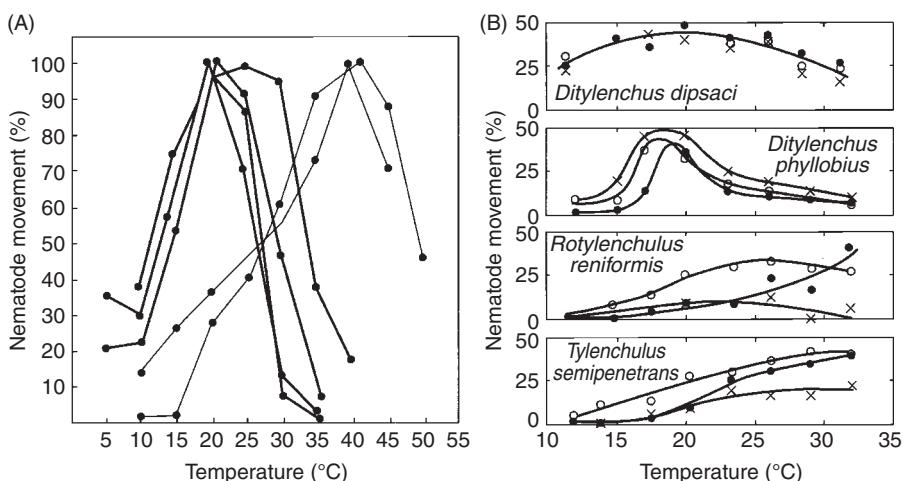


Fig. 8.4. Effects of temperature on nematode activity. A: Motility of skin penetrating stages of parasites of warm-blooded hosts (thin lines) contrasted with plant parasites (bold lines) (from Croll, 1975). B: Random dispersal on agar by foliar and stem parasites (*Ditylenchus dipsaci* and *D. phyllobius*) contrasted with root parasites (*Rotylenchulus reniformis* and *Tylenchulus semipenetrans*) from the same region of Texas. (From Robinson, 1989.)

(Alexander, 2002), and further enhanced in the infective stages of plant-parasitic species through the space-efficient storage of food reserves as lipid rather than glycogen (Barrett and Wright, 1998; Barrett, 2011; see Chapter 7).

In vitro, solutions with high osmotic pressure rapidly desiccate most nematodes. However, as will be discussed, except in highly saline soils, osmotic pressure within soil and plant tissue is unlikely to influence the water balance of plant-parasitic nematodes as the soil dries because most water will be extracted from nematodes by other forces before osmotic pressure is physiologically important. Moreover, the surface tension of soil water will bind nematodes tightly to the soil before physiologically critical levels of osmotic pressure in the soil develop. Limited data available for effects of low oxygen tension on plant-parasitic nematodes indicate that activity of most species appears unaffected until the ambient partial pressure (normally 20%) drops below 1 or 2%. Thus, over most of the range of oxygen tension that plant-parasitic nematodes are likely to encounter within plant tissue and agricultural soil, activity is unlikely to be suppressed or stimulated. However, symptoms of carbon dioxide (CO_2) intoxication in plant-parasitic nematodes have been reported at concentrations as low as 5%, which is well within the range that can be expected in waterlogged soils with a high biological oxygen demand. Although many saprophytic nematodes appear able to withstand strongly hypoxic, reducing conditions, plant-parasitic nematodes as a general rule are quickly intoxicated and rendered immobile by oxygen deprivation or high ambient concentrations of CO_2 and nitrogenous waste products.

Plant-parasitic nematodes typically move spontaneously in water and agar except during moulting lethargus. Although reports of environmental cues triggering activity are generally lacking for plant-parasitic nematodes, it is not known to what extent exogenous stimuli may regulate the level of activity of plant-parasitic nematodes when within opaque matrices such as soil and plant tissue.

8.3.2. Physical factors influencing the occurrence and rate of movement

Substrates through which nematodes move may be porous or non-porous and porosity is scale-dependent. For example, freshly prepared agar is non-porous from a nematode's perspective but porous at the molecular level. Soil is particulate and is effectively solid for a nematode if pores are too small or too dry to permit nematode movement. Pores may be partly or completely filled with water. Yeates (2004) noted that nematodes can be considered **interstitial** or **pellicole**, based on the water content of the substrates they typically occupy. Interstitial nematodes occupy interstices completely filled with water or other fluid (marine sediments and many animal tissues), whilst pellicole nematodes occupy pores or channels filled only partly with water or other fluid (moist soil, spongy foliar tissue, leaf galls). Substrates with partially filled interconnected pores lined with water films allow rapid diffusion of gases and volatiles over relatively long distances through the substrate and expose nematodes to powerful surface tensions within water films.

Any substrate on which nematodes move can be considered an interface, which on either side may be solid, semi-solid, porous, liquid or gas. As movement along an interface constrains motion to one or two dimensions, the rate of random dispersal along an interface should be faster than random dispersal in three dimensions. When moving on an interface, such as the root epidermis or the surface of stems and leaves,

topology is very important. Generally a moisture film must also be present and nematodes can be drawn into depressions such as embryonic leaf folds where moisture films recede as the surface dries. Obviously, some regions constrain motion largely but not entirely to one or two dimensions. For example, a moist stem surface covered with trichomes (leaf hairs) through which nematodes thread themselves is a two-dimensional interface but might more accurately be considered a narrowly bounded porous substrate where space for movement is less than 1 mm radially, and 10 mm circumferentially and 100 mm axially. Thus, the expected magnitude of random dispersal in those directions is greatly influenced by topology.

All soil classification systems recognize that soil particles fall into three classes: (i) those that are colloidal (stay suspended by Brownian movement) and have electrically charged surfaces largely due to laminated aluminium silicates (clay); (ii) those that are relatively large and primarily uncharged silicon dioxide particles (sand); and (iii) those that are between these two in size and charge characteristics. The quantity of clay and silt in most agricultural soils predicts that if small particles did not bind together only soils comprised largely of medium to coarse sand would offer sufficiently large pores between particles to permit passage by most kinds of soil-inhabiting nematodes. However, this is clearly not the case for reasons that we shall examine next.

The energy required by plants to extract water from soil depends on the Gibb's free energy of soil water, which is a cumulative measure of the osmotic pressure of soil water, the pull of gravity and the powerful attraction of water to charged soil particle surfaces, known by plant physiologists as matric potential. For plants, the soil water matric potential is the primary factor affecting plant-water status. In agricultural soils it typically ranges from 0 to negative 15×10^5 Pa, the so-called permanent wilting point for plants. When soil is irrigated, a wetting front moves down through the soil profile saturating the soil interstices. In time, gravity partially drains the soil pores and air channels form, connecting the soil with the atmosphere. At this point, the soil is said to be at field capacity water content, which usually occurs at a matric potential near 5×10^3 Pa.

Classical experiments by Wallace in the 1950s and 1960s (summarized by Robinson, 2004) demonstrated, firstly, that it was not the soil texture that determined the suitability of soil for nematode movement but rather the soil crumb size, i.e. the porosity of the soil from a nematode's perspective (Fig. 8.5). He found, secondly, that the total amount of water per se in soil was not critical; what really mattered was the matric potential and the optimum matric potential was that observed at field capacity (Fig. 8.5). This is very important because finely textured soils can retain several times as much water as sandy soils at the same matric potential. Thus, the truly useful predictor of the suitability of the soil-moisture level for nematode movement was not the water content but the energy required to extract water, which is directly related to the surface tension and thickness of water films within the soil. Wallace also found that nematode movement within soil was restricted to a narrow range of matric potentials compared with the range over which plants can extract water and survive, and it was already well established that, except in highly saline soils, increases in osmotic pressure in soil water as a result of evaporation is physiologically insignificant to plants compared with the soil matric potential. Subsequently, other work demonstrated that osmotic pressure was of no importance to nematode movement in natural soils (Fig. 8.5).

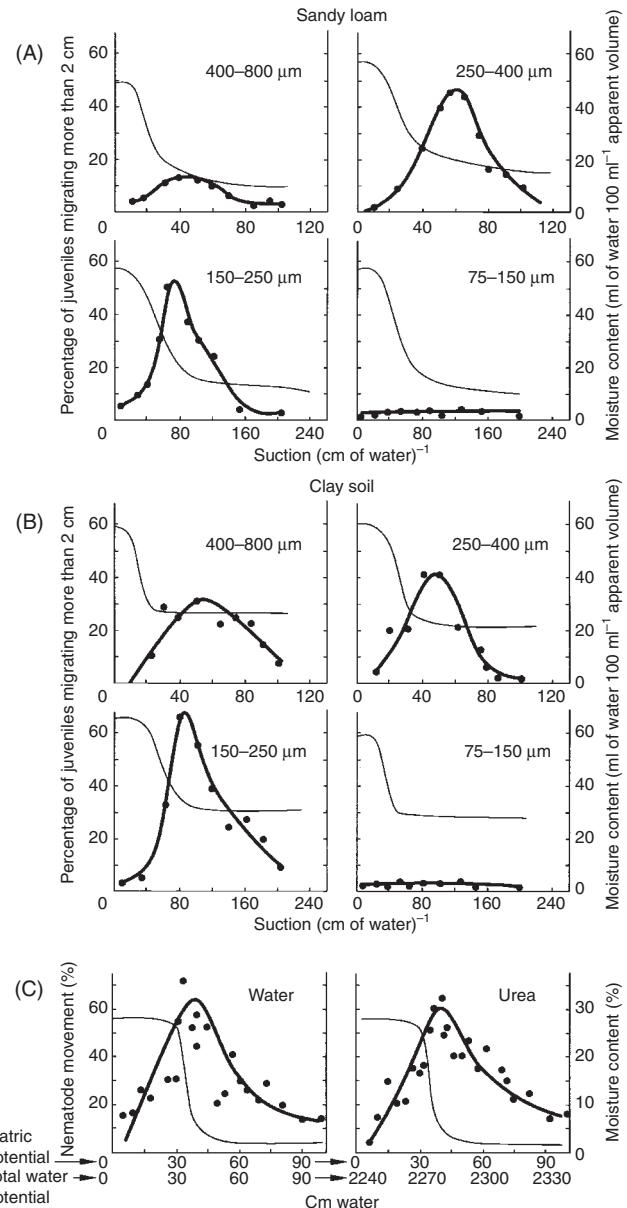


Fig. 8.5. Factors affecting nematode movement through soil (Wallace, 1968). A, B: Soil texture is only indirectly important when compared with soil structure and matric potential. Similar effects in texturally contrasting sandy and clay soils are observed when movement by *Globodera rostochiensis* is compared in relation to soil structure (crumb diameter in μm) and water potential (cm of water). C: Matric potential is far more important than osmotic pressure over ranges that occur in natural soils. The marked influence of soil matric potential on movement by *Ditylenchus dipsaci* in sand wetted with water (left) is unaltered when 0.1 M urea is substituted for water to elevate osmotic pressure independently of soil matric potential (the energy required to extract water mechanically). (From Blake, 1961.)

Electrical charge separation on soil particles, particularly in the clay fraction, is of great significance to agricultural soil fertility and nematodes. Surface charges bind ionic species in the soil, providing a reservoir of plant nutrients and facilitating the establishment and collapse of gradients of ionic nematode attractants and repellents because specific ions are removed and added to different regions of the soil by plant roots, biodegradation, leaching, irrigation and fertilizer applications. Many nematodes are attracted and repelled by various salts, and attraction of root-knot nematodes to roots can be enhanced by addition of small amounts of clay to pure sand. Surface charges on soil particles also favour plants and nematodes by binding soil particles together to create a structure consisting of larger diameter aggregates than would exist otherwise, thus increasing aeration, decreasing resistance to water percolation, and providing larger pores for nematodes to move through.

The pH of soil is typically buffered by ammonium, carbonates, sulphates and phosphates often bound to or comprising soil particles. Their concentrations are changed by plant roots and the soil microflora and vary markedly with depth and distance from roots. Thus, pH and any substance whose concentration is pH-dependent (such as CO₂, see Section 8.4.3) are likely to establish concentration gradients in the soil providing information regarding depth, vertical orientation and the location of roots.

8.4. Movement in Response to Stimuli

In Section 8.3, we discussed physiological and physical factors that influence the activity of plant-parasitic nematodes. In this section, the response of plant-parasitic nematodes to various stimuli in the soil is examined. While in the soil, plant-parasitic nematodes are dependent on their food reserves and need to locate a host rapidly in order to feed and develop. For example, under optimal conditions for movement, J2 of *Globodera rostochiensis* have an infective life of only 6–11 days after hatching. Around actively growing roots there exist several gradients of volatile and non-volatile compounds, including amino acids, ions, pH, temperature and CO₂. It is evident that nematodes orientate towards the roots using at least some of these gradients and this enhances the chances of host location and reduces the time without food.

Evaluating the reality of the attractiveness or otherwise of an individual compound is difficult. Much of the available information is based on *in vitro* behavioural bioassays, which bear little if any resemblance to the situation in the soil; care must therefore be exercised in extrapolating from such assays to the field situation (Spence *et al.*, 2009). However, some generalizations can be made and certain gradients are strongly implicated in orientating nematodes to the roots. Perry (2005) separated gradients into three types: (i) long-distance attractants that enable nematodes to move to the root area; (ii) short-distance attractants that enable the nematode to orientate to individual roots; and (iii) local attractants that are used by endoparasitic nematodes to locate the preferred invasion site. In the following sections, some of the major stimuli in soil will be discussed and related to these three types.

8.4.1. Fundamental concepts and terms in nematode behaviour

The terminology used here to define responses of organisms to environmental cues is that of Burr (1984) as modified by Dusenberry (1992) (Box 8.3). The most important concepts are **migration**, **taxis** and **kinesis**. All are exhibited by nematodes. **Migration** is movement of an individual or population in a direction oriented with respect to a stimulus field and can be accomplished by taxis or kinesis. **Taxis** results from orienting the body to the stimulus direction, whereas **kinesis** results from undirected responses to changes in stimulus intensity, for example a change in forward speed (orthokinesis) or in random turn frequency (klinokinesis). Orientation can result from comparing stimulus intensity sequentially at two or more points in time (klinotaxis) or simultaneously at two points in space (tropotaxis) or many points in space (teleotaxis). Orientation can be positive, negative or transverse. Prefixes can denote operative stimuli (Box 8.4). When applying these concepts to nematodes, one should remember the dorsal–ventral orientation of body waves as well as the bilateral placement of amphids and the hexaradiate placement of papillae at the anterior end (see Section 8.1). Also, when nematodes are provided with freedom of movement in three

Box 8.3. Behaviour terminology.

Kinesis. Behaviour comprised of undirected responses that are dependent on the intensity or temporal change in intensity of a stimulus. Undirected responses are unrelated to the orientation of the stimulus or stimulus field.

Orthokinesis. A kinesis in which translational motion (i.e. speed) is affected.

Klinokinesis. A kinesis in which rotational motion (i.e. direction) is affected.

Taxis. Migration oriented with respect to the stimulus direction or gradient which is established and maintained by directed turns. Directed turns are ones that are biased in some way with respect to the orientation of the stimulus field.

Klinotaxis. A taxis which results from directed responses to sequential samples of stimulus intensity or direction.

Tropotaxis. A taxis which results from directed responses to two (or in three dimensions, three) simultaneous samples of stimulus spatial distribution or directional distribution.

Teleotaxis. A taxis due to directed response to information gathered by a raster of many receptors. Directed turning occurs until the fixation area of the raster is exposed to the directional stimulus. An extreme form of tropotaxis. (Added by Dusenberry, 1992.)

Box 8.4. Prefixes denoting stimuli investigated for effects on nematode behaviour.

Chemo-	Chemical	Photo-	Light
Galvano-	Electric field	Rheo-	Fluid flow
Geo-	Gravity	Thermo-	Temperature
Magneto-	Magnetic field	Thigmo-	Touch

dimensions, conclusions based on patterns of forward movement on agar may not be valid because within soil and plant tissue muscle innervation of the anterior end permits orientation and movement in any plane, allowing nematodes to steer laterally in response to different concentrations of chemicals at the two amphids (Fig. 8.6).

8.4.2. Orientation to temperature

It is well established that plant-parasitic nematodes migrate in response to temperature but it is unclear why, although some interesting theories have been proposed. For example, metabolic heat from roots could be a local attraction for nematodes (Perry, 2005). In addition, temperature might be the most consistent cue to differentiate up from down within soil where there is no light and gravitational effects are minuscule compared with surface tension.

At least nine species of nematodes have been found to exhibit a ‘preferred temperature’ (temperature preferendum) toward which they migrate when placed on a temperature gradient plate *in vitro*. In most cases where tested, the preferendum is shifted partly or completely in the direction of a new adaptation temperature within several hours. One of these nematodes is the J2 of *M. incognita*. The threshold ambient temperature change eliciting a detectable change in the rate of movement of these nematodes is less than 0.001°C (Robinson, 2004), which is near the theoretical limit of temperature sensation in animals. In migration experiments, gradients sufficient to achieve a maximal response by *M. incognita* and other species, including *G. rostochiensis*, *D. dipsaci*, *T. semipenetrans* and *D. phyllobius*, have been found to lie between 0.01 and $0.1^\circ\text{C cm}^{-1}$.

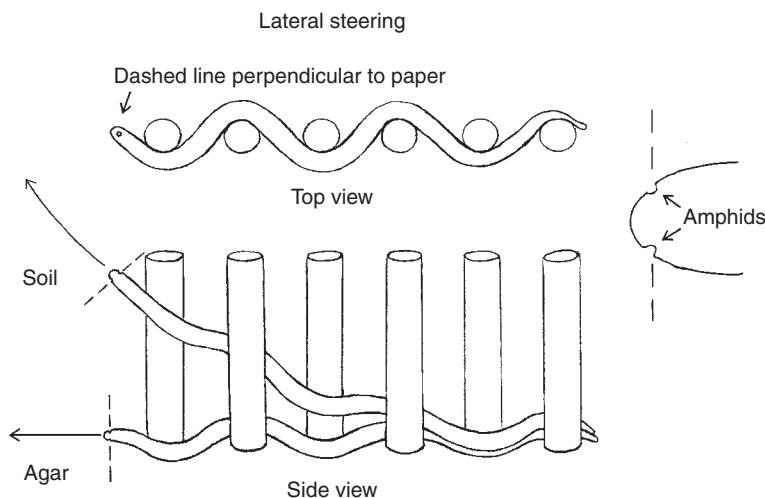


Fig. 8.6. Lateral movement of the anterior end of a nematode within a three-dimensional matrix, in theory, can permit orientation to gradients perceived by comparing sensory input of the two amphids, whereas amphids are necessarily positioned at the same concentration of chemicals in gradients on the surface of agar, because nematodes on the surface of agar lie on their sides.

Of what value can these kinds of responses possibly be to plant-parasitic nematodes? Can they assist them to find roots? El-Sherif and Mai (1969) found two species in Petri dishes to be attracted to heat released by germinating alfalfa seedlings, demonstrating that this was possible. However, in soil, root metabolism is a small heat source compared with solar radiation, which in many latitudes can elevate the soil surface temperature by 20°C or more in several hours. Diurnal surface heating and cooling also sends a heat wave down through the soil every day (Fig. 8.7). The wave characteristically starts with maximum amplitude at the surface during the afternoon, dampening as it moves downwards 2–3 cm h⁻¹. Robinson (1994) measured magnitudes of vertical gradients in a cotton field in Texas during the afternoon on a typical sunny day and found them to be 0.3 and 0.15°C cm⁻¹ at depths of 10 and 25 cm, respectively. Thus, behaviourally effective gradients extended deep into the soil. In addition, hourly collection of temperature data at 2.5 cm increments down to 60 cm across most months of the year clearly showed gradient inversions and other perturbations indicative of rainy periods, cold fronts and other weather patterns throughout most of the root growth zone (Fig. 8.7).

As soil temperatures change constantly and gradients invert daily while nematodes are constantly adapting and migrating in response to gradients, it is exceedingly complex to predict the net result. Robinson (1994) subjected two root parasites, *M. incognita* and *R. reniformis*, and one foliar parasite, *D. phyllobius*, in the laboratory to gradient fluctuations in soil that precisely mimicked those measured previously in cotton fields. Nematodes in the laboratory were equilibrated for 24 h under fluctuating temperatures identical to those at the same depth in soil where they were released; at intervals following release their distributions were determined. The two root parasites consistently moved in opposite directions; moreover, movement of *R. reniformis* was down and *M. incognita* was up, consistent with their known vertical distributions in cotton fields. The foliar parasite appeared to move toward cool regions regardless, consistent with previous observations on agar, and consistent with its need to be attracted to the soil surface during rainy periods to ascend cool, moist foliage.

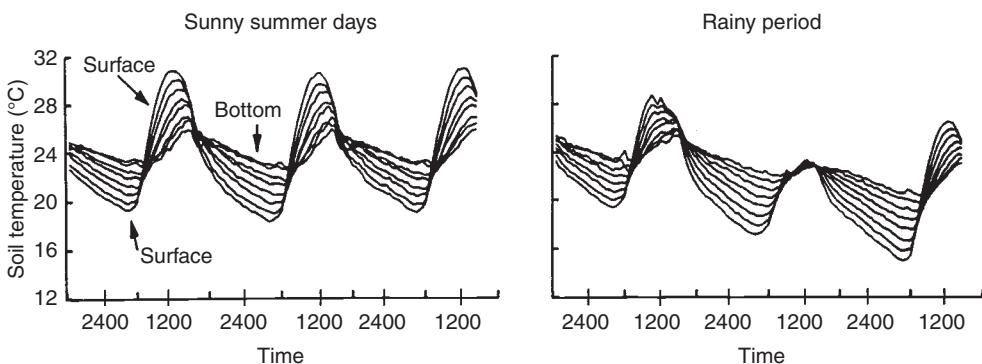


Fig. 8.7. Diurnal heat wave propagation down through soil in a cotton field during late summer in eastern Texas. Temperatures were measured with thermocouples planted 5–25 cm deep at 2.5 cm intervals.

8.4.3. Response to carbon dioxide

CO₂ strongly attracts a number of species *in vitro* (Box 8.5) and may be the most common and potent nematode attractant (Perry, 2005). It is released abundantly by living and decaying plant and animal tissues, providing an obvious cue to the possible presence of food. Plant-parasitic nematodes are confronted with the problem of distinguishing roots from decaying material from which they would be unable to obtain food. It is possible that other substances modulate nematode attraction to CO₂. For example, *M. incognita* is repelled by ammonia and several nitrogenous salts released by decaying material. It has also been proposed that CO₂, like temperature, may serve as a collimating stimulus, providing a vertical directional reference to soil organisms.

The attraction of plant-parasitic nematodes to CO₂ *in vitro* was first observed in the 1960s. Not everyone who looked for the response observed it and failures to detect it in some cases may have resulted from CO₂ toxicity because pure CO₂ at high flow rates was used. Concentrations significantly exceeding 5% suppress motility. Robinson (1995) used infusion pumps to deliver small amounts of CO₂ over extended periods of time through needles positioned within tubes of moist sand. The optimal release rate for attracting *M. incognita* and *R. reniformis* to a point source was 15 µl min⁻¹, a minuscule rate indeed. Intervals of 40 and 29 h were needed to attract most nematodes within 52 mm of the source and the total amount of gas that was needed to be released over this very long period of time (about 10 cm³) was calculated to be equivalent to that released by a germinating sunflower seed.

Pline and Dusenberry (1987) elegantly showed that the threshold at which *M. incognita* responds to CO₂ varies with ambient concentration; that is, nematodes become more sensitive as the ambient concentration drops and, thus, can detect about the same relative change at any ambient concentration. This effect is very important ecologically because it means that nematodes can detect gradients at far greater distances from the source than would be possible with a fixed concentration differential threshold. The threshold for *M. incognita* corresponded to a relative change of about 3% cm⁻¹. It is very important to understand that this does not mean 3% CO₂ in air but rather 3% of the concentration of CO₂ in air, whatever that concentration might be, but typically less than 1%. Results from other studies are consistent with Pline and Dusenberry's conclusions. The minimum effective gradient measured in the first study on *D. dipsaci* on agar was approximately 0.1% cm⁻¹. *Meloidogyne incognita* and *R. reniformis* were strongly attracted to CO₂ in sand, migrating up a 0.2% cm⁻¹ gradient at a mean ambient CO₂ concentration of 1.2%, which was a 16% cm⁻¹ relative change or 1% per nematode body length.

Box 8.5. Plant-parasitic nematodes attracted to CO₂

Aphelenchoides fragariae; *Bursaphelenchus xylophilus*; *Ditylenchus dipsaci*; *Heterodera schachtii*; *Meloidogyne hapla*; *M. incognita*; *M. javanica*; *Pratylenchus neglectus*; *P. penetrans*; *Rotylenchulus reniformis*

However, interpreting the significance of attraction to CO₂ released by roots is confounded by effects of CO₂ on gradients of redox potential, pH, carbonic acid, bicarbonate and carbonate in the soil. CO₂ attracts nematodes to roots and either dissolved CO₂ or carbonic acid is the attractive species. Wang *et al.* (2009a) considered that the observed attraction of root-knot nematodes to CO₂ may be due to acidification of solutions by dissolved CO₂ rather than to CO₂ itself.

8.4.4. Response to plant attractants

Long-distance attractants, such as CO₂, may enable the nematode to move to the root area but other gradients are important to ensure the nematodes locate individual roots. These short-distance attractants have received most attention and there is evidence that, in some instances, the attractiveness of a host to the pest species is correlated with its efficiency as a host.

Research on *G. rostochiensis* has demonstrated that diffusates from the roots of the host plant, potato, increase the activity of the infective J2 and also attract them to the roots. This potato root diffusate (PRD) is required to stimulate hatching of the majority of J2 of the potato cyst nematodes *G. rostochiensis* and *G. pallida* (see Chapter 7), but work by Devine and Jones (2002) has shown that the chemicals in PRD responsible for hatching differ from those responsible for attracting the J2 to the root. Electrophysiological analysis of sensory responses (Perry *et al.*, 2004) demonstrated that spike activity of J2 of *G. rostochiensis* increased on exposure to PRD but not to root diffusate from the non-host sugar beet, thus indicating that responses to diffusates may be host-specific. Two *Solanum* species, *Solanum tuberosum* and *S. sisymbriifolium*, respectively a susceptible and a trap crop for *G. rostochiensis* and *G. pallida*, exude different hatching factors but send common signals to attract the J2 towards the root area (Sasaki-Crawley *et al.*, 2010).

Research evaluating the attraction of *M. graminicola* and *M. incognita* for cereals or dicotyledonous roots supports the role of root diffusates in regulating host specificity. This research showed that these species take the most direct route to their preferred host but often take the longest route towards the poor host (Dutta *et al.*, 2011). When in the rhizosphere, plant-parasitic nematodes have to navigate their way to roots amongst a complex of repellent and attractant chemicals and gradients of these chemicals might influence the interaction between *Meloidogyne* spp. and their hosts, and Dutta *et al.* (2011) suggested that chemical differences in complexes of ‘short-distance attractants’ seem to define host preference for these root-knot nematodes. The role of amphids in chemoreception has been demonstrated in experiments where exposure of J2 of *M. javanica* and *G. rostochiensis* to antibodies to amphidial secretions blocked the response to host root allelochemicals. However, responses were not permanently blocked as, after a period of between 0.5 and 1.5 h, turnover of sensilla secretions presumably ‘unblocked’ the amphids (Perry and Maule, 2004; Curtis, 2008).

The orientation of J2 of endoparasitic nematodes to the preferred invasion site, the root tip, is well established but the active factors in root diffusates that constitute these ‘local attractants’ are unknown. It is possible that specific allelochemicals are responsible but it is also possible that the nematodes orient to an electrical potential

gradient at the elongation zone of the root tip. For example, low pH amongst other local attractants might contribute to gather root-knot nematodes at the zone of elongation as *M. hapla* is attracted to, and aggregates between, pH 4.5 and 5.4, which corresponds to the acidic pH gradient encountered in the zone of elongation (Wang *et al.*, 2009a). Although this pH range was attractive to all tested root-knot nematode isolates and species, the level of aggregation depended on the species/strain assessed.

Root diffusates change in response to plant growing conditions. For example, the form of nitrogen supplied to rice plants directly affected the movement and invasion of *M. graminicola*. Rice plants grown in hydroponics containing ammonium nitrate or ammonium chloride repelled J2 and invasion was reduced, whereas plants grown in calcium nitrate attracted J2, resulting in increased invasion (Patil *et al.*, 2013a,b).

The relative importance of electrical and chemical attractants for location of roots and, especially, root tips is unknown. Local attractants from root cap exudates from peas increased motility and attracted J2 of *M. incognita*. However, when in contact with root border cells and mucilage, J2 of *M. incognita* entered a reversible state of immobility. These nematodes are able to recover fully and can find and penetrate root tips within 24 h after recovery from immobility (Curtis *et al.*, 2011). This ability of the root cap to deliver products that temporarily immobilize nematodes may have an important role in protecting the root tip from infection by acting as a natural trap for pathogenic organisms.

Once nematodes are near or at the root surface, local root signals induce the nematode to adopt an exploratory behaviour with rhythmic stylet movements, aggregation, increase of motility, production of pharyngeal secretions and changes to their surface cuticle (Curtis, 2008). Before root invasion root-knot nematodes are able to perceive signals in root diffusates by changing behaviour and gene expression (Teillet *et al.*, 2013). These events are likely to be important for preparing nematodes for root invasion and possibly allowing their survival inside the host tissue (Curtis *et al.*, 2009, 2011). *Caenorhabditis elegans* also detects environmental signals present in root diffusates that lead to a change in the surface cuticle. Detection of plant signals by chemosensory organs of plant-parasitic nematodes might be important to protect the nematode surface from biological or chemical attack in the rhizosphere (Davies and Curtis, 2011).

Understanding the complexity of the molecular signal exchange and response during attraction and invasion, as well as the nature of root diffusates affecting nematode behaviour, is important. This knowledge can reveal targets for chemical or genetic intervention to control plant-parasitic nematodes that can be used to disorientate nematodes and disrupt host recognition. Once the nematode migratory phase in the rhizosphere is prolonged with a concomitant increased depletion of food reserves, there will be a reduction in their infectivity and an increase in their exposure to natural enemies.

8.4.5. Response to pheromones

Of the wide variety of pheromones known to influence behaviour of invertebrates, only sex pheromones have been studied extensively in plant-parasitic nematodes (Perry and Aumann, 1998). Most studies have been on cyst nematodes and have established that sex pheromones have volatile and non-volatile components.

Electrophysiological analysis of the responses of males of the potato cyst nematodes to non-volatile components of female sex pheromones showed that *G. rostochiensis* males exhibited specific mate recognition, whilst *G. pallida* males responded to the sex pheromones from females of both species. However, the homospecific response of *G. pallida* was much greater and may be dominant in the soil environment. The sex pheromone of *G. rostochiensis* is composed of several polar, weakly basic compounds. Chemical characterization of these compounds, and the two polar compounds in the sex pheromone of *H. schachtii*, is required. Only one male attractant has been identified for a plant-parasitic nematode: vanillic acid for *H. glycines* (Jaffe *et al.*, 1989).

Wang *et al.* (2009a) found that nematodes aggregated when suspended in pluronic gel without roots; when a coverslip was placed on the gel it served as a focus for the aggregation, suggesting that lower oxygen or a volatile attractant is involved in this aggregation behaviour. However, the possibility of a nematode pheromone being involved in this aggregation phenomenon should not be excluded; aggregation pheromones have been reported previously for some animal-parasitic nematodes.

8.5. Nematode Feeding and Movement within Plant Tissue

Plant-parasitic nematodes have adapted in diverse ways to feed on almost every part of the plant. When the life cycle of a given nematode species is compared on various plant hosts, a strongly characteristic pattern is typically observed in its migration route and feeding behaviour irrespective of the host (Wyss, 2002). Evidence accumulated across numerous taxa in all major taxonomic groups has shown that it is the specific combination of behavioural, anatomical, developmental, biochemical and molecular features that makes the particular feeding pattern and pathology induced by each plant-parasitic species unique. Anatomical variables influencing migration and feeding behaviour include the dimensions of the body, stylet lumen and length, and pharyngeal glands. Developmental variables include the migratory potential of the adult female (vermiform and motile as in *Pratylenchus*, vermiform but sedentary as in *Criconemoides*, or saccate and sedentary as in *Meloidogyne*), the type of reproduction (amphimictic vs parthenogenetic because the enclosure of females within galls may prevent copulation), the number of eggs produced, and the occurrence of metamorphosis during male development. Biochemical and molecular variables include timing and capacity of dorsal and subventral pharyngeal glands to produce enzymes involved in plant cell wall degradation, cytoplasm pre-digestion or feeding tube formation, and elicitors triggering development of nurse cells and syncytia.

The behavioural repertoire of plant-parasitic nematodes moving through and feeding on plant tissue is restricted to a small number of simple activities that are exhibited to varying degrees by all species. However, the manner in which they are timed and coordinated with other events results in behaviours and effects on the plant that are highly consistent within nematode taxa across many plant hosts, yet sufficiently variable across nematode groups to enable utilization of flowers, foliar galls, stems, root hairs, root cortex and various cell layers within the root vascular system as food sources. Simple and consistent patterns of behaviour within a species or taxonomic group in response to an environmental trigger have been termed releaser responses of fixed action patterns (Sukhdeo *et al.*, 2002).

A number of discrete behavioural patterns are exhibited by plant-parasitic nematodes while moving towards and through plant tissues (Box 8.6). The sequence and duration of these activities tends to be highly consistent within species; however, across species activities may be elaborated, sequences may be completely different or cyclic, and the duration of each activity may range from a few seconds or minutes to several days. Plant-parasitic nematodes can be placed in at least ten categories based on their migration and feeding patterns within plant tissue and usually the groups are taxonomically consistent. Since stylets evolved independently several times in plant-parasitic nematodes, it is prudent to examine the behaviour of different groups separately. Therefore, first we will examine the trichodoridae and then longidoridae. After that, we will compare cyst and root-knot nematodes, followed by discussions of other sedentary root parasites, foliar parasites and insect-vectored plant parasites.

Box 8.6. Behavioural patterns exhibited by plant-parasitic nematodes while moving toward and through plant tissues.

1. Undulatory locomotion through the soil, either toward specific regions of roots or to stem bases and then onto stem surfaces.
2. Lip rubbing of host cell surfaces, often with gentle stylet probing.
3. Orientation of the anterior body perpendicular to the host epidermal wall.
4. Rapid forceful thrusting jabs of the stylet until the cell wall is penetrated, often along a line of holes that merge to form a slit (this behaviour occurs during hatching, epidermis penetration and penetration of cells during intracellular migrations through the root cortex).
5. Migration within the host, either intracellularly or intercellularly, depending on the nematode species; some species move more or less randomly in the cortex and others follow a specific migration route through certain tissues to come to rest at a specific cell layer or region of tissue.
6. Periods of total inactivity.
7. Periods of inactivity with partial protrusion of the stylet and release of pharyngeal gland secretions.
8. Basal or metacorpal bulb pumping and ingestion of cellular constituents.
9. Withdrawal from the feeding site.

8.5.1. Trichodoridae

The trichodoridae nematodes (approx. 90 species in five genera) are moderate-sized (<1.5 mm long), strictly epidermal feeders (Fig. 8.8, 1A) that can consume several hundred cells in a few days (Hunt, 1993; Wyss, 2002). The trichodoridae exemplify the consistency of nematode feeding patterns within taxa. The trichodoridae stylet is a ventrally curved, grooved tooth, termed the onchium, which uniquely permits them to ingest entire cell organelles. They feed on cells quickly (<4 min cell⁻¹), always exhibiting characteristically continuous stylet thrusting while feeding. Moreover, for every cell fed upon, five distinct feeding stages are seen (Box 8.7).

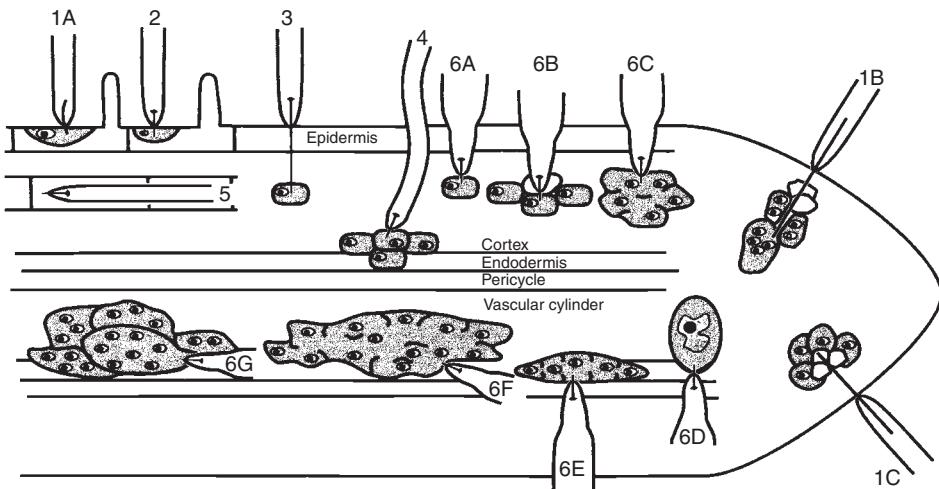


Fig. 8.8. Feeding sites of selected root parasitic nematodes (from Wyss, 1997).

(Note: 1A, 1B and 1C are all dorylaimid migratory ectoparasites and all others are tylench nematodes.) 1A: *Trichodorus* spp. 1B: *Xiphinema index*. 1C: *Longidorus elongatus*. 2: *Tylenchorhynchus dubius* (migratory ectoparasite). 3: *Criconemoides xenoplax* (sedentary ectoparasite). 4: *Helicotylenchus* spp. (migratory ecto-endoparasite). 5: *Pratylenchus* spp. (migratory endoparasite). 6A: *Tryptotylenchulus obscurus*. 6B: *Tylenchulus semipenetrans*. 6C: *Verutus volvingensis*. 6D: *Cryphodera utahensis*. 6E: *Rotylenchulus reniformis*. 6F: *Heterodera* spp. 6G: *Meloidogyne* spp. (6A–6G are all sedentary endoparasites).

Box 8.7. Feeding stages of trichodorids (Wyss, 2002).

1. Cell wall exploration with lip rubbings.
2. Wall perforation consisting of 1 min of forceful rapid stylet thrusting concurrent with contraction of radial muscles of the basal bulb.
3. Salivation or release of pharyngeal gland secretions through the stoma while thrusting continues and cytoplasm 'liquefies' (the longest phase).
4. Food ingestion (20% of total feeding behaviour).
5. Withdrawal from feeding site.

8.5.2. Longidoridae

In contrast to trichodorids, the longidorids are large (up to 12 mm long) with long slender stylets, including about 240 species of *Xiphinema* and 100 of *Longidorus*; only a few of either group have been studied extensively. Several distinct feeding patterns have been noted in longidorids (Fig. 8.8, 1B and 1C) but they are generally similar in many ways and all are markedly different from that in trichodorids. Several *Xiphinema* spp. feed on differentiated vascular tissue but those studied the most feed on root tips where they cause galls that eventually attract other nematodes. Root tip feeding has been carefully studied for *X. index* and *X. diversicaudatum* on *Vitis* spp. (grape) and *Ficus carica* (fig), where five feeding stages are evident (Box 8.8).

Box 8.8. Feeding stages of longidorids (Wyss, 2002).

1. Exploration via lip rubbings.
2. Insertion of stylet 2–3 cells deep via quick repeated jabs.
3. Progressive insertion of stylet into deeper cells, withdrawing food from each for several minutes, followed by withdrawal of stylet and reinsertion at a new point, with the same process then repeated.
4. Long quiescent period with no bulb pumping invariably followed by a prolonged period of food removal lasting up to 30 min.
5. Continued feeding leading to binucleate cells surrounding necrotic spaces that transform into multinucleate feeding cells (only in susceptible plants).

In *Longidorus* species, which only feed on root tips, *Longidorus caespiticola*, *L. elongatus* and *L. leptocephalus* have been studied *in vitro* on perennial ryegrass (*Lolium perenne*). Five feeding phases are noted: (i) as in *X. index*; (ii) as in *X. index* except rapid thrusting continues until the stylet is fully protracted; (iii) always a period of inactivity up to 1 h when pharyngeal secretions are thought to be released; (iv) continuous food ingestion for several hours (i.e. longer than in *X. index* and up to 40 cell volumes may be removed); and (v) continued feeding leading to galling and cell wall dissolution; however, nuclear divisions without cytokinesis as observed in *X. index* are generally not characteristic.

8.5.3. General aspects of tylench tissue migrations and feeding behaviour

Tylench nematodes can be divided into those that feed on roots and those that feed on above-ground plant parts. All nematodes in both groups are vermiform as juveniles and those feeding above ground generally are vermiform throughout life. Root parasites may be vermiform or saccate as adults (Fig. 8.8). Gravid females as a general rule are sluggish and, during the physiological events of moulting, preceding ecdysis (the physical shedding of the cuticular remnant), all nematodes exhibit quiescent behaviour termed moulting lethargus. Aside from these periods, most plant-parasitic nematodes that are vermiform as adults are motile throughout the life cycle, with the arguable exception of Paratylenchinae and Criconematinae (Siddiqi, 2000; Wyss, 2002).

8.5.4. Tylench root parasites with vermiform adults

The browsing ectoparasite *Tylenchorhynchus dubius* feeds on root hairs and epidermal cells (Fig. 8.8, 2). Feeding lasts 10–12 min cell^{-1} and is marked by four phases: (i) lip rubbings with gentle stylet probings; (ii) vigorous stylet thrusts; (iii) salivation; and (iv) ingestion (85% of total, or about 9 min). Thus, the period of ingestion for *T. dubius* lasts about ten times as long as in *Trichodorus* spp., perhaps because the former feeds through a narrow stylet lumen, whereas the latter feeds via the buccal cavity. The ecto-endoparasites (e.g. *Helicotylenchus* spp., *Hoplolaimus* spp. and

Rotylenchus spp.) move partly or, in some cases, entirely into the cortex (Fig. 8.8), feeding on individual cells for long periods. By comparison, migratory endoparasites (e.g. *Pratylenchus* spp., *Hirschmanniella* spp. and *Radopholus* spp.) feed on root hairs when in the J2 and third-stage juvenile (J3) stages and later stages continuously migrate intracellularly in the cortex as they develop (Fig. 8.8), most often parallel to the root axis, leaving extensive tracks of destroyed cells and necrotic tissue. Cortical migrations are periodically interrupted by quiescent periods.

8.5.5. Tylench root parasites with saccate adults

By far the most commonly encountered plant-parasitic nematodes with sedentary, saccate adults are the root-knot nematodes (see Chapter 3) and the cyst nematodes (see Chapter 4). Our knowledge regarding feeding behaviour of root-knot nematodes comes from studies of *M. incognita* and of cyst nematodes from studies on *G. rostochiensis*, *H. schachtii* and *H. glycines*; thus, generalizations should be made with caution.

In both cyst and root-knot nematodes the J2 is the motile, infective stage, which after infection settles in the vascular region where it elicits highly specialized plant cell responses resulting in the development of feeding sites comprised of enlarged hyper-active cells upon which the nematode permanently feeds (Fig. 8.8). Behaviourally, the two groups differ in the migration root taken by J2 through root tissue, the type of feeding site induced and, most importantly perhaps, in the structure and function of the stylet and pharyngeal glands utilized to enable root migrations and feeding cell establishment (see Chapter 9).

Cyst nematode J2 have a relatively robust stylet that allows them to enter the root, mainly in the zone of elongation, via repeated forceful jabs of highly coordinated stylet thrusts that produce a line of merging holes to form a slit, thereby cutting through the tough epidermal wall and subsequently other cell walls that they encounter as they move intracellularly to the vascular tissue. Here they initiate feeding on cells that, in *Arabidopsis thaliana*, have been confirmed consistently to be cambial or procambial. Root-knot nematode J2 have a more delicate stylet and enter the root closer to the meristematic region. Root penetration involves continuous head rubbings and stylet movement, including occasional stylet protrusions followed by metacorporeal bulb pumpings of a few seconds, indicating cell wall degradation enzymes are probably involved. The epidermal cells eventually soften and lyse. Once inside, the J2 initially migrate acropetally and intercellularly (in contrast to cyst nematodes, which migrate intracellularly) through the relatively soft tissues of the cortical parenchyma until reaching the apical meristematic region, where they turn around and enter the newly formed vascular cylinder, moving basipetally several body lengths before coming to rest and initiate feeding. There is biochemical and molecular evidence for cell wall degrading enzymes in the sub-ventral gland secretions of both groups (see Chapter 9).

The destructive behaviour pattern of the cyst nematode J2 during migration changes into a subtle exploratory pattern upon reaching the vascular cylinder. A single cell, termed the initial syncytial cell (ISC) is then perforated by careful stylet thrusts, after which the stylet tip remains protruded a few micrometres deep for 6–8 h (the so-called preparation period); no obvious changes in the ISC are

observed nor are there any metacorpal bulb pumpings. However, in addition to the gradual increase in the dorsal and decrease in the sub-ventral glands, there are a few sudden shrinkages of the body, when the J2 defecates. Subsequently, the ISC enlarges and feeding begins, which occurs in repeated cycles, each consisting of three distinct phases: (i) nutrients are withdrawn by continuous rapid pumping of the metacorpal bulb with no forward flow of pharyngeal gland secretory granules; (ii) stylet retraction and reinsertion; and (iii) continuous forward movement of secretory granules.

When a J2 of root-knot nematodes reaches the cambial region where the eventual multinucleate nurse cells (called giant cells) form, forward motion stops but the head continues to move in all directions, exhibiting the same stylet tip protrusions and metacorpal bulb pumping as during migration; however, these periods increase until it is apparent the J2 are extracting food from the enlarging cells surrounding the anterior end.

Additional tylench nematodes with saccate adults are found in the families Hoplolaimidae and Tylenchulidae. Knowledge regarding tissue migrations in these nematodes comes primarily from histopathological observations rather than direct observations of the infection process *in vitro*, and much more information is available for some than others due to their greater economic importance. In species of *Rotylenchulus* and *Tylenchulus* the adult vermiform female is the infective stage and penetrates the root cortex of a largely differentiated zone, eliciting formation of a feeding cell and (or) syncytium without migrating longitudinally through the root. Thus, the initial and final orientation of the nematode is perpendicular to the root axis. The male of *R. reniformis* has a weak stylet and does not feed. However, the vermiform female of *R. reniformis*, which has a robust stylet similar to that of cyst nematode J2, penetrates the root cortex intracellularly, perpendicular to the root axis in a largely differentiated zone, coming to rest and feed, usually on an endodermal cell, less commonly on a pericycle or cortical cell on which it feeds permanently. A syncytium interconnecting the feeding cell with a curved sheet of hyperactive pericycle cells extending part way around the vascular cylinder through partial dissolution of common radial walls is characteristic in many plant species. In *T. semipenetrans*, the vermiform female also is infective and infection is similar to that in *Rotylenchulus*, but feeding almost invariably involves cortical cells. *Nacobbus aberrans* differs in that the J2 and J3 feed as migratory endoparasites, much like *Pratylenchus* spp. The adult females, however, are saccate as in *Rotylenchulus* and *Tylenchulus* and establish a syncytium where they feed as sedentary parasites in the cortex.

8.5.6. Above-ground parasites of plants

Nematodes parasitizing aerial plant parts occur in the infraorder Tylenchomorpha, and include species in the Sphaerularioidea and Aphelenchoidea. Within Sphaerularioidea, aerial parasites are common among the 150 species in the family Anguinidae, and particularly among the 100 or so species of *Anguina*, *Subanguina* and *Ditylenchus*. Within Aphelenchoidea, aerial parasites of plants include at least three of the 138 species of *Aphelenchoidea*, namely *Aphelenchoides besseyi* (white tip of rice nematode), *A. fragariae* (strawberry crimp nematode) and *A. ritzemabosi* (chrysanthemum nematode).

Aphelenchoidea also includes at least two insect-vectored wood inhabitants of major economic importance that have life cycles completely different from other aerial plant parasites in Tylenchomorpha. Both feed facultatively on fungi in the plant host and elicit the production by the tree of tyloses and autotoxins that contribute to tree death. These nematodes are *Bursaphelenchus cocophilus* (coconut red ring disease nematode) and *B. xylophilus* (pine wilt nematode).

Bursaphelenchus cocophilus is vectored endophoretically by the palm weevil *Rhynchophorus palmarum* (see Chapter 5). The J3 of *B. cocophilus* enter weevil grubs burrowing through palm tissue through the spiracles and mouth and migrate to the haemocoel, where they remain without developing until the insect undergoes metamorphosis and emerges as an adult female weevil. The J3 then concentrate in the ovipositor and when the weevil moves to another tree to oviposit they are transferred with the eggs. The nematodes migrate intercellularly through all the parenchymatous tissue of the palm, but in the trunk are largely restricted to the 2–4 cm wide ‘red ring’, which is about 3–5 cm beneath the trunk surface (Hunt, 1993).

Bursaphelenchus xylophilus is vectored ectophoretically primarily on two long-horn beetles, *Monochamus alternatus* in Japan and *M. carolinensis* in North America, although many other related beetles can carry the nematode. The fourth-stage juveniles (J4) are carried from tree to tree under the elytra and in the tracheae. In susceptible tree species, juveniles enter feeding wounds made by the insect and gain access to the resin canals of the shoots where they feed on epithelial cells. Feeding inside the resin canals causes a pronounced decrease in oleoresin production allowing the nematodes to migrate throughout the dying tree and reproduce. Dying trees in turn attract insect vectors. Experiments *in vitro* showed the J4 to be attracted to CO₂ from the vector and to terpenes predominant in the vector’s diet, especially β-myrcene. *Bursaphelenchus xylophilus* can nictate (standing vertically out from the substrate) and jump, behaviours generally not found among plant-parasitic nematodes. With some entomopathogenic nematodes, nictation often is stimulated by vibration, CO₂ or other cues from the insect.

Infective stages of aerial plant parts move faster than infective stages of root parasites. Body undulations in J2 of *D. phyllobius*, for example, exceed 1 wave s⁻¹ and direct comparisons showed *D. phyllobius* and *D. dipsaci* J4 to disperse on agar 6–20 times as fast as infective stages of *T. semipenetrans* and *R. reniformis* (Robinson, 2004). Aphelenchoidids in general are also considered highly active nematodes, and *A. besseyi* along with *D. angustus* are considered likely to be the best swimmers among plant-parasitic nematodes (Hunt, 1993). Such fast movement undoubtedly increases the chances that infection sites on aerial parts can be reached before moisture films required for migration dry.

Aphelenchoides besseyi, *A. fragariae* and *A. ritzemabosi* are usually described to feed both ecto- and endoparasitically on buds, stems, leaves and inflorescences. There are at least four reports of direct observations of entry and exit of *A. fragariae* and *A. ritzemabosi* through leaf stomata, and CO₂ has been demonstrated to lure *A. fragariae* through stomata-size pores in plastic film. However, it is clear that many nematodes commonly penetrate soft regions of the foliar epidermis directly, especially in the tender folds between embryonic leaves and floral structures within buds. For example, in a comparison of *D. dipsaci* and *A. fragariae* on alfalfa seedlings in pots, *D. dipsaci* penetrated through the epidermis at the base of cotyledons forming cavities in cortical parenchyma within 12 h of inoculation, whereas *A. fragariae* fed primarily

ectoparasitically. *Ditylenchus phyllobius* similarly penetrates the epidermis of *Solanum elaeagnifolium* directly, but only in the leaf folds in buds above ground or on shoots produced by rhizomes as they grow up through the soil. This nematode induces massively galled leaves weighing up to 10 g, but these develop from leaves infected when at the embryonic stage and infection appears never to occur after leaves have expanded. Direct observations of nematode feeding within foliar galls are lacking but a number of histological studies have shown induction of putative nurse cells and associated hyperplastic tissue similar to that observed in root parasites and it can be surmised that the types of feeding behaviours directly observed for root parasites *in vitro* are probably shared by these foliar feeding species.

8.6. Other Types of Movement and Behaviour

Touch receptors, running along the body length (Jones, 2002), are presynaptic to many other neurons, including motor neurons, and detect gentle mechanical stimuli. A model for the functioning of these receptors (Driscoll and Kaplan, 1997) in *C. elegans* proposes that the cuticle is linked, via proteins, to a movement-sensitive ion channel. Microtubules within the receptor cell may also be linked, via other proteins, to the ion channel. Ion flow could be stimulated by movement of proteins or microtubules.

Adverse environmental conditions engender behavioural responses of nematodes, frequently associated with survival and entry to a dormant state (Chapter 7). For example, coiling and clumping by some species of nematodes, such as *D. dipsaci*, occur in response to gradual removal of water. Coiling and clumping both reduce the surface area of the nematode that is exposed to drying conditions and reduces the rate of water loss, a slow rate of dehydration being a critical factor for survival. In *Anguina amsinckia* and *A. tritici*, aggregations occur within galls. Within the galls induced by *A. amsinckia* are hundreds of desiccated adults and juveniles of all stages, many of which are coiled. By contrast, the galls induced by *A. tritici* contain tightly packed aggregates of J2 only, each of which remains uncoiled when dry. Mass movement or swarming found, for example, in *Aphelenchus avenae* is probably a behavioural response to lack of food or toxic products from decaying hosts.

Several hundred species within the Criconematidae have backwardly angled cuticular outgrowths, such as scales or spines, or retrorse annules in juvenile stages, which probably provide purchase for a peculiar form of travel. The criconematids are noted for moving forwards via sluggish, anteriorly propagated peristaltic contractions, in contrast to the backwardly propagated undulations in other nematodes (Siddiqi, 2000). This behaviour has received little study relative to its probable ecological importance (Burr and Robinson, 2004).

9

Molecular Aspects of Plant–Nematode Interactions*

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9.1. Nematode Parasitism of Plants

The relationships between plant-parasitic nematodes and their hosts are diverse. Plant-parasitic nematodes may be sedentary or browsing and can be ectoparasites or endoparasites. For some nematodes, including most migratory ectoparasites, plants simply provide an ephemeral food source and the interaction between the nematode and the plant is limited. For other nematodes the interactions are far more complex and long-lasting. The most economically damaging plant-parasitic nematodes are biotrophic and induce changes in the roots of their hosts to form feeding sites that serve to supply the nematode with a rich and continuous food source.

Nematodes are well equipped for parasitism of plants. Plant parasitism has arisen independently at least three times in the Nematoda (see Fig. 2.9) but despite the fact that plant parasites are not always directly related in phylogenetic terms, some structural features are always present. All plant-parasitic nematodes have a strong, hollow, needle-like structure that is used to pierce plant cells (Fig. 9.1). This structure (stylet, onchiostyle or odontostylet in the various taxonomic groups) is used to introduce nematode secretions into plant tissues (see below) and to remove plant cell contents during feeding. Stylets vary in shape and size according to the feeding strategy of the nematode; for example, nematodes such as *Trichodorus* that feed on epidermal cells have short onchiostyles, whereas those such as *Xiphinema* or *Longidorus* have considerably longer odontostylets (Fig. 9.1) and can feed on cells deeper within the plant. In many plant-parasitic nematodes the secretions of the pharyngeal gland cells play important roles in the host–parasite interaction. Plant-parasitic nematodes have two sets of these gland cells, dorsal and sub-ventral. The precise number and arrangement of each cell type varies. In Tylenchoidea (including the major sedentary endoparasites) there are two sub-ventral gland cells and one dorsal gland cell (Fig. 9.2). The sub-ventral gland cells are large and full of secretory granules in the invasive second-stage juvenile (J2) after hatching and during the earliest stages of parasitism. These cells become smaller in the third- and fourth-stage juveniles and adult females. By contrast, the dorsal gland cell increases in size throughout the life cycle. These structural studies are mirrored by studies using antisera that recognize the various gland cells. Antibodies that bind to the sub-ventral gland cells give a strong signal in J2 and little or no signal in adult females. By contrast, antibodies that recognize the dorsal gland cell give a weak signal in J2 but a much stronger signal in adult females. These observations show that the gland cell products are developmentally regulated and suggest that the products of the sub-ventral gland cells are important in the early stages of parasitism, during invasion and perhaps during feeding site induction, while the products of the dorsal gland cell play a role later in the parasitic process, probably in feeding site development or maintenance.

Nematode feeding sites (NFS) are incredibly diverse and can be induced in a variety of root tissues (Fig. 9.3) but have some conserved features (Wyss, 2002). All have structural characteristics of metabolically active tissues including cytoplasm highly enriched in subcellular organelles. Many show signs of DNA replication and may have enlarged or multiple nuclei. The simplest feeding sites are composed of single modified cells. *Trophotylenchulus* feeds from a single cell that appears metabolically active and contains one enlarged nucleus. Other nematodes, such as *Tylenchulus*, feed on clusters of such cells. *Cryphodera* feeds from a single greatly enlarged cell that

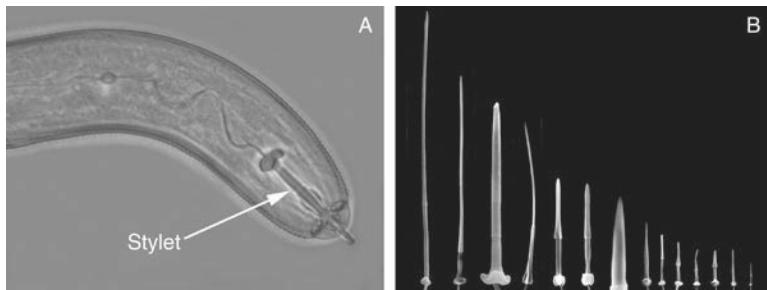


Fig. 9.1. Stylets of plant-parasitic nematodes. A: Stylet at the anterior tip of a second-stage juvenile of the beet cyst nematode *Heterodera schachtii*. B: Scanning electron micrograph of stylets dissected from various plant-parasitic nematodes.

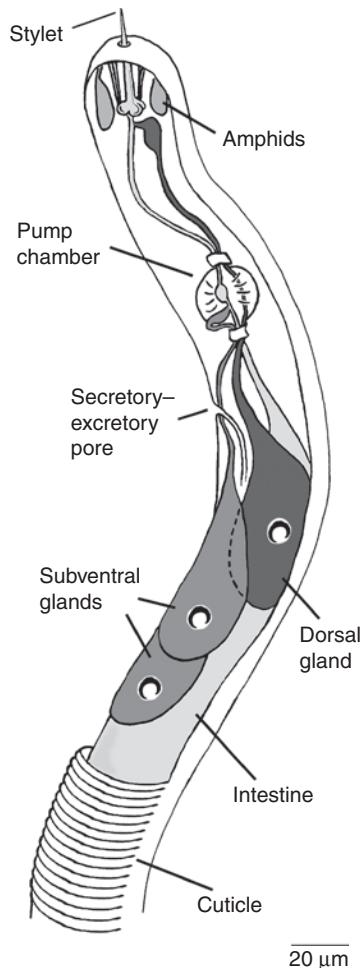


Fig. 9.2. Sketch of the anterior end of a tylench plant-parasitic nematode showing the relative positions of various nematode structures including the sub-ventral and dorsal pharyngeal gland cells.

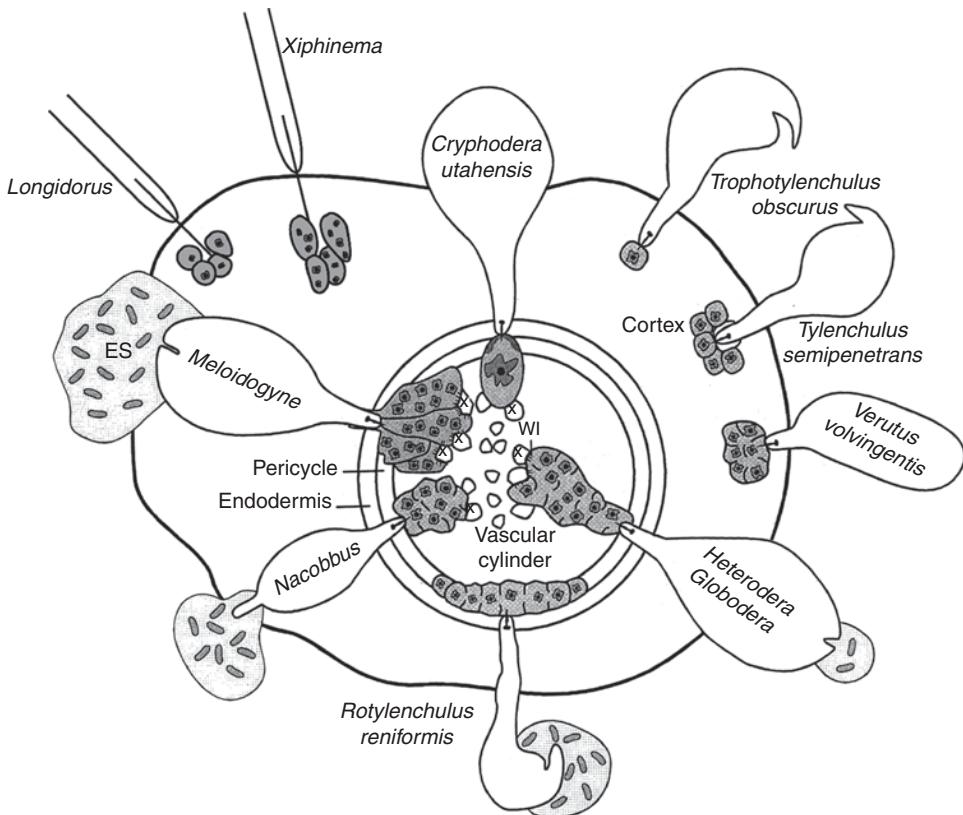


Fig. 9.3. Schematic diagram of the range of feeding sites induced by various plant-parasitic nematodes. X, xylem; WI, cell wall ingrowths; ES, egg sac.

contains one enlarged nucleus. More complex feeding sites fall into two categories: syncytia and giant cells. Syncytia are large multinucleate cells formed by breakdown of plant cell walls and fusion of adjacent protoplasts. The most complex syncytia are induced by cyst nematodes (*Heterodera* and *Globodera*). Giant cells of root-knot nematodes are formed through repeated rounds of nuclear division and cell growth in the absence of cytokinesis. The nematodes that induce these complex feeding sites cause severe damage to plants and are the most economically important of the plant-parasitic nematodes. These nematodes will form the basis for most of the rest of this chapter.

9.2. Invasion and Migration

Endoparasitic nematodes need to invade host tissues and migrate to where they feed. Plants have evolved complex defences to protect themselves against attack by nematodes and other pathogens (see Sections 9.3 and 9.8). One of the major physical barriers encountered by any invading pathogen is the plant cell wall. Each plant cell is surrounded by a cell wall and the middle lamella forms the interface between adjacent

cell walls (Fig. 9.4). The plant cell wall is a complex but highly organized composite of polysaccharides and proteins. Cellulose, formed by β 1–4-linked chains of glucan sugars, is the most abundant polymer in the plant cell wall. Cellulose exists in the cell wall in the form of microfibrils that are formed from dozens of individual polysaccharide chains, hydrogen bonded to one another along their length. These microfibrils are crosslinked to one another by hydrogen bonded crosslinking glycans, the most important of which are the xyloglucans and glucuronoarabinoxylans. This network of cellulose and crosslinking glycans is embedded within a matrix formed from pectins. Pectins serve a variety of other roles in the plant cell wall including control of cell wall porosity and cell–cell adhesion at the interface with the middle lamella and are formed from highly branched chains of polysaccharides rich in D-galacturonic acid. Although the carbohydrates described above form the major part of the plant cell wall, two major classes of structural protein are also present. Hydroxyproline-rich glycoproteins such as extensin and glycine-rich proteins may form networks in the plant cell wall. The interrelationships of the cell wall components are summarized in Fig. 9.4. The plant cell wall presents a formidable barrier and any invasive pathogen needs to have mechanisms that allow it to overcome this obstacle.

Plant-parasitic nematodes, particularly the endoparasites, migrate for considerable distances within the plant before settling to feed. Intriguingly, there are differences in the way in which various nematodes migrate within the plant. Cyst nematodes enter the root, mainly at the zone of elongation, and migrate destructively through cortical cells until they reach a position near the differentiating vascular cylinder. Root-knot nematodes enter the root and migrate intercellularly, first heading towards the root tip until they reach the root apex. They then turn around and migrate (again intercellularly) back up the root until they reach a site near the vascular cylinder suitable for feeding site induction. It is possible that this behaviour of migrating between plant cells, which prevents extensive damage to the host during migration, is partly

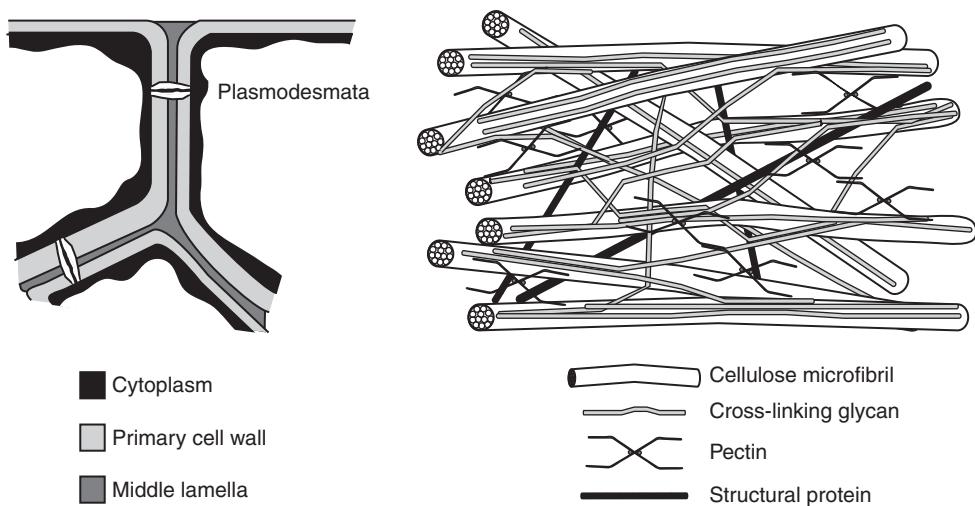


Fig. 9.4. Schematic diagrams of the plant cell wall (left) and of the molecules that make up the primary cell wall (right).

responsible for the huge host range of root-knot nematodes, which encompasses many thousands of flowering plant species. Both the cyst and root-knot nematodes use the stylet during invasion and migration as a cutting tool. This physical disruption of plant tissues is thought to be more important for cyst nematodes (which are equipped with a strong stylet) than root-knot nematodes.

Detailed examination of the behaviour patterns of nematodes migrating through plant roots led to the suggestion some time ago that cell wall degrading enzymes might be used by plant-parasitic nematodes during migration. For example, root-knot nematodes within roots show stylet movements and protrusions that are followed by pumping of the metacorporeal bulb. This prediction was proved correct when the first nematode pathogenicity factor, a secreted protein produced by the pharyngeal gland cells, was identified as a cellulase (see Box 9.1). Cellulases break the β 1–4 links within the cellulose polysaccharide chain and a family of these proteins, which has probably arisen by gene duplication, is now known to be present within plant-parasitic nematodes. The genes that encode these proteins are expressed specifically in the sub-ventral gland cells and the cellulase proteins are secreted into the plant from the stylet during migration. Subsequent studies have shown that this is not the only plant cell wall degrading enzyme produced by plant-parasitic nematodes. Pectate lyases, xylanases, polygalacturonases, arabinases and arabinogalactan galactosidases have been reported from various phylogenetic groups. It has also been shown that plant-parasitic nematodes secrete expansins during migration. These enzymes are thought to disrupt the non-covalent interactions (hydrogen bonds) between cellulose chains and between cellulose and crosslinking glycans. The action of expansins greatly enhances the accessibility of the other polysaccharide chains to the activity of the cellulases and pectate lyases. Analysis of the genomes of two root-knot nematodes sequenced to date (*Meloidogyne incognita* and *M. hapla*) shows that six different gene families representing a total of up to 60 proteins (for *M. incognita*) are present; plant-parasitic nematodes therefore secrete a cocktail of enzymes that degrade the plant cell wall and allow migration to where the feeding site is induced (Haegeman *et al.*, 2011).

The presence of these cell wall degrading enzymes in nematodes is remarkable for several reasons. Plant cell wall degrading enzymes had previously only been shown to be produced by plants themselves, and by pathogenic bacteria and fungi, with no reports of their production by animals. Almost all other animals use endosymbiotic bacteria for production of the enzymes and, before 1998, when the first nematode cellulases were described, it was believed that no animals contained endogenous genes of this type. The nematode genes, when compared to other sequences in databases, are most similar to cell wall degrading enzymes from bacteria. However, several convincing lines of evidence show that these are endogenous nematode genes. The genes are expressed solely in the sub-ventral gland cells, which do not contain symbiotic bacteria. Analysis of genomic sequences of the genes shows that they contain introns, which are not present in bacterial DNA. In addition these introns have sequence features found in other nematode introns and are spliced in the way that is expected for nematode introns. Since these genes are not present in other (non-plant-parasitic) nematodes or almost all other invertebrate animals, the most logical explanation for their presence in plant-parasitic nematodes is that they have been acquired by horizontal gene transfer from bacteria.

The acquisition of genes encoding plant cell wall degrading enzymes by an ancestor of the plant-parasitic nematode species present today was a key event in the evolution

Box 9.1. How do we identify nematode genes involved in parasitism?

Plant-parasitic nematodes are extremely awkward experimental animals. They are inconveniently small, difficult to produce in large numbers and many of the most interesting parasitic stages live deep within plant roots. However, nematode genes and proteins have been identified using several different approaches.

Perhaps the most direct approach has been to collect nematode secretions and analyse the proteins present. This approach requires many millions of nematodes and was facilitated by the discovery that certain chemicals and plant root diffusates stimulate release of secreted materials. Once secreted proteins have been collected they can be analysed directly using two-dimensional gel electrophoresis. In this technique, proteins are first separated on an immobilized pH gradient on the basis of their isoelectric point. The separated proteins are then run on a large gel, which separates the proteins on the basis of their size. The result is a large rectangular gel in which individual proteins appear as spots. Spots can be excised from the gel and short sequence tags can be obtained using mass spectrometry. This allows protein identification. A more recent development in proteomics has been used to identify secreted proteins from *M. incognita*, where collected secretions were chromatographically separated before being directly analysed by mass spectrometry (Bellafiore *et al.*, 2008). As with all protein-based approaches, this method requires extensive genome information to give the best chance of identifying proteins.

Antibodies raised against secreted proteins can also be used to identify nematode parasitism genes, either by screening of a cDNA library (see below) or by purifying the protein from nematode homogenate. This approach was used for identification of nematode cellulase encoding genes.

One successful route for gene identification in nematodes has been the use of expressed sequence tags (ESTs). A cDNA library (which can be viewed as a catalogue of the genes expressed in the tissue or organism at the time of RNA extraction) is first made from a life cycle stage or tissue of interest. Genes are sequenced at random from this library and the resulting sequence tags are subjected to bioinformatic analysis in order to try to determine the identity of the genes from which they are obtained. cDNA libraries for EST analysis can be generated from whole nematodes or they can be made from the pharyngeal gland cells that are thought to be the source of the secreted proteins of most interest. Both approaches have advantages and disadvantages. For analysis of whole nematode libraries only abundantly expressed gland cell components will be present at levels allowing them to be detected readily when compared to the genes expressed in the rest of the body. However, analysis of whole nematode libraries provides greater information on genes expressed in all body tissues (e.g. the hypodermis) that may play roles in the host-parasite interaction. On the other hand, making good quality cDNA libraries from gland cells is extremely technically challenging but the genes encoding proteins important in parasitism are likely to be present in abundance.

As DNA and RNA sequencing has become less expensive, analysis of full genome or transcriptome sequences for secreted proteins has become more common. Next-generation sequencing technologies, such as 454 FLX and Illumina allow generation of many gigabases of sequence information at low cost, making genomics accessible for all organisms. Secreted proteins can be identified from these resources using various bioinformatic approaches.

Continued

Box 9.1. Continued.

Functional analysis of nematode genes has been greatly facilitated by the development of RNA interference (RNAi). Exposure of an organism to double-stranded RNA (dsRNA) generated from a gene of interest causes rapid degradation of the endogenous mRNA from which the dsRNA was made. This causes a drop in the level of the corresponding protein. The effects of the removal of the protein on the normal biology of the nematode can then be observed, allowing the function of the original gene product to be investigated.

of plant parasitism. Many of the Tylenchoidea plant parasites investigated to date have these genes present, including migratory endoparasites such as *Pratylenchus* and *Radopholus*. It seems likely that several transfer events have occurred during the diversification of the Tylenchoidea that have allowed nematodes to live within and to exploit plant tissues. Horizontal gene transfer may have driven the evolution of plant parasitism by nematodes on more than one occasion. Another plant-parasitic nematode, *Bursaphelenchus xylophilus*, a member of the Aphelenchoidea rather than the Tylenchoidea, has been shown to contain cellulase and pectate lyase (Kikuchi *et al.*, 2011). However, in this nematode the cellulase appears to have been acquired from fungi, supporting the concept of multiple independent horizontal gene transfer events from a range of sources.

9.3. Wound and Defence Responses of the Plant

Nematode infection results in wounding of plant tissues, particularly during the migration phase but also during expansion of the feeding site. Root-knot nematodes avoid eliciting strong plant responses by migrating between the root cells. By contrast, cyst nematodes migrate straight through the cortical cells towards the centre of the root, causing necrosis from their entrance point to the site where they settle to feed (Wyss, 2002).

Plants are usually able to recognize and react to parasites by activating various defence responses. Defence responses include the production of toxic oxygen radicals and systemic signalling compounds as well the activation of defence genes that lead to the production of structural barriers or other toxins designed to harm intruding pathogens (see below). The plant defence (immune) system is thought to consist of two layers of responses and is summarized in both functional and evolutionary terms by the ‘zigzag’ model (Jones and Dangl, 2006; Fig. 9.5). Plants detect highly conserved pathogen molecules (pathogen-associated molecular patterns, PAMPs) through cell surface pattern recognition receptors (PRRs) and trigger pattern-triggered immunity (PTI), also referred to as basal defences. Although little is known about PAMPs in nematodes, in other plant pathogens they are highly conserved, specific to the pathogen and are usually structural molecules essential for pathogen survival. PTI is relatively durable as the pathogens are not readily able to evolve changes to PAMPs. All successful biotrophic pathogens need to be able to suppress PTI in order to infect plants.

This is achieved using effectors, secreted proteins introduced into the host, which target the signalling pathways invoked during PTI. However, plants have subsequently evolved a further layer of defences known as effector-triggered immunity (ETI). ETI is mediated by the products of resistance genes and these recognize the effectors or, more frequently, the changes to host metabolism induced by the effectors. ETI is frequently mediated by a strong localized cell death response (the hypersensitive response), which targets the tissues on which the pathogen is trying to feed. Further details of resistance genes and the effectors that they recognize are provided in Section 9.8.

Changes in gene expression correlated with wound or defence responses have been studied in several plant–nematode interactions. Just 12 h after inoculation of tomato roots with root-knot nematodes, general plant defence genes are upregulated (Williamson and Hussey, 1996). Most of these genes are induced in both the compatible and the incompatible interaction, albeit with differences in levels and timing. Genes encoding direct defence proteins that are activated include peroxidase, chitinase, lipoxygenase, extensin and proteinase inhibitors. Genes that encode enzymes in pathways that result in synthesis of other defence compounds are also activated during plant defence responses. For example, genes encoding enzymes that lead to synthesis of phytoalexins (such as glyceollin in soybean), or deposition of physical barriers such as callose and lignin, are induced in the early phases of the nematode infection process.

The application of high throughput molecular analyses (micro-arrays, RNA sequencing) to plant–nematode interactions has enabled a more comprehensive overview of the plant response to nematode invasion, indicating that the molecular defence

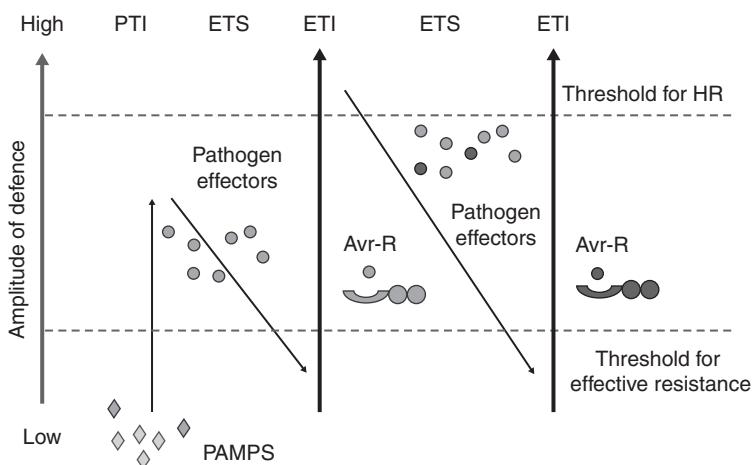


Fig. 9.5. The zigzag model (Jones and Dangl, 2006). Conserved pathogen molecules (pathogen-associated molecular patterns, or PAMPs) are detected by host cell surface pattern recognition receptors (PRRs), which activate pattern-triggered immunity (PTI). Adapted pathogens deliver effector proteins inside host cells to suppress these defences, thus inducing effector-triggered susceptibility (ETS). To counter pathogen-induced PTI suppression, plants possess a second layer of immune receptors to detect the presence of effectors, leading to effector-triggered immunity (ETI). An evolutionary battle then follows with pathogens evolving new effectors that evade detection and plants evolving new resistance genes. HR, hypersensitive response.

response to nematode infection is very similar to the response to many other pathogens. These responses include activation of genes encoding pathogenesis-related (PR) proteins and defence signalling compounds, as well as general stress response genes and genes associated with the hypersensitive response. However, specific plant defence pathways may be locally suppressed in the feeding sites. Several genes encoding key enzymes in jasmonic acid biosynthesis were found to be downregulated in syncytia induced by *H. glycines* in soybean (Ithal *et al.*, 2007) and some genes for pathogenesis-related proteins were downregulated in *Arabidopsis* giant cells (Barcala *et al.*, 2010).

In addition to these local responses at the infection site, defence responses are often systemically induced in remote plant tissues after pathogen attack. As a result of this systemically acquired resistance (SAR), the plant is better prepared for attack by nematodes on other roots. Nevertheless, in some cases plant defence in systemic tissues is suppressed, possibly causing the plant to be more susceptible to leaf pathogens after nematode attack. During *H. schachtii* infection of *Arabidopsis*, the expression of *PR-1*, *PR-2* and *PR-5*, considered to be markers for salicylic acid (SA)-dependent systemic-acquired resistance (SAR), was induced in roots and leaves. The expression of *PR-3* and *PR-4*, which are used as markers for jasmonic acid (JA)-dependent SAR, was not altered in roots, but *PR-3* was induced, whereas *PR-4* was downregulated in the leaves of *H. schachtii*-infected plants (Hamamouch *et al.*, 2011). However, all tested *PR* genes (*PR-1* to *PR-5*) were downregulated in the leaves of *M. incognita*-infected plants, suggesting the suppression of both SA- and JA-dependent SAR. Similarly, many defence-related genes were suppressed in shoots of root-knot nematode infected rice at 3 days after infection, but not in shoots of rice infected by cyst or migratory nematodes (Kyndt *et al.*, 2012a). These results suggest that root-knot nematodes are not only specialized in avoiding plant defence responses (see above) but actively suppress the host defence.

9.4. Suppression of Host Defences and Protection from Host Responses

Plant-parasitic nematodes, like other plant pathogens, have evolved a series of physical and biochemical adaptations that help them to either avoid eliciting a host response, suppress host defences or to reduce the toxic effects of any plant defence response.

9.4.1. Avoidance of host defence responses

The nematode cuticle is a complex multilayered structure (see Chapter 1 and Box 1.1). Two main structural layers (the basal zone and the cortex) provide strength and flexibility to the cuticle. However, microscopical and immunological studies have shown that the cuticle surface is overlaid with a surface coat made up largely of proteins and glycoproteins. The origins of the surface coat are uncertain. The genes encoding some of the proteins present in the surface coat are expressed in the hypodermis. However, it has also been suggested that the gland cells surrounding the amphids may be the source of surface coat material at the anterior end of the nematode, while in other studies the secretory-excretory system has been shown to be a

source of surface coat material. The surface coat is a dynamic structure that is continuously shed and replenished. This can be demonstrated by the fact that it is replaced after being removed with protease enzymes or detergents. In addition, antibodies or lectins that recognize the surface coat bind to material shed behind *M. incognita* as it moves within plants. This shedding of the surface coat may help to protect plant-parasitic nematodes in two ways. For motile nematodes it may mean that where a defence response is initiated it is targeted at the shed surface coat, leaving the nematode itself unimpeded. It has also been shown that epitopes present within the surface coat of *M. incognita* are also present within other plant tissues. The surface coat may therefore mimic host tissues in an attempt to avoid eliciting a resistant or defence response.

9.4.2. Suppression of host defences

Although it is likely that the principles of the zigzag model apply to the interactions between biotrophic nematodes and their hosts, few details of the mechanisms by which nematodes suppress PTI are known. A large family of proteins, the SPRYSECs, has been identified from the potato cyst nematodes *G. rostochiensis* and *G. pallida* and it has been shown that at least one of these proteins is important in suppression of host defence responses (Postma *et al.*, 2012). A secreted calreticulin of *M. incognita* has been shown to suppress PTI, possibly by preventing calcium influx from the apoplast (Jaouannet *et al.*, 2012). In addition, constitutive expression of a *H. schachtii* effector similar to annexins in *A. thaliana* promoted hypersusceptibility to nematode infection. This annexin was shown to interact with an oxidoreductase, which promotes susceptibility to oomycetes. Both lines of evidence are consistent with the annexin playing a role in suppression of host defences.

Another effector that may interfere with host defences, by possibly disturbing the SA pathway, is 10A06 from *H. schachtii*. Constitutive expression of 10A06 in *A. thaliana* enhances susceptibility to the nematode but also to unrelated pathogens such as the bacterium *Pseudomonas syringae* and cucumber mosaic virus, indicating that this effector targets a general component of the plant immune system. This effector protein stimulates polyamine biosynthesis through its interaction with spermidine synthase 2 (SPDS2) causing upregulation of antioxidant genes and the disruption of SA-mediated defence signalling (Hewezi *et al.*, 2010).

9.4.3. Protection from host defence responses

The proteins that are present within the secretions of plant-parasitic nematodes (reviewed in Haegeman *et al.*, 2012) include proteins designed to protect the nematode from host defence responses. For example, plant-parasitic nematodes produce glutathione-S-transferase (GST) and one GST of *M. incognita* is expressed in the pharyngeal gland cells from where it is presumably secreted into the host. Knockout of the transcript encoding this gene led to a decrease in parasitism, suggesting it plays an important role in the nematode life cycle (Dubreuil *et al.*, 2007). In many animal-parasitic organisms, including nematodes, GSTs detoxify endogenous and xenobiotic compounds using a range of biochemical mechanisms. It is possible that GSTs may

have a similar role in the interactions between nematodes and plants, detoxifying some of the wide range of secondary metabolites that the plant uses to deter invaders.

One of the major responses of plants to nematode infection is production of damaging free radicals, including hydrogen peroxide. In order to overcome this threat, plant-parasitic nematodes secrete enzymes that can break down this hydrogen peroxide in their surface coat. A peroxiredoxin (thioredoxin peroxidase) that specifically metabolizes hydrogen peroxide has been shown to be present in the surface coat of *G. rostochiensis*. In addition a secreted glutathione peroxidase is expressed in the hypodermis of this nematode and may also be secreted to the parasite surface. This protein does not metabolize hydrogen peroxide but has a preference for larger, lipid hydroperoxides. These compounds may also be produced by the plant as direct anti-pathogen factors and can also form part of plant defence signalling pathways. *Globodera rostochiensis* also secretes other proteins that may inhibit defence signalling pathways. Linoleic and linolenic acids are metabolized by lipoxygenase in the first steps of the jasmonic acid signalling pathway. Activation of this pathway leads to changes in gene expression associated with systemic defence responses. The surface coat of *G. rostochiensis* contains a secreted lipid binding protein (GpFAR-1) that can bind linoleic and linolenic acids and it has been shown that the binding activity of GpFAR-1 can inhibit lipoxygenase-mediated peroxidation of these compounds. Plant-parasitic nematodes, therefore, secrete proteins that inhibit defence signalling pathways as well as proteins that can metabolize the toxins produced by plants as part of defence responses.

The presence of peroxiredoxin, glutathione peroxidase, GST and secreted lipid-binding proteins within the secretions of plant-parasitic nematodes shows that there are parallels in the ways in which plant and animal parasites protect themselves from their hosts. Whilst defence mechanisms of plants and animals are different, some common components are present. Both plants and animals respond to an invader by producing free radicals and both have signalling pathways that include lipid molecules. Like plant parasites, some animal-parasitic nematodes produce peroxiredoxins, glutathione peroxidases, GST and lipid-binding proteins in their secreted proteins. These substances are not present in secretions of non-parasitic nematodes such as *Caenorhabditis elegans*. Their presence in secretions of a range of parasites is therefore a remarkable example of convergent evolution, where the site of expression of similar standard nematode housekeeping proteins is changed in order to provide protection from similar host defences.

9.5. Molecular and Cellular Aspects of the Development of Nematode Feeding Cells

9.5.1. Cellular changes

Nematodes that feed for a prolonged period from the same cell induce cytological modifications that increase the metabolic and transport capacities of the cell (Fig. 9.3). The precise mechanism underlying feeding cell formation is unknown but there is growing evidence that the secretions from the nematode's pharyngeal glands have a pivotal role in this process (Haegeman *et al.*, 2012). When a cyst nematode

selects a competent root cell, this cell responds by gradually widening some of the plasmodesmata that link it to neighbouring cells. The protoplasts of the initial syncytial cell and its neighbours fuse through these developing wall openings. At later stages, cell wall openings are formed *de novo* and the syncytium continues to expand by incorporating hundreds of adjacent cells. The first sign of giant cell induction by a root-knot nematode is the formation of several binucleate cells (Jones and Payne, 1978; Fig. 3.5). Additional nuclear divisions (mitoses) uncoupled from cell division result in several large multinucleate cells being formed. At the site of cell plate formation in developing giant cells, vesicles accumulate but the formation of the cell plate is aborted, preventing cell division (Jones and Payne, 1978). These acytokinetic mitoses result in polyploid cells with up to 100 nuclei. In addition, cells that surround the giant cell divide and swell to form the typical gall or root knot.

Despite their different ontogeny, many of the physiological functions (such as nutrient supply to the nematodes) and underlying cellular features of these two types of feeding cells are similar. Both have multiple enlarged nuclei, small vacuoles and show proliferation of smooth endoplasmic reticulum, ribosomes, mitochondria and plastids. In both giant cells and syncytia the high metabolic activity of the feeding cells is reflected in the upregulation of mitochondrial and ribosomal genes, genes encoding components of the proteasome pathway (for protein turnover) and genes involved in carbohydrate metabolism (Box 9.2; Gheysen and Mitchum, 2009).

Although many different methods can be used to study plant gene expression (see Box 9.2), the comprehensive analyses were only feasible with the use of micro-arrays containing thousands of genes. These micro-arrays can be incubated with labelled mRNA copies to compare nematode-infected and control roots. Puthoff *et al.* (2003) have used *A. thaliana* infected with *H. schachtii* and as a control with *H. glycines*. This latter nematode cannot induce syncytia in *A. thaliana* roots efficiently because of non-host resistance. In this way genes could be identified that are specifically upregulated 3 days after inoculation in the *H. schachtii*-infected roots. These genes are interesting candidates for involvement in the early stages of syncytium formation. Many micro-array experiments have been performed since and not only on *A. thaliana* but also on soybean and on tomato (for a comprehensive review see Escobar *et al.*, 2011). Next-generation sequencing is opening up even more possibilities to analyse in detail differences in expression patterns between control and infected plant tissues.

9.5.2. Cell wall and membrane modifications

Nematode-induced feeding sites develop through cell expansion, which requires cell wall (Fig. 9.5) loosening and remodelling. This is reflected in the upregulation but also sometimes suppression (reviewed in Gheysen and Mitchum, 2009) of endogenous plant genes encoding cell wall modifying proteins including expansins, β 1–4 endoglucanases, polygalacturonase and pectin acetyl esterase. It is evident that feeding cell formation is a complex process that requires the coordinated regulation of several different types of plant cell wall modifying proteins, as well as the differential expression of individual gene family members. In syncytia, cell wall modifying proteins are

Box 9.2. Identification of plant genes that are responsive to nematode infection.

Cellular and biochemical changes in plants are caused by changes in a variety of proteins including structural proteins such as cytoskeleton compounds or enzymatic proteins such as kinases involved in cell cycle regulation. Proteins are made by genes through the production of an intermediate messenger, the mRNA which is transcribed from the gene after activation of the promoter. A study of the molecular response of the plant can therefore be performed at different levels: analysis of promoter activity, mRNA differences or protein patterns. Indeed, the study of the plant response to nematode infection has been done at these different levels and using a variety of methods (Escobar *et al.*, 2011). Examples include studies:

1. At the protein level using two-dimensional gel electrophoresis (see Box 9.1) and antibody labelling of plant tissue sections.
2. At the mRNA level: mRNA has been extracted from infected roots or from nematode feeding sites (NFS) and comparisons were made with mRNA from non-infected roots. These methods (see also Box 9.1) comprise analysis of cDNA libraries, quantitative reverse-transcriptase PCR, micro-arrays and RNA sequencing.
3. At the promoter level: a reporter gene was used to analyse activity in NFS of known or unknown promoters.

Data collection and interpretation on plant gene expression after nematode infection has profited from the use of the model plant *Arabidopsis thaliana*. A model organism is one studied by many scientists because it has features that make it easy to study and it is similar enough to other organisms for extrapolation of conclusions. *Arabidopsis thaliana* is a small weed of the cabbage family (Brassicaceae) and it has some key characteristics that make it suitable as a model organism for plant studies:

- Small genome size, 120 million base pairs (compared to, e.g. soybean 1100 million and maize 2500 million base pairs), which has allowed determination of the whole DNA sequence.
- Small size, many plants can be grown in a growth chamber.
- Short life cycle, from seed to next generation seed in 6–8 weeks.
- Many small seeds are produced that can easily be stored.
- Production of mutants is easy by chemical, radiation or insertion mutagenesis.
- Transformation is easy and rapid, e.g. the floral dip method does not require tissue culture facilities or sophisticated equipment.
- The thin transparent roots facilitate following the nematode infection process.

Since the first use of *A. thaliana* as a model species for plant nematode research (Sijmons *et al.*, 1991), most molecular data on the plant response have been obtained from this model species. Nevertheless, it is still necessary to analyse crop species to verify important conclusions, and therefore soybean, tomato, potato and more recently *Lotus* and rice have also been studied (Gheysen and Fenoll, 2011).

Some nice examples of the advantages of working on a model species include the analysis of hormone mutants (see Box 9.3) and the possibility of analysing expression of all plant genes in one experiment by using micro-arrays. Because all the genes of *A. thaliana* had been sequenced, micro-arrays that carry unique fragments of all these genes were available first for this plant species.

also involved in the cell wall breakdown that occurs between adjacent cells, allowing the cytoplasm to move freely throughout the syncytium. Nematodes appear to enhance this process by secreting proteins that activate cell wall modifying proteins from the plant. A cellulose binding protein (CBP) from *H. schachtii* was shown to interact with a pectin methylesterase from *A. thaliana*, thereby activating and potentially targeting this enzyme to aid cyst nematode parasitism (Hewezi *et al.*, 2010).

In both syncytia and giant cells, the cell wall adjoining the xylem increases its thickness by forming finger-like wall invaginations lined with plasma membrane (Jones and Northcote, 1972). A similar process occurs in plant transfer cells and it is likely to facilitate water transport from the xylem into the feeding cell. It has been estimated that each developing juvenile of *H. schachtii* withdraws an amount of solute equivalent to four times the total syncytial volume from the syncytium every 24 h. Genes encoding water channel proteins are upregulated in giant cells but similar genes do not appear to be upregulated in syncytia. However, a syncytium is one huge cell that may consist of up to 250 fused root cells, whereas giant cells are interconnected separate cells. Therefore, giant cells may require water channels to redistribute the water rapidly, even though they are interconnected with each other through plasmodesmata.

While water is taken from the xylem, nutrients are retrieved from the phloem. However, it has been shown that syncytia are initially symplastically isolated from surrounding tissues, including the phloem. Assimilates must therefore be imported into the syncytium via the apoplast. The sucrose carrier AtSUC2 is responsible for the active import of sucrose into sink tissues and is highly expressed in syncytia. Therefore, it may have a role in forming or maintaining the metabolic sink activity in syncytia. The activity of sugar or other transporters is dependent on the energy generated by H⁺-ATPases, which have also been shown to be upregulated in NFS. At later stages, functional plasmodesmata are being established and at 15 days after infection all syncytia are symplastically connected to the phloem (reviewed in Grundler and Hofmann, 2011).

9.5.3. The cytoskeleton

Sedentary endoparasitic nematodes induce long-term rearrangements of the cytoskeleton during the infection process. Actin genes are highly expressed in NFS, as would be expected in large expanding cells that need extensive internal transport (de Almeida-Engler *et al.*, 2004). Tubulin genes are slightly upregulated in syncytia and highly upregulated in giant cells, probably reflecting the need for a mitotic cytoskeleton for the rapidly dividing nuclei in the latter. Analysis of the cytoskeleton by immunolocalization of actin and tubulin proteins and by the use of GFP fusions revealed that the cytoskeleton was strongly disrupted in syncytia (de Almeida-Engler *et al.*, 2004). In giant cells, although disturbed compared to normal root cells, actin and microtubular fibres are visible. Also a functional mitotic apparatus, consisting of microtubules forming spindles (for separating the chromosomes during nuclear division) and phragmoplasts (which initiate the cell plate), are present in developing giant cells (de Almeida-Engler *et al.*, 2004). As nematode feeding involves the retrieval of large volumes of cytoplasm, a degree of cytoskeleton fragmentation may facilitate uptake during nematode feeding.

9.5.4. Nuclear changes and cell cycle activation

Several cell cycle genes are among those that are induced in the early stages of NFS formation. Both types of feeding cells undergo multiple rounds of shortened cell cycles leading to genome amplification, but do so in different ways (Fig. 9.6). Thus, giant cells go through repeated (acytokinetic) mitoses, whereas nuclear divisions have never been observed inside syncytia, which repeatedly go through S (synthesis)-phase (repeated DNA synthesis or endoreduplication), apparently shunting mitosis. The absence of clear differences in expression of cell cycle-related mRNAs between giant cells and syncytia (even when specifically analysing genes involved in endoreduplication), in spite of the differences in ontogeny of these structures, can be explained in several ways. It is possible that the genes involved specifically in endoreduplication or in acytokinetic mitosis have not yet been identified, or that these processes are controlled by subtle differences in expression time or levels. It is also possible that regulation occurs primarily at the post-transcriptional level, by regulation of protein turnover or activity.

9.5.5. Signal transduction pathways and transcription factors

Transcription factors and protein kinases involved in signal transduction pathways are crucial in cell differentiation processes. Those that have been found to be up- or

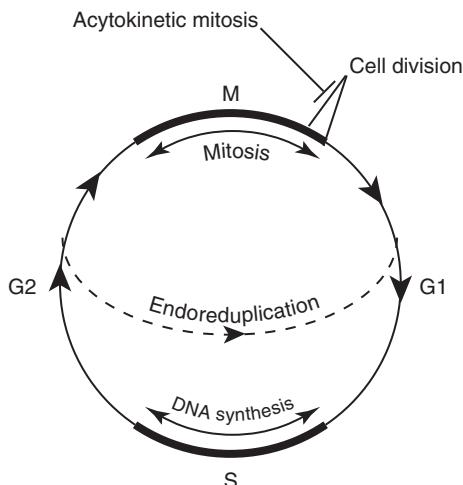


Fig. 9.6. The cell cycle. The standard eukaryotic cell cycle consists of four successive stages. The nuclear DNA is replicated during S (synthesis) phase and the nucleus divides in the M (mitosis) phase. The interval between the completion of mitosis and the beginning of DNA synthesis is called the G1 phase (G = gap) and the interval between the end of DNA synthesis and the beginning of mitosis is the G2 phase. At the end of the mitotic phase, the cell divides by a process called cytokinesis. Cyst nematodes activate the cell cycle in the feeding cells they induce, but no mitosis occurs. Instead the cells repeatedly go through the S phase (endoreduplication), probably also through (part of) G1 and G2 phases but bypass mitosis. Root-knot nematodes induce repeated nuclear division but no cell division; this is called acytokinetic mitosis.

downregulated in NFS may therefore merit particular attention. Because these genes belong to gene families and are often involved in complex pathways, their specific roles in NFS formation are difficult to grasp without detailed functional studies. Some transcription factors, however, have been analysed thoroughly (reviewed in Gheysen and Mitchum, 2009). For example, the KNOX transcription factor is highly expressed in giant cells while its role in uninfected plants is to maintain meristem function and for development of lateral organs. Disruption of auxin transport was found to mimic KNOX overexpression and a strict correlation between KNOX expression and elevated cytokinin levels has been observed. KNOX expression is in turn regulated by the transcription factor PHAN, which is also highly expressed in giant cells. Another transcription factor that is highly expressed in both early giant cells and syncytia is WRKY23. This gene is induced by auxin and is important for primary root development and nematode infection (Grunewald *et al.*, 2008, 2012). For more details on the involvement of hormones in NFS development, see below and Box 9.3.

A transcription factor that is upregulated in the incompatible soybean–*H. glycines* interaction but downregulated in the compatible interaction, is an EREBP-domain protein (EREBP, ethylene-responsive element-binding protein) (Mazarei *et al.*, 2002). Overexpression of this transcription factor causes upregulation of PR proteins, indicating that its downregulation in the compatible interaction may inhibit the plant defence response to facilitate infection (Mazarei *et al.*, 2007).

9.5.6. Functional analysis

Cataloguing plant genes that are up- or downregulated upon nematode infection is only the first step. Many genes may be activated because they happen to have the appropriate regulatory signals that are recognized in developing NFS and not because they play an essential role. To understand the molecular basis of NFS ontogeny, functional analysis of the plant genes expressed in NFS is crucial. However, the consequence of knocking out a gene function (or overexpressing it) on NFS formation has been investigated in only a few instances (reviewed in Gheysen and Mitchum, 2009). For example, overexpression of ENOD40 (see below) gave rise to plants that supported significantly higher numbers of galls (Favery *et al.*, 2002). *TobRB7* and *PHAN* antisense plants are less susceptible to root-knot nematode infection, as are *rpe* homozygous mutants. Knocking down the expression of WRKY23 resulted in lower infection of the cyst nematode *H. schachtii* (Grunewald *et al.*, 2008). Mutants in cell wall modifying proteins such as the *Arabidopsis Cel2* mutant resulted in a reduced number of developing *H. schachtii* females (Wieczorek *et al.*, 2008). Another method for functional analysis of the cell biology of the NFS is the use of chemical inhibitors that interfere with a specific process. Blocking the cell cycle with specific inhibitors, for example, has been shown to lead to an arrest in NFS development.

Comparisons with other, better understood, systems of plant cell differentiation and organ formation can also be helpful in developing our understanding of NFS development. Root-knot nematodes and endosymbiotic rhizobia induce similar structures within plant roots (galls and nodules, respectively) and this has inspired studies comparing gene expression in the two processes. The genes encoding the transcription factors PHAN and KNOX, the early nodulin gene ENOD40 and the cell cycle gene CCS52 are induced in both nodules and giant cells compared to control roots.

Box 9.3. Plant hormones play an important role in feeding site development.

Plant hormones are biochemicals that act specifically on plant cells and tissues to regulate growth and development at low concentrations. Plant hormones can be classified into auxins, cytokinins, gibberellins, ethylene, abscisic acid, polyamines, brassinosteroids, strigolactones, peptides and oligosaccharins, each of these regulating a variety of processes. Evidence has accumulated for the involvement of auxins, cytokinins and ethylene in supporting a successful nematode infection.

Auxins influence cell elongation, cell division and differentiation, and apical dominance. They are mainly produced in the plant apex and are transported to the root tip. This polar auxin transport is important for growth and development, for example in lateral root formation. Ethylene is involved in fruit ripening, senescence and abscission, and its biosynthesis is stimulated by auxin. Cytokinins promote cell differentiation and division, often acting in association with auxin. Their synthesis occurs in various tissues and they are inactivated by oxidation.

Some examples of the complex correlation between nematode infections, cytokinin and ethylene are illustrated below. For a comprehensive overview of this topic, see Goverse and Bird (2011).

1. In tomato, cytokinin activity was lower in resistant than in susceptible cultivars, and exogenously supplied cytokinins shifted the response of resistant tomato plants towards susceptibility.
2. The cytokinin-responsive promoter ARR5 is activated in young galls induced by *M. incognita*, and expression of a cytokinin oxidase results in lower gall numbers (Lohar *et al.*, 2004).
3. In the early infection stages of root-knot nematodes on rice, genes involved in the cytokinin pathways seem to be repressed (Kyndt *et al.*, 2012b).
4. Biologically active cytokinins are produced by *Meloidogyne* spp.
5. Ethylene-overproducing *A. thaliana* mutants attract more second-stage juveniles and result in larger syncytia and females. The enhanced syncytial cell wall breakdown in these mutants indicates that ethylene-induced cell wall degradation is involved in syncytium development, pointing to similarities between cell wall alterations in syncytia and during fruit ripening. Ethylene can also induce endoreduplication.
6. Ethylene biosynthesis inhibition and impaired ethylene signalling result in a higher susceptibility of rice toward root-knot nematodes. The fact that ethylene was not detected in galls at 1–2 days post-infection (Glazer *et al.*, 1983), in combination with the transcriptional patterns observed (Barcala *et al.*, 2010; Nahar *et al.*, 2011), argue against a crucial role for ethylene-activated pathways during early giant cell differentiation.

However, a larger scale comparison (Favery *et al.*, 2002) revealed that similarities between nodules and galls may be limited. Only two of 192 genes expressed in nodules in *Medicago truncatula* were upregulated upon root-knot nematode infection. Subsequent studies have shown that the numbers of both nodules and galls are comparably decreased in roots expressing cytokinin oxidase, while lateral root formation is greater in these roots, again indicating similarities in symbiotic and parasitic organ formation (Lohar *et al.*, 2004).

Several types of plant hormones have been studied since the middle of the last century, with cytokinins, auxins and ethylene among the best characterized. These hormones (see Box 9.3) have also long been proposed to be relevant in establishment

of NFS. Despite their strong record of scientific analysis and recent molecular evidence for their role in NFS formation, it is still very difficult to propose a unifying model, because of pleiotropic effects and interactions of these hormones and contradictory results about their hypothetical roles in nematode infection. For the involvement of auxin in syncytium formation, however, a clear picture has emerged. The auxin-responsive promoter *DRC5* is rapidly and transiently activated during NFS initiation by *Meloidogyne* and *Heterodera* (Karczmarek *et al.*, 2004). Disturbance of auxin gradients with a polar auxin transport inhibitor results in abnormal feeding sites, and strong auxin-insensitive mutants barely support cyst nematode reproduction (Goverse *et al.*, 2000). The importance of polar auxin transport and of the PIN efflux transport proteins in syncytium formation have been elucidated (Grunewald *et al.*, 2009). PIN1 is necessary for transporting auxin from the plant shoot to the infection site, and its expression is downregulated in the initial syncytial cell, preventing pumping out of auxin. This, together with the upregulation/activation (see also Section 9.6.1) of auxin import proteins AUX1 and LAX3 in the syncytium results in accumulation of auxin in the early syncytium (Lee *et al.*, 2011).

9.6. Nematode Signals for Feeding Site Induction and Other Processes

Understanding how nematodes induce the changes in plant root cells that lead to feeding site formation has long been one of the holy grails of plant nematology. In recent years the number of nematode molecules identified that may play a role in host–parasite interactions has increased dramatically (Haegeman *et al.*, 2012), mainly due to the development of new technologies for gene isolation (Box 9.1). Although there have been numerous suggestions as to how these molecules might affect plant metabolism, the mechanisms used by nematodes to parasitize plants still remain largely unknown.

9.6.1. 19C07

The identification and characterization of the Hs19C07 effector protein secreted by *H. schachtii* indicates that the nematode can modify the cellular partitioning of auxin in part by targeting one of the auxin importers of the AUX1/LAX family in *A. thaliana* (Lee *et al.*, 2011). Hs19C07 has been shown to interact with the LAX3 auxin transporter and can stimulate its activity. This work is important as it represents the first direct link between a nematode effector and the plant signalling pathways that control auxin in roots.

9.6.2. Chorismate mutase

Cyst nematodes and root-knot nematodes both secrete chorismate mutases. The proteins are produced in one or both of the pharyngeal gland cell types and, like cell wall degrading enzymes, are not usually present in animals and are thought to have been acquired by horizontal gene transfer from bacteria. Chorismate mutase converts

chorismate to prephenate. Compounds derived from chorismate include auxin, an important plant hormone that has been implicated in early feeding site development (see Section 9.5 and Box 9.3), whilst a variety of secondary metabolites are derived from prephenate. Expression of the nematode chorismate mutase in soybean hairy roots gives rise to a remarkable phenotype. Normal vascular tissue development is suppressed and lateral roots do not appear to develop normally. Both these phenotypes are indicative of an auxin defect, and exogenous application of auxins reverses these observed phenotypes. It has been proposed that nematode chorismate mutases may deplete levels of auxins in the early stages of feeding site development by removal of the chorismate precursor within the cytoplasm of the plant cell. However, other studies have suggested an important role for auxins in early feeding site development (see Section 9.5.6 and Box 9.3). An alternative role for chorismate mutase, supported by its presence in migratory nematodes and fungal pathogens, is the suppression of plant defence (Djamei *et al.*, 2011). One potential fate of chorismate is conversion, via a two-step reaction, to the plant defence signalling compound salicylic acid (SA). Therefore, it is possible that CM could reduce the pool of chorismate available for conversion to SA, thus preventing normal activation of host defences.

9.6.3. CLAVATA3-like peptides

Studies on *A. thaliana* (see Box 9.2) have revealed that a transmembrane receptor-like kinase, CLAVATA1, regulates the balance between cell differentiation and cell division in the shoot apical meristem. CLAVATA1 and another protein, CLAVATA2, are thought to dimerize and to bind a small secreted peptide, CLAVATA3. Binding of CLAVATA3 to the extracellular side of this receptor complex may allow activation of the intracellular protein kinase domain and subsequent activation of signalling pathways. A bioinformatic analysis of cDNAs present in a cDNA library made from gland cells of *H. glycines* has shown that a gene encoding a short peptide with similarity to the CLAVATA3 ligand is present in this nematode (Olsen and Skriver, 2003). This protein has a signal peptide, suggesting it is secreted from the nematode into the plant. The gene encoding the *H. glycines* CLAVATA3 peptide has been shown to complement *cle3* mutants of *A. thaliana*, confirming that the nematode protein is a functional analogue of the plant peptide. The nematode peptide has been shown to bind to the CLE receptors in plants and to alter root development, providing compelling evidence that nematode CLAVATA peptides play a role in manipulation of the host (Guo *et al.*, 2011). Interestingly, recent studies suggest that plant CLAVATA peptides may also trigger innate immunity, raising the possibility that the nematode peptides may play a role in this process.

9.6.4. The ubiquitination pathway

Ubiquitin (UBI) is a small protein present in all eukaryotic cells that is used to tag other proteins for a variety of cellular processes, the best studied of which is proteolytic degradation. There is mounting evidence that cyst nematodes exploit the ubiquitination pathway in order to manipulate plant metabolism. Several cyst nematodes produce a UBI-like protein in their dorsal gland cells. These proteins appear not to be

present in root-knot nematodes. The cyst nematode UBI-like proteins are secreted from the stylet and have a C-terminal extension that, in the case of *H. schachtii*, targets a fused GFP protein to the plant nucleolus *in vivo*.

Other proteins similar to those involved in ubiquitination pathways are also present in cyst nematodes. S-phase kinase associated proteins (SKP-1) and RING-H2 proteins, which are subunits of the complex that transfers UBI tags to target proteins, are expressed in gland cells of *H. glycines* and may be secreted into plants.

Although understanding the role of the ubiquitination pathway in syncytium development still requires more research, it is intriguing to note that changes in ubiquitination pathways can affect cell cycle regulation in some organisms, giving rise to cells that go through multiple S phases with no intervening mitosis. This results in hypertrophic nuclei similar to those found in syncytia. Alternatively, the putative members of the UBI-proteasome complex secreted into plant tissues might represent a mechanism of cellular regulation and mitigation of host defences to promote parasitism by nematodes. In many pathosystems, the ubiquitination system is exploited by pathogens in order to suppress host defences.

9.6.5. Cytokinins

Cytokinins are plant hormones that have a variety of roles in plant development, often acting in concert with auxins (Box 9.3). Secretions of root-knot nematodes contain cytokinins at biologically significant levels. Thus, it is feasible that root-knot nematodes directly introduce cytokinins into plant cells and that these molecules influence plant developmental pathways. The major cytokinins present in root-knot nematode secretions are zeatins. These molecules are present at far lower levels in cyst nematodes. Zeatins are known to influence the G2 to M phase transition of the cell cycle. Formation of cyst nematode feeding sites does not involve mitosis, whereas root-knot nematode giant cells show mitosis uncoupled from cytokinesis. It is possible that zeatins are secreted by root-knot nematodes in order to drive the cell cycle to another phase and that the absence of these molecules from cyst nematodes is correlated with the different developmental patterns observed in syncytia.

9.6.6. Nodulation factors

It has been shown that there are some structural and physiological parallels between nodules and developing giant cells induced by root-knot nematodes. More detailed analysis has shown that similar regulatory pathways are induced in these structures (see Section 9.5.6). Although it is dangerous to try to generalize about the similarities between giant cells and nodules, there are clearly intriguing parallels and it is interesting to note that root-knot nematodes have been shown to express a nodulation L (NODL)-like protein. This protein is one of a cascade present in nodulating bacteria that synthesize the nodulation factors responsible for inducing formation of nodules in compatible plants. Its biochemical role is to acetylate a polyglucosamine chain. Nematodes are known to produce such polymers in chitin and it is therefore feasible that root-knot nematodes can synthesize some form of nodulation (NOD)

factor-like molecule. Studies have shown that root-knot nematodes produce a secreted compound that induces changes in *Lotus japonicus* root hairs that are diagnostic of responses to nodulation factors. Further studies showed that these changes were not induced in response to nematode secretions by mutant lines of *L. japonicus* defective in the ability to detect nodulation factors. Some of these mutant lines also displayed a reduced capacity to support nematode infection (Weerasinghe *et al.*, 2005). Taken together these findings suggest that signalling pathways used by root-knot nematodes when infecting plant roots overlap with those exploited by nodulating bacteria and that compounds similar to nodulation factors may be produced by these nematodes.

9.6.7. Others

Whilst it is possible to speculate on functions for the proteins and compounds described above, plant-parasitic nematodes also produce other secreted proteins in their pharyngeal gland cells for which it is more difficult to ascribe a putative role in the host–parasite interaction. Root-knot nematodes have been shown to produce a secreted 14-3-3-like protein in their pharyngeal gland cells and this protein has been detected in purified stylet secretions. However, this protein has a wide range of biological roles and its function, if any, in terms of host–parasite interactions is unknown. In addition, analysis of ESTs from gland cells of both cyst and root-knot nematodes has shown that both produce a large number of proteins with signal peptides that have no matches to any other proteins in any databases. Although some of them have been functionally analysed (see 10A06 in Section 9.4.2 and 19C07 in Section 9.6.1), the function of many of these proteins is still unknown and, given the difficulties of elucidating the biological role of entirely novel proteins, this is likely to remain the case for some time to come.

9.7. Comparisons Between Cyst and Root-knot Nematodes

It is clear that there are intriguing parallels as well as clear differences between the invasion process and feeding sites of cyst and root-knot nematodes and that these parallels and differences are reflected in the substances secreted by the two groups of nematodes. The main features are summarized in Table 9.1. Phylogenetic analysis shows that cyst and root-knot nematodes are not directly related to each other, with each seeming to have evolved independently from different groups of migratory endoparasites (see Section 2.11.3). This implies that the ability to induce feeding sites has also evolved independently within each group. A comparison of the proteins secreted by each group is in agreement with this idea; the only effectors that are common between cyst and root-knot nematodes are also present in migratory endoparasites. These include cell wall degrading enzymes and chorismate mutase. Other effectors, including the large numbers of ‘pioneer’ sequences identified from gland cell ESTs, are generally specific to cyst or root-knot nematodes.

Table 9.1. Comparison of some secreted proteins and feeding sites of cyst and root-knot nematodes.

	Cyst nematodes	Root-knot nematodes
Nematode secretions		
Sub-ventral glands		
Cell wall degrading enzymes		
β 1,4-endoglucanase	+	+
Pectate lyase	+	+
Polygalacturonase	N	+
Expansin	+	+
Xylanase	N	+
Cellulose-binding domain	+	+
Calreticulin	N	+
Chorismate mutase (also in dorsal gland in some species)	+	+
Dorsal gland		
SPRYSEC	+	N
Ubiquitin extension	+	N
CLAVATA3	+	N
14-3-3	N	+
Other		
Plant hormones	N (very low levels)	+
Feeding sites		
Origin	Syncytium by fusion of protoplasts	Giant cells by acytokinetic mitosis
Cytology	Dense cytoplasm with many organelles	Dense cytoplasm with many organelles
Nuclei	Many large nuclei	Many large nuclei
Cell wall	Cell wall ingrowths close to xylem Cell wall degradation	Cell wall ingrowths close to xylem —

N = not found; + = present; — = not upregulated.

9.8. Resistance and Avirulence Genes

Many plant resistance genes have been cloned and the encoded proteins fall into six classes, four of which (like the majority of plant resistance genes) consist of proteins with leucine-rich repeats (LRRs), structural motifs that have been shown to mediate protein–protein interactions (reviewed in Hammond-Kosack and Jones, 1997). Some encode transmembrane and mainly extracellular proteins, whilst some encode entirely cytoplasmically located proteins. It is most likely that the localization of the *R*-gene product reflects the site of the cellular location of the interaction with the avirulence product. For example, a viral AVIR protein will be present inside the plant cell whilst a fungal AVIR protein may be localized in the intercellular plant space. Many *R*-proteins have a TIR or serine/threonine protein kinase domain, domains involved in intracellular signal transduction. Each *R*-gene is thought to have at least two functions: recognition of a specific AVIR-derived signal and activation of downstream signalling pathways to trigger the various defence responses, ultimately resulting in localized plant cell death.

Plant resistance genes to nematodes are present in several crop species and wild relatives (see Chapter 14) and their identification and incorporation into commercially viable cultivars are important factors in breeding programmes of tomato, potato and soybean, among others. Many of these resistance genes have been mapped at precise positions on plant chromosomes to facilitate the breeding process, and several of these genes have now been cloned (reviewed in Caromel and Gebhardt, 2011, and Kaloshian *et al.*, 2011).

The first nematode resistance gene to be cloned was the *Hs1^{pro-1}* gene (from wild beet), which confers resistance to *H. schachtii*. This gene is different from any other resistance gene cloned from plants and in backcrosses the presence of *Hs1^{pro-1}* does not correlate with a resistant phenotype. Consequently, some controversy as to its precise role remains. The gene encodes a relatively small protein (282 amino acids) that does not contain a leucine-rich repeat, the typical hallmark of most resistance genes (see Fig. 9.7). Two *rhg* genes, which confer resistance against *H. glycines* in soybean, have been cloned and also show highly unusual properties compared with other resistance genes. Resistance conferred by the *rhg1* gene is due to the presence of multiple copies of a segment of DNA containing several different genes, none of which show a typical NB-LRR structure (Cook *et al.*, 2012). In addition, the *rhg4* gene has been identified as a serine hydroxymethyl transferase (Liu *et al.*, 2012). Details of the mechanisms underlying the resistance provided by either of the *rhg* genes are not yet known.

The best studied nematode resistance gene is the tomato gene *Mi-1.2*, which confers resistance to three species of root-knot nematodes. It is rather unusual compared to other resistance genes because it also confers resistance to three other very different organisms, the potato aphid *Macrosiphum euphorbiae*, the white fly *Bemisia tabaci* and the tomato psyllid *Bactericercus cockerelli*. The *Mi* gene encodes a 1257 amino acid protein that is a member of the LZ-NBS-LRR family (Fig. 9.7). Swaps of

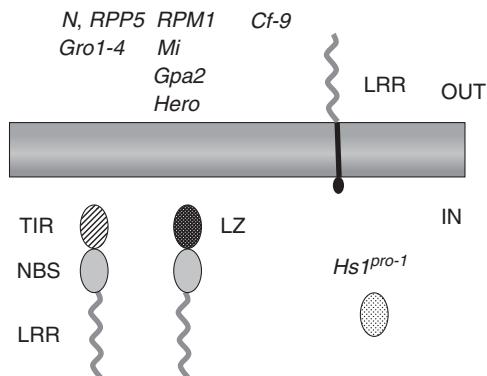


Fig. 9.7. Classes of plant resistance genes. The nematode genes that have been cloned to date fall into one of three classes: (i) TIR-NBS-LRR family to which *Gro1-4* and, among others, the virus resistance gene *N* and the bacterial resistance gene *RPP5* belong; (ii) LZ-NBS-LRR family to which three cloned nematode resistance genes and, among others, the bacterial resistance gene *RPM1* belong; and (iii) *Hs1^{pro-1}*, which is very different from all other resistance genes identified to date. Another important class of plant resistance genes has an extracellular LRR domain; the fungal resistance gene *Cf-9* belongs to this family. No nematode resistance genes have yet been identified that belong to this class although there is evidence that some of the soybean cyst nematode resistance genes might do so. (Abbreviations: LZ, leucine zipper; LRR, leucine-rich repeat; NBS, nucleotide binding site; TIR, Toll/interleukin-1/resistance domain.)

protein domains between *Mi-1.2*, the functional gene and *Mi-1.1*, a non-functional related protein, have shown that the LRR region has a role in the signalling process that leads to plant cell death (typical for the hypersensitive response) and that the N-terminal part of the protein controls this cell death. This model implies that the N-terminus of the protein keeps the LRR in an inactive state, unless an avirulence signal from the pathogen releases this inhibition.

Two other cloned genes, *Gpa2* (a potato gene conferring resistance to *G. pallida*) and *Hero* (a tomato gene conferring resistance against *G. pallida* and *G. rostochiensis*) also belong to the LZ-NBS-LRR family (Fig. 9.7) but their overall sequence is not very similar to *Mi*. *Gro1-4* confers resistance to *G. rostochiensis* and belongs to the TIR-NBS-LRR class of resistance genes.

Studies on nematode genes that may encode the avirulence proteins recognized by resistance genes have not progressed as far as studies on similar proteins from other pathogens. At present three nematode effectors have been identified that may represent avirulence determinants. The root-knot nematode gene *Mi-Cg1* is required for *Mi-1.2* to confer resistance in tomato to *M. incognita* (Gleason *et al.*, 2008). Knocking-down expression of the *Mi-Cg1* gene by RNAi allows avirulent individuals to become virulent, suggesting that its product is recognized by *Mi-1.2*. However, the *Mi-Cg1* transcript does not seem to encode a secretory protein capable of interacting with an immune receptor. It is possible that this transcript is involved in regulation of an effector that is recognized by *Mi-1.2*. cDNA-AFLP analysis was used to compare near isogenic lines of *M. incognita* virulent and avirulent against *Mi-1.2* and a novel gene (*Map-1*) was identified encoding a secreted protein. Expression analysis suggested that variants of the protein containing fewer repeat sequences are expressed only in avirulent nematode species/lines. However, no experimental analysis showing that expression of *Map-1* in the presence of *Mi-1.2* leads to a hypersensitive response (HR) has been published. A more complete example of a nematode avirulence gene is the *Gp-RBP-1* SPRYSEC gene from *G. pallida* (Sacco *et al.*, 2009). Transient co-expression of *Gp-RBP-1* and the nematode resistance gene *Gpa2* in leaf tissues induces a specific HR, making this effector the likely cause of avirulence in nematode populations.

In recent years it has become clear that the majority of plant resistance genes do not interact directly with pathogen avirulence factors. Instead, it is thought that the majority of resistance gene products monitor (or guard) a host protein and detect pathogen-induced changes in this host protein. This guard hypothesis neatly explains why some resistance genes (such as *Mi-1.2*) provide resistance against diverse pathogens; each of the pathogens targets the same host protein to promote infection and *Mi-1.2* detects these changes as it guards this common host target.

Acknowledgements

Figure 9.1 contains images from Nemapix 1 (Mactode Publications, <http://www.dreamwater.com/biz/mactode>) originally produced by J.D. Eisenbach. Figure 9.3 is reproduced from Wyss (2002) with the permission of the publishers (Taylor and Francis Ltd). Figure 9.6 is reproduced from Gheysen and Fenoll (2002) with permission from Annual Reviews. The authors thank Bartel Vanholme for Fig. 9.2 and for comments on an earlier draft of this chapter.

Part III Quantitative Nematology and Management

Preventing the spread of nematodes through effective quarantine legislation and prophylactic measures is essential to avoid previously uncontaminated areas from becoming infested. Once plant-parasitic nematodes are present in an area, effective sampling within cost and personnel constraints is necessary to define the extent of their spread. Limiting the damage caused by plant-parasitic nematodes depends on knowledge of nematode distribution patterns, population dynamics and their effects on plant growth. Sampling and damage assessment are the criteria for determining the economic rationale for management and control in relation to the economic benefits of increased crop returns. Integrated management strategies involve the use of several control components, including cropping schemes, biological control, the judicious use of chemicals and resistant cultivars. With the rapid advances in molecular information, development of resistant cultivars using a genomic approach is being actively pursued. The seven chapters in this section reflect each of the quantitative assessments of nematode populations and distribution, and each component of the overall management strategies, including the burgeoning field of genetic engineering.

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10 Plant Growth and Population Dynamics*

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10.1. Introduction

The main purpose of quantitative nematological research is to achieve an optimal economical protection of crops against plant-parasitic nematodes. To accomplish this, the costs of control measures must be adjusted to the costs of the expected yield reduction compared to the yield in a situation without the need for control. Such an adjustment requires quantitative knowledge of:

1. The relationship between a measure for the nematode activity (in practice mostly their population density at the time of planting) and plant response.
2. The population dynamics of nematodes in the presence of food sources (of different quality) and in the absence of food.
3. The effect of control measures on plant response and nematode population dynamics. The control measures may range from pesticide treatment, crop rotation and cultivation of crops that vary in suitability as a food source for nematodes.
4. Cost/benefit of the control measures.

10.2. Relationships of Nematodes with Plants

The majority of species of plant-parasitic nematodes live on or around plant roots (see Chapter 8). Nematode species can be divided into four types according to the plant parts they infest: (i) species that form galls in ovaries and other above-ground plant parts, e.g. *Anguina tritici* in wheat; (ii) leaf nematodes (*Aphelenchoides*) infesting leaf buds and causing malformations and necrosis in leaves of many ornamental plants and in strawberries; (iii) stem nematodes (*Ditylenchus dipsaci*) causing malformations, swellings, growth reduction and dry rot in above- and underground parts of plant stems such as onions, bulbs, rye, wheat, beet, potatoes and red clover; and (iv) root nematodes causing growth reduction in whole plants and malformations in underground plant parts (*Meloidogyne* spp., *Rotylenchus uniformis*), root necrosis and growth reduction (*Pratylenchus penetrans*, *Tylenchulus semipenetrans*) or growth reduction without any symptoms (*Globodera rostochiensis*, *G. pallida*, *Tylenchorhynchus dubius*).

In most cases of infestations by stem and root nematodes, the nematodes were already present in the soil at the time of planting. Damage in red clover and lucerne often is the result of seed infested with stem nematodes. Stem nematodes introduced into a field with infested onion seed are not known to reach densities that are high enough to cause immediate visible infestations. The introduction of nematodes on planting material such as bulbs and tubers and the spread of nematodes by machinery and other vectors is discussed in detail in Chapter 11. *Bursaphelenchus cocophilus* in coconut and oil palm and *B. xylophilus* in various pine species are unusual. Both are transmitted by a beetle. In fact, several species of beetle support the life cycle of *B. xylophilus*: pine sawyer beetles (*Monochamus* spp.) transport the nematodes to the pine trees where they feed on blue stain fungi. Bark beetles help to introduce the blue stain fungi into the trees and thus allow the nematodes to feed and multiply. *Bursaphelenchus xylophilus* (and possibly one or two other species) can feed on live trees as well as fungi.

10.3. Predictors of Yield Reduction

10.3.1. Symptoms

Figure 10.1 gives some examples of visible symptoms of nematode infestations. Some of them are very conspicuous but others are hardly visible. In some cases visible symptoms in plants can be used as a measure of yield reduction. For example, symptoms of nematode infestations in above- and underground parts of the stem are often easy to recognize, and yield reduction is closely related to the extent of the phenomena. Some root nematodes inflict conspicuous deterioration in roots or underground plant parts: *Meloidogyne* spp. cause root knots, and species of *Longidorus* and *Xiphinema* are responsible for bent, swollen root tips. If these plant parts are marketable products, the symptoms are closely related to yield reduction. If not, the relationship between symptoms and yield is much more complex.

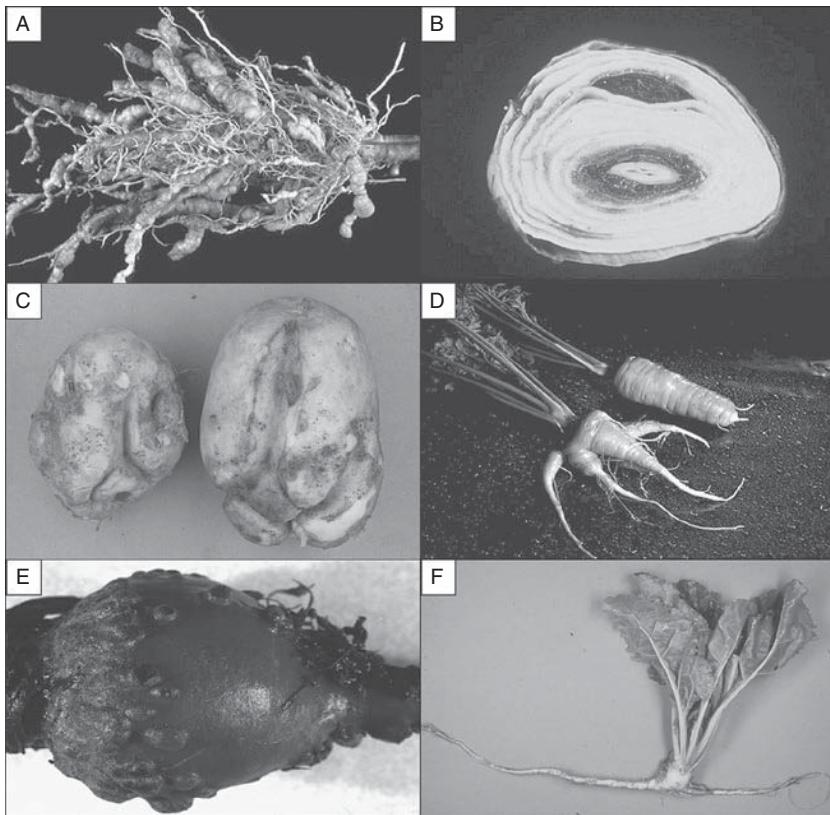


Fig. 10.1. Visual symptoms of nematode attack. A: *Meloidogyne incognita* on tomato roots. B: *Ditylenchus dipsaci* in onions. C: Deformations in potatoes caused by *Pratylenchus* spp. D: *Meloidogyne* in carrots. E: *Meloidogyne* in table beets. F: Deformation in sugar beet caused by trichodoridae.

By contrast, other nematode species cause hardly any specific or recognizable symptoms and yet reduce yields severely. For example, *P. penetrans* causes root necrosis, and low to medium numbers of cyst nematodes, *T. dubius*, *R. uniformis*, *Helicotylenchus* spp., *P. crenatus* and *P. neglectus* cause no, or hardly any, symptoms, yet these species can cause considerable growth reduction in the plants they attack. *Longidorus elongatus* in some cases causes swollen root tips and a smaller root weight, but does not affect the above-ground plant parts. Therefore, in general, visible symptoms of nematode infestation can seldom be used as a measure for growth and yield reduction.

10.3.2. Pre-plant density (P_i)

Only leaf and bud nematodes and *Bursaphelenchus* spp. can multiply fast enough to cause considerable damage very shortly after the first infection, even at very low densities. For these species, damage or yield reduction is almost independent of the numbers of nematodes at the time of planting (P_i). To control these nematodes, all sources of infection, such as infested plants, must be removed and breeding material must be free from nematodes. By contrast, the multiplication of most root nematodes is relatively slow, even on good hosts. Root nematodes only cause yield reduction when harmful densities are already present in the soil at the time of planting of a sensitive crop. On a small scale, root nematodes are distributed in the soil according to a negative binomial distribution with an aggregation coefficient (k) larger than 40 for most nematode species. This distribution is regular enough to assume that growing plant roots are continuously exposed to the attack of a nematode population with about the same density and, therefore, that the growth of annual plants (or the growth of perennial plants during the first year) is retarded at a constant rate.

10.3.3. Multiplication

Nematode damage to plants can have some influence on nematode multiplication but only when large nematode densities reduce the food source (often the root system) in sensitive plants. Conversely, multiplication of nematodes is of little importance to the growth and yield reduction they cause; the same amount of yield reduction may occur in resistant (non-host) and susceptible plants. Examples are *G. pallida* and *G. rostochiensis*, causing the same damage in resistant and susceptible potato cultivars (Fig. 10.2), *Meloidogyne naasi* and *M. hapla* damaging beet during the first months after sowing, and damage by stem nematodes in flax, yellow lupin, maize and sun spurge. In these latter crops, marked yield reductions or growth aberrations have been found without any nematodes being detected in the plants. Therefore, the host status of a plant and its susceptibility to damage must be treated as independent qualities. The reason for this independence is that only a very small part of the damage caused by nematodes is caused by food withdrawal from host plants and the main part by the biochemical and mechanical disruptions that nematodes bring about in plants.

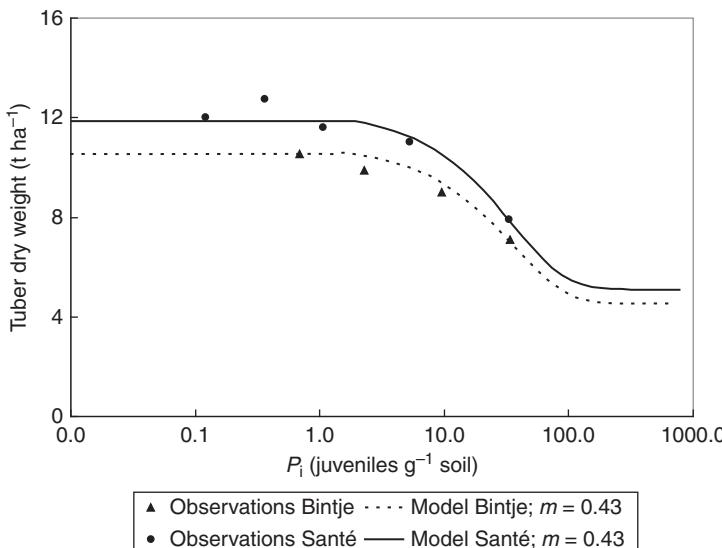


Fig. 10.2. The potato cultivars Bintje (susceptible) and Santé (resistant) were grown in a field infested with *Globodera pallida*. Although the yield potential of Santé (11.9 t ha^{-1}) is greater than that of Bintje (10.6 t ha^{-1}), the effect of the nematodes on tuber dry weight is the same: both cultivars have a relative minimum yield (m) of 0.43 and a tolerance limit T of 18 juveniles g^{-1} of soil. (L. Molendijk, unpublished data.)

10.4. Different Response Variables of Nematodes

Crop returns are reduced by nematode attack as a result of reduction of crop weight per unit area, which is mostly equivalent to average weight of marketable product per plant, and reduction of the value of the product per unit weight. For example, carrots attacked by root-knot nematodes (*Meloidogyne* spp.) may be worthless because of branching and deformation of the taproot (Fig. 10.1D), although they have the same weight per unit area as carrots without nematodes. Onions of normal weight but infected with a few stem nematodes (*D. dipsaci*) at harvest will, nevertheless, be lost in storage. Attack of potatoes by *G. rostochiensis* and *G. pallida* not only reduces potato tuber weight but also may reduce tuber size. However, small and medium densities of potato cyst nematodes attacking potatoes and almost all root-infesting nematodes attacking crop plants of which the above-ground parts are harvested, hardly ever affect the value per unit weight of harvested product. Therefore, prediction of crop reduction by these nematodes can, in general, be based on models of the relation between nematode density at planting (P_i) and average weight of single plants (y) at harvest. In the following sections, the term ‘yield’ will be avoided. The yield in the agronomic sense must be derived from individual plant weights.

10.5. Stem Nematodes (*Ditylenchus dipsaci*)

To construct a model of the relationship between initial population density (immediately before planting), P_i , and the proportion, y , of uninfected plants (onions, flower

bulbs), a theory is required concerning the mechanisms involved. The theory has to be translated into a mathematic model so that it can be tested. In fact, a mathematical analogue of the theory is formulated. To test or validate the mathematical model (and the theory), it is compared with mathematical patterns that are distinguishable in data derived from observations. At the same time, the values of system parameters are estimated under various experimental conditions. P_i and y are called system variables because they have different values in each experiment, in contrast to system parameters, which are constants. They have only one value in a certain experiment but they can vary between experiments because of changes in external conditions.

Seinhorst (1986) presented a competition model for the relationship between stem nematode densities (P_i) and the proportion of infested onion plants. As only nematode-free onions are marketable and the degree of infestation of single plants is irrelevant, only infested and non-infested onions were distinguished. To formulate the model, three assumptions were needed:

1. The average nematode is the same at all densities. This means that initial population density (P_i) does not affect the average size or activity of the nematodes.
2. Nematodes do not affect each other's behaviour. They do not attract or repel each other directly or indirectly.
3. Nematodes are distributed randomly over the plants in a certain small area.

It is postulated that at $P_i = 1$ a proportion d of the onion plants is infected and that, therefore, a proportion $1 - d$ is left non-infected. Then, at density $P_i = 2$, a proportion d of already damaged plants is attacked (which has no additional effect as onions, once attacked, are worthless), plus a proportion d of the still non-infected proportion $(1 - d)$. So at $P_i = 2$ a proportion $d + d(1 - d)$ onions is attacked and $1 - d - d(1 - d) = (1 - d) - d(1 - d) = (1 - d)^2$ of the plants is left non-infected. At $P_i = 3$, again a proportion d of already damaged onions is damaged, which has no effect, and a proportion d of the non-infected plants $(1 - d)^2$ is attacked. Summing it all up, we see that at $P_i = 3$ the proportion of infected onions amounts to $d + d(1 - d)^2$ and that the proportion of non-infected onions is $1 - d - d(1 - d)^2 = (1 - d) - (1 - d)^2 = (1 - d)^3$. Schematically:

Population density, $P_i =$	Proportion of infected onions	Proportion of non-infected onions
1	d	$1 - d$
2	$d + d(1 - d)$	$1 - \{d + d(1 - d)\} = (1 - d)^2$
3	$d + d(1 - d) + d(1 - d)^2$	$1 - \{d + d(1 - d) + d(1 - d)^2\} = (1 - d)^3$
P		$(1 - d)^P$

In general: a nematode density $P_i = P$ leaves a proportion

$$y = (1 - d)^P = z^P \quad (10.1)$$

of the onions non-infected. In Eqn 10.1 P is an integer, y is a variable (like P_i) and z is a parameter. The parameter z must be estimated. The expected value of z and its variance must be estimated in field experiments; the population density P_i can be estimated by taking soil samples with an appropriate sampling method (see Chapter 11). In Fig. 10.3 values of y ($= z^{P_i}$) are plotted for three different values of z . The values of y are not plotted against P_i , but against $\log P_i$. This log-transformation of P_i not

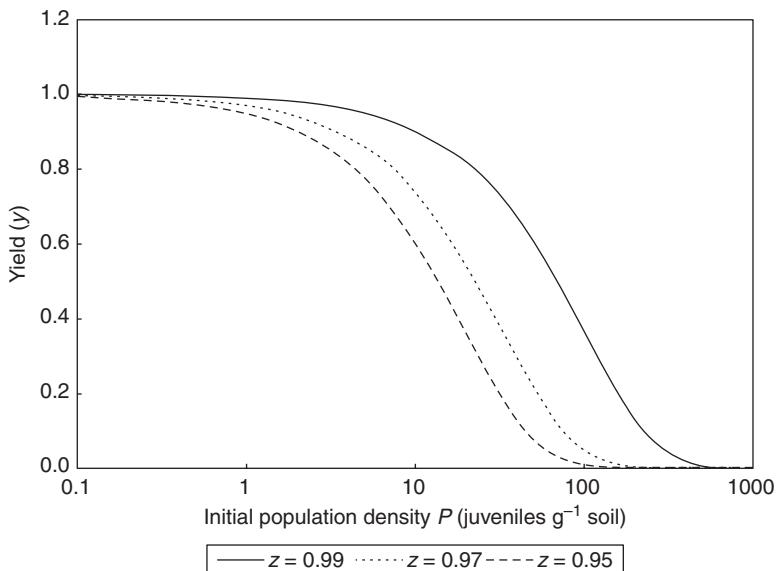


Fig. 10.3. Yield relation for stem nematodes according to equation $y (= z^{P_i})$ with three different values for z ($z = 0.99, 0.97$ and 0.95). The smaller z , the greater the activity of the nematodes and the smaller the yield.

only has the advantage that the shape of the curves is the same for all z , but also that, if P_i is estimated by counting nematodes from a soil sample, the variance of $\log P_i$ is constant (and independent of P_i), provided that P_i is not very small. The value of the parameter z is determined by conditions that influence the efficiency of nematodes in finding and penetrating plants. In patchy infestations of stem nematodes these conditions for attack appear to be more favourable in the centre of the patch than towards the borders. This results in an increase of z with increase of the distance from this centre and, thus, in persistency of the patchiness. The model also applies when nematodes spread from randomly distributed infested plants to neighbouring ones, leading to overlapping patches of infested plants.

10.6. Root-invading Nematodes

Root-knot nematodes, *Pratylenchus* spp. and cyst nematodes are considered to be the most important tylench root-invading nematodes. Although some of these nematodes, especially root-knot nematodes, can also inflict qualitative damage in underground plant parts, they generally reduce crop yield in a less direct way than stem nematodes. Often there are no visible symptoms. Only the rate of growth and development of attacked plants is reduced, resulting in lower weight compared to plants without nematodes. To put it simply: in plants with nematodes the same thing happens later. In exceptional cases, nematode-infested plants reach the same final weight as plants without nematodes, but at a later stage. In general, such a delay results in ripening of the crop being prevented by external conditions at the end of the plant growing season.

Seinhorst (1986) based a growth model on two simple concepts: (i) the nature of the plant (an element that increases in weight over time); and (ii) the nature of the plant-parasitic nematode (elements that reduce the rate of increase of plant weight and, in principle, the more so the larger the population density). To formulate the model for root nematodes three extra assumptions must be added to those made for stem nematodes (Section 10.5):

4. Root-infesting nematodes are distributed randomly in the soil.

5. Nematodes enter the roots of plants randomly in space and time. Therefore, the average number of nematodes entering per quantity of root and time is constant. This number is proportional to the nematode density P (number of nematodes per unit weight or volume of soil).

6. The growth rate of plants at a given time t after planting is the increase in total weight per unit time (dy/dt). Let this growth rate be r_0 for plants without nematodes and r_p for plants at nematode density P . According to Fig. 10.4, $r_0 = \tan(\alpha) = \Delta y/\Delta t_0$ and $r_p = \tan(\beta) = \Delta y/\Delta t_p$. Thus, for plants of the same total weight (and, therefore, of different age) with nematode density P and without nematodes, the ratio r_p/r_0 is constant during the growing period. Therefore,

$$r_p/r_0 = t_0/t_p \quad (10.2)$$

The relationship between population density of the nematodes and its total effect on the growth rate of the plants accords with Eqn 10.1. Equation 10.1 is a continuous function for $0 \leq P \leq \infty$. There is one complication: all accurate observations on the relationship between the population density P of various nematode species and weight of various plant species indicate that there must be a maximum density, T ,

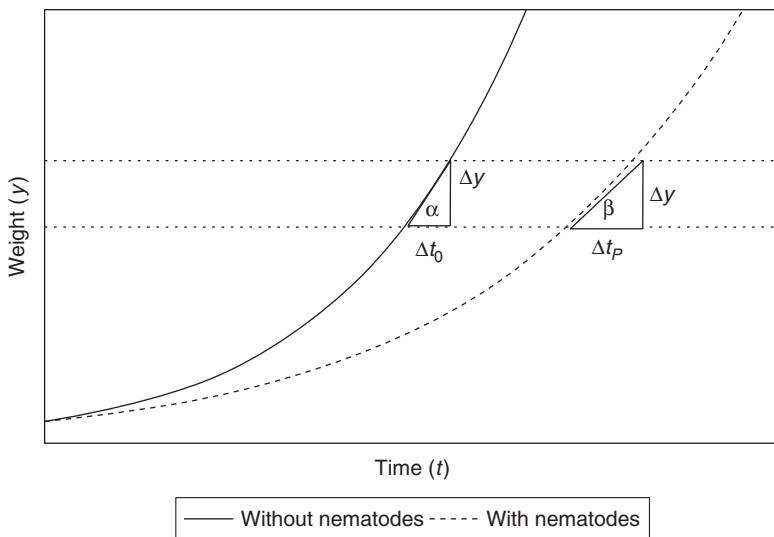


Fig. 10.4. Growth curves of plants without nematodes and with nematodes at density P ; y = plant weight and t = time after planting. The variables t_0 and t_p are the times that plants without nematodes and at density P need to reach the same total weight y , respectively; $r_0 = \tan(\alpha) = \Delta y/\Delta t_0$ and $r_p = \tan(\beta) = \Delta y/\Delta t_p$ are the growth rates of plants without nematodes and at nematode density P , respectively.

below which the nematodes do not reduce plant weight. Therefore, Eqn 10.1 is adapted by replacing P by $P - T$ and we have to deal with a discontinuous function. In practice the transition between $P > T$ and $P \leq T$ will be smoother than it is in theory. Further, only in very few experiments were large nematode densities able to reduce plant weight to zero, whilst growth rates of attacked plants were never reduced to zero. Therefore, a second adaptation in Eqn 10.1 was the introduction of the minimum relative growth rate $k = r_p/r_0$ for $P \rightarrow \infty$. The equation constituting the growth model for plants attacked by root nematodes then becomes:

$$r_p/r_0 = k + (1-k) \cdot z^{P-T} \quad \text{for } P > T \quad (10.3)$$

and

$$r_p/r_0 = 1 \quad \text{for } P \leq T$$

T , z and k are parameters (constants); r_p , r_0 and P are variables. The parameters z and k are constants smaller than 1. The value of the parameter k is independent of nematode density and time after planting but may vary between experiments. Growth curves of plants for different nematode densities can be derived from a growth curve of plants without nematodes with the help of Eqns 10.2 and 10.3. These curves may vary in shape but they must answer two conditions: (i) they must be continuous; and (ii) the growth rate must decrease continuously from shortly after planting. The frequently used logistic growth curve complies with these conditions. Figure 10.5 gives an impression of the three-dimensional model with axes for total plant weight y , relative nematode density P/T and time t after planting.

From the model it can be deduced that nematodes reduce growth rates of plants by the production of a growth-reducing substance only during penetration in the roots but not when they have settled. This hypothesis is supported by Schans (1993), who observed stomatal closure in potato plants infested by *G. pallida* during the time of penetration and concluded that disturbance of cell development just behind the root tips interferes with the production of abscisic acid, which is known to act as a messenger for stomatal closure. Because of the constant number of nematodes penetrating per unit quantity of root and per unit duration of time, the growth-reducing stimulus will then remain constant per unit weight of plant.

For nematodes that do not move once they have initiated a feeding site within the root, such as root-knot and cyst nematodes, z^P can be interpreted as the proportion of the food source that is left unoccupied by nematodes at density P . For species that are mobile during their whole life cycle, $1 - z^P$ is a measure of the ratio between the feeding times at density P and the maximum feeding times at $P \rightarrow \infty$. Therefore, $1 - z^P$ is proportional to a hypothetical growth-reducing substance that nematodes bring into the plants during penetration. The parameter k means that nematodes cannot stop plant growth completely and that, even at very large nematode densities, some growth is left: $r_p/r_0 = k$.

10.6.1. A simple model for nematode density and plant weight

The model described in Section 10.5 makes good biological sense but is not easy to use in everyday nematological practice. The primary results of experiments are almost

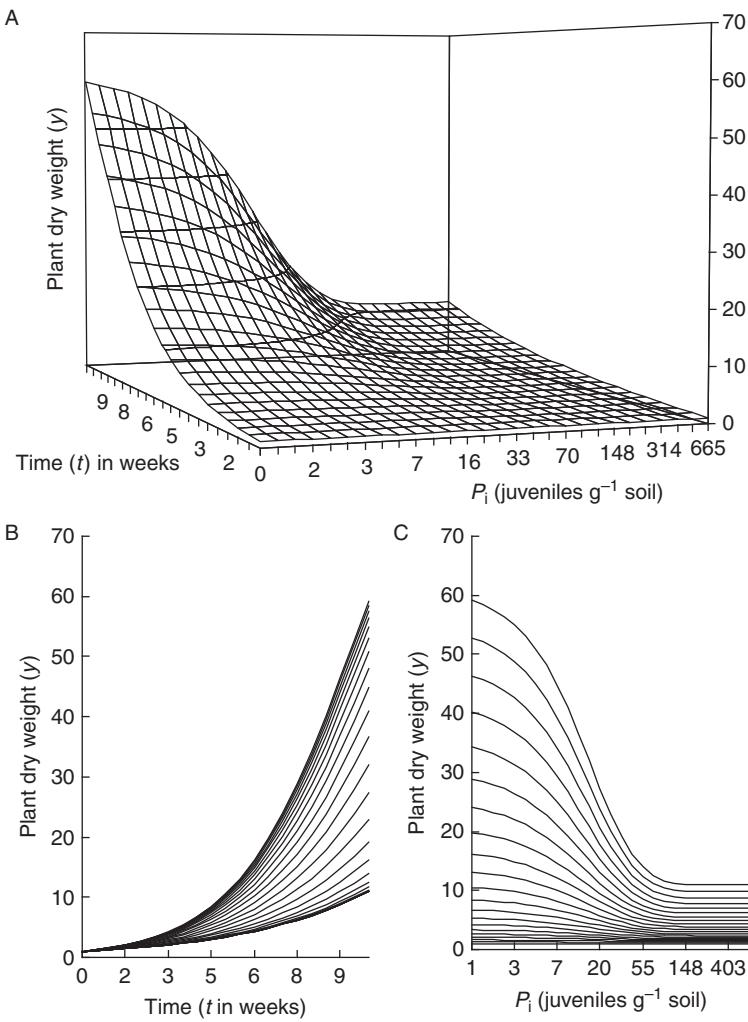


Fig. 10.5. Surface plots of the three-dimensional model (Eqns 10.2 and 10.3) representing the relation between weight, the relative nematode density P/T and time t after planting: A, at 230 degrees rotation; B, at 0 degree rotation, showing the relation between plant weight and t at different nematode densities; C, at 270 degrees rotation, showing the relation between plant weight and P_i .

always weights of plants attacked by known nematode densities at a given time after sowing or planting. To investigate whether these relationships are in accordance with the growth model, they must be compared with cross-sections orthogonal to the time axis (Fig. 10.5), through growth curves of plants for ranges of densities P/T and different values of k . If we describe these cross-sections mathematically, they appear to be in close accordance with Eqn 10.4:

$$y = m + (1 - m) \cdot z^{P-T} \quad \text{for } P > T \quad (10.4)$$

$$y = 1 \quad \text{for } P \leq T$$

The parameter m is the minimum relative plant weight and usually slightly larger than k , the parameter z is a constant <1 with the same or a slightly smaller value than in Eqn 10.3 and the parameter T is the tolerance limit with the same value as in Eqn 10.3.

As in most experiments z^T deviates little from 0.95, Eqn 10.4 can be transformed into Eqn 10.5 for fitting the model to data:

$$y = m + (1 - m) \cdot 0.95^{(P-T)/T} \quad (10.5)$$

Another mathematic formula to describe the relationship between population density and plant yield is the equation of Elston *et al.* (1991):

$$y = \frac{Y_{\max}}{1 + \frac{P_i}{c}} \quad (10.6)$$

where c is the parameter for tolerance and Y_{\max} the yield at $P_i = 0$. At smaller densities, and if the tolerance limit T is negligible, predictions by Eqns 10.5 and 10.6 do not deviate much. However, as Eqn 10.6 lacks a minimum yield, it overestimates yield reduction at medium and high nematode densities. The main shortcoming of Eqn 10.6 is that it cannot be translated into biological processes and, therefore, does not contribute to theory building.

10.6.2. Mechanisms of growth reduction

We can discriminate three kinds of growth reduction caused by nematodes: the ‘first mechanism of growth reduction’ operating at all population densities, and a ‘second mechanism of growth reduction’ additional to that of the first mechanism, with a noticeable effect only at medium to large nematode densities. ‘Early senescence’ of plants, attacked by high densities of nematodes, can be considered as a third mechanism.

In the first mechanism, Eqns 10.3 and 10.4 only apply to growth reduction that retards growth of plants at a constant rate and, occasionally, increases haulm length of plants. As long as only the first mechanism is active, water consumption during short periods is proportional to plant weight and, therefore, relative water consumption at different nematode densities and times after sowing or planting is a measure of relative plant weight. Actual plant weights are these relative weights times the actual weights of plants of the same age without nematodes, determined at the same time (Seinhorst, 1986).

In the second mechanism, water consumption per unit plant weight and the (active) uptake or excretion of K^+ and Na^+ are reduced, and the (passive) uptake of Ca^{2+} and the dry matter content of plants are increased (Seinhorst 1981; Been and Schomaker, 1986). It also tends to advance the development and ripening of seed to a certain limit. There is probably a negative correlation between age of the plant and the smallest P_i where the second mechanism manifests itself, i.e. the younger the plant, the smaller the P_i where the second mechanism can be noticed. For potato cyst nematodes, this density is rarely as small as $16T$ but more commonly, for other nematode species on other plant species, the density is $>32T$.

Early senescence was first observed in potato plants attacked by large numbers of *G. pallida*. Early senescence is a sudden ending of the increase of haulm length and weight and results in an early death of plants. There are indications that ‘early

'senescence' coincides with initiation of tuber growth and, because of that, is negatively correlated with nematode density. The earliest occurrence was 9 weeks after planting in the early potato cultivar Ehud and the lowest nematode density of $25 \times T$ (about 45 nematodes g^{-1} of soil) in the potato cultivar Darwina. Not all cultivars are equally sensitive. Early senescence was also observed in plants exposed to air pollution. So far, the causes are unknown.

10.6.3. *T* and *m* as measures of tolerance

Tolerance in plants can be quantified by expressing it in values of the tolerance limit *T* and the minimum yield *m*. The parameter *T* manifests itself at small nematode densities, *m* at larger ones. Yet we need a whole range of pre-plant nematodes densities (P_i) to estimate either one of the parameters.

The value of the tolerance limit, *T*, seems unaffected by differences in external conditions and can, therefore, be determined in pot experiments in both glasshouse and (more laborious and less accurate) field experiments. The only requirement of glasshouse tests is that large enough pots are used to guarantee about the same root density in the soil as in the field and to prevent the plants from becoming pot-bound. The latter affects the relationship between nematode density and plant weight and obscures the true value of *T* (Seinhorst and Kozlowska, 1977). The accuracy of the estimates is mainly a matter of uniformity of plant material and growth conditions (light, water content), and carefully and uniformly filling and handling of the pots.

The minimum yield, *m*, is more sensitive to external conditions than *T*. Values for potato cyst nematodes on potatoes varied between 0 on cultivar Bintje and 0.8 on cultivar Agria. Estimates of *m* for *Heterodera schachtii* on red beet varied between 0.3 and 0.65 and on Brussels sprouts between 0.32 and 0.65. More estimates of *T* and *m* are given in Table 10.1. Differences in tolerance in, for example, plant cultivars, should be established in one experiment under the same conditions and preferably conducted in a glasshouse. In addition, a sufficient number of values of *m* must be estimated in field experiments to establish a distribution function of *m*.

Estimation of *T* and *m* in field experiments is much more laborious than in pot experiments (Box 10.1). A full range of nematode densities, from 0 to at least 250 nematodes g^{-1} of soil, is needed and nematode density must be the only variable. For some species (e.g. potato cyst nematodes), ranges of nematode densities in infestation foci come closest to this requirement. Unfortunately, ranges of nematode densities cannot be created by applying different dosages of a nematicide as this has unpredictable effects on crop yield in addition to those caused by killing nematodes. The ranges of nematode densities needed to estimate the parameters in question must be determined in samples large enough to guarantee a coefficient of variation (standard deviation/average) of egg counts less than 15%. At this coefficient of variation, density differences of 1:2 are just distinguishable. Soil samples of 4 kg plot^{-1} are then needed to estimate population densities of 1 egg g^{-1} of soil, given a coefficient of variation of the number of eggs per cyst of 16% and a negative binomial distribution of egg densities in samples from small plots with a coefficient of aggregation of 50 for 1 kg soil. As the coefficient of variation per unit weight of soil is negatively correlated

Table 10.1. Data of 30 plant–nematode combinations (14 nematode species and 27 plant species/cultivars) out of 29 experiments on the relationship between P_i and total weight y , with $z^T = 0.95$.

Nematode species	Plant species, cultivar	T^a	m^b
<i>Globodera pallida</i> and <i>G. rostochiensis</i>	<i>Solanum tuberosum</i>	1.8	0.2–0.6
<i>Heterodera avenae</i>	<i>Triticum sativum</i>	0.3	0
<i>H. avenae</i>	<i>Avena sativa</i>	1.4	0.27
<i>H. avenae</i>	<i>A. sativa</i>	0.85	0.05
<i>H. avenae</i>	<i>A. sativa</i>	0.35	0.6
<i>H. avenae</i>	<i>A. sativa</i>	1	0.6
<i>H. carotae</i>	<i>Daucus carota</i>	0.7	0
<i>H. ciceri</i>	<i>Cicer arietinum</i>	1.3	0.16
<i>H. trifolii</i>	<i>Trifolium repens</i>	0.8	0
<i>H. trifolii</i>	<i>T. repens</i>	0.7	0.12
<i>H. goettingiana</i>	<i>Pisum sativum</i>	3.4	0.28
<i>Longidorus elongatus</i>	<i>Fragaria vesca</i>	0.09	0.3
<i>Meloidogyne artiellia</i>	<i>Cicer arietinum</i>	0.13	0.1
<i>M. incognita</i>	<i>Beta vulgaris</i>	1.1	0.1
<i>M. incognita</i>	<i>Brassica oleracea</i>	0.5	0.05
<i>M. incognita</i>	<i>Capsicum annuum</i> (susceptible)	0.74	0.1
<i>M. incognita</i>	<i>C. annuum</i> (resistant)	0.74	0.4
<i>M. incognita</i>	<i>Coffea arabica</i>	1.4	0.4
<i>M. incognita</i>	<i>Cucumis melo</i>	0.19	0
<i>M. incognita</i>	<i>Helianthus annuus</i>	1.85	0.25
<i>M. incognita</i>	<i>Solanum lycopersicum</i> (resistant)	0.5	0.7
<i>M. incognita</i>	<i>S. lycopersicum</i> (susceptible)	4	0
<i>M. incognita</i>	<i>Nicotiana tabacum</i>	2	0
<i>M. incognita</i>	<i>Solanum melongena</i>	0.054	0.05
<i>M. hapla</i>	<i>Trifolium repens</i>	1.3	0.43
<i>M. javanica</i>	<i>Coffea arabica</i>	1.15	0.4
<i>M. javanica</i>	<i>Helianthus annuus</i>	0.25	0.43
<i>M. javanica</i>	<i>Oryza glaberrima</i>	2.2	0.035
<i>M. javanica</i>	<i>Oryza sativa</i>	0.18	0.04
<i>Pratylenchus penetrans</i>	<i>Daucus carota</i>	1.4	0.49
<i>P. penetrans</i>	<i>Digitalis purpurea</i>	5.6	0.16
<i>P. penetrans</i>	<i>Malus</i>	1.5	0.44
<i>P. penetrans</i>	<i>Vicia faba</i>	6.2	0.43
<i>P. penetrans</i>	<i>V. faba</i>	1.3	0.43
<i>Tylenchorhynchus dubius</i>	<i>Lolium perenne</i>	1.6	0.6
<i>T. dubius</i>	<i>L. perenne</i>	1.6	0.22
<i>Xiphinema index</i>	<i>Vitis vinifera</i>	0.15	0.05

^aNematodes g⁻¹ of soil.

^bFraction of yield without nematodes.

Box 10.1. Experimental set-up to estimate T and m .

Pot or microplot experiments

Pot experiments can be conducted in glasshouses where external conditions, especially soil moisture and temperature, are controlled. Most nematodes die when temperatures rise above 30°C and both nematode multiplication and plant growth are reduced if soil moisture drops below 10% of the soil dry weight. If climate-controlled glasshouses are not available, microplot experiments in the open air with inoculated soil is the best alternative. In both types of experiments the volume of soil has to be chosen so that the root system has the same space as in normal agricultural conditions. If the pots are too small, the plants will resume their normal growth rate (the same as without nematodes) as soon as the pot boundaries are reached and the nematodes are depleted. To estimate T and m , nematode densities must be created according to a log series 2^x , x being whole negative and positive numbers. If x is chosen between -3 and 9 the density series: $2^{-3}, 2^{-2}, 2^{-1}, 2^0, 2^1, 2^2, 2^3, 2^4, 2^5, 2^6, 2^7, 2^8, 2^9$ is obtained which equals 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, 512 nematodes g^{-1} of soil. The ratio between two succeeding nematode densities in this series is 2, which means that a $^2\log$ series is attained. As mentioned, a log series is required to plot P_i against y . The number of nematodes that is needed in an experiment with a $^2\log$ series of nematode densities is about twice the number of nematodes needed at the highest density. In an experiment with the above-mentioned series, using 1 l pots and five replicates $2 \times 515 \times 1000 \times 5 = 5,150,000$ nematodes are required. In theory, there is no objection against log nematode series with a base different from 2, as long as it is smaller. In practice it is not easy to create a dilution series with, for example, a factor 1.7 between succeeding nematode densities. During inoculation, care must be taken that the nematodes are distributed randomly through the soil, for example by placing them in well-distributed channels throughout the pot using syringes (Been and Schomaker, 1986). It is also important that the nematodes are present in the soil at the time of planting or sowing. If the soil is inoculated with nematodes days or weeks after planting, an artificial situation is created by introducing plants with an established, but non-infected, root system; this does not equate to the normal agricultural situation and minimum yield and plant tolerance will be increased.

Field experiments

In field experiments it is almost impossible to create densities so pre-sampling is necessary to select plots with the required densities. Another requirement of a suitable experimental field is that the target nematode is the only variable that causes variation in yield. To estimate T and m a field with both very small and large densities and some intermediate densities is needed. Fields with infestation foci are often very useful for experiments because of their gradually increasing population densities. In this case, the sampling errors can further be reduced by regression, making use of the focus model (linear regression on log nematode numbers and distance; see Chapter 11). The nematode densities according to the model are considered to be the 'true' densities. The deviations of the original data points from the model represent the variation due to sampling and laboratory procedures and can be removed. In fields where nematodes are distributed more uniformly, plant weights per plot often vary

Continued

Box 10.1. Continued.

considerably between plots that have approximately the same nematode density. This makes curve fitting very difficult. This situation can be improved by dividing nematode densities into classes where ratios between minimum and maximum limits are less than 1.7. Average plant weight per density class is then plotted against average ${}^{10}\log$ nematode density. Per class, average nematode density $P_i = P$ is estimated as the antilog of P , which is 10^P . The chosen base of the logarithm is arbitrary. If an ${}^e\log$ (or natural logarithm) is chosen, the antilog is e^P (also written as $\text{EXP}(P)$). The sample sizes needed to estimate small, medium and large nematode densities accurately are discussed in Chapter 11.

with nematode density, so is the required sample size. For example, to estimate densities of 0.5 or 0.25 eggs g⁻¹soil with the same accuracy, eggs from soil samples of 10 and 20 kg, respectively, must be counted. Another requirement is that plots must on the one hand be small (e.g. 1 m²) to reduce the effect of medium-scale density variation (see Chapter 11), whereas on the other hand a large enough area per small density interval must be available to guarantee a small variability of tuber weight per unit area. Again, there must be a sufficient number of plots at densities smaller than T to obtain the best estimate of the maximum yield with the smallest variance. It is most efficient to estimate T and m , as much as possible, from glasshouse experiments. Field trials are best used to confirm or reject these estimates for different combinations of pathotypes and cultivars under more natural environmental conditions. In Box 10.2 the estimates of T and m from data originating from field trials is presented.

10.6.4. Growth reduction in perennial plants

During the first year after planting, effects of nematode attack on perennial plants can be investigated in the same way as with annual plants. We cannot yet answer the question whether a reduction in growth and productivity should be expected in the second and subsequent years; this depends on nematode densities at planting, especially small densities. On citrus, *Radopholus similis* spreads from old roots to new roots at the periphery, thus rapidly increasing nematode numbers. The same tendency for numbers to increase rapidly and for migration is observed for stem nematodes in red clover and lucerne, in these cases via moist surfaces of plant leaves. Nematodes often cause no specific disease symptoms and annuals are more tolerant to second and later generations of nematodes than to a first generation present at planting (Seinhorst, 1995). Therefore, it is not certain that the presence of large nematode numbers in orchards with old trees, for example, will cause substantial reductions in productivity. Increase in productivity after treatment with contact nematicides or nematostatics is no proof of nematode damage, as yield increases in treated fields even when damaging species of nematodes are absent. To investigate growth reduction of perennial plants by increasing nematode populations, patterns of weight of the total plant and its fruits must be studied at sufficiently wide ranges of nematode densities and at regular time

Box 10.2. Fitting the Seinhorst model to data.

Pot experiments

Suppose the following data are the results from a pot experiment that is performed to estimate tolerance of a cultivar for a nematode species:

P_i	0	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
y_i	10.4	10.2	10.3	10.6	10.5	10.3	10	9.5	9	7.7	7	6.2	6.1	6.2

P_i is nematode density at the time of planting; y_i the average plant weight at population density P_i . For data analysis, the choice is basically between analysis of variance (ANOVA) and regression analysis. Regression is the preferred method if the predictor variable (P_i) represents a series and an increase or decrease in the response variable (y_i) is noticed. Moreover, ANOVA cannot estimate tolerance parameters. This dataset has an increasing series of P_i values and a decreasing series of y_i , and an estimate of T and m is needed. Therefore, regression analysis is chosen. Several methods for regression are available and the most simple and accurate is **least squares** (LS) with trial and error, provided that two conditions are satisfied: the response variable y must be normally distributed and its variation must be constant. In field experiments there is the added complication that the predictor (P_i) has to be estimated and, therefore, P_i is also subject to variance. Care must be taken to keep this variance small, close to normally distributed and constant. This can be achieved if the requirements for field sampling (see Chapter 11) are followed. When LS is used, T and m are chosen so that the sum of squares (SS) of the deviations of y_i from the model (Eqns 10.5 and 10.6) is minimal. Expressed as a formula:

$$SS_i = \sum \left[y_i - y_{\max} \cdot \left(m + (1-m) \cdot 0.95^{\frac{(P_i-T)}{T}} \right) \right]^2 \quad \text{when } P_i > T \quad (10.7)$$
$$SS_i = \sum [y_i - y_{\max}]^2 \quad \text{when } P_i \leq T$$

y_{\max} is the average plant dry weight at $P_i \leq T$ and equals 10.38 in this particular experiment. We choose z^T to be 0.95 because that is the most probable value in relationships between P_i and plant weight (see Section 10.2.8). As the model is non-linear, the minimum value for SS and the best fit must be found by numerical trial and error. One can make the iterations in Excel, which may be laborious, or sophisticated computer software can be used. In this example, Excel is chosen because it is readily available. In Fig. 10.6A, y is plotted against $\log P_i$ to get an impression of a general pattern in the observations and to make the first estimates of the parameters y_{\max} , T and m .

Remembering that T is the largest nematode density P_i without yield reduction, an educated guess would be that T lies somewhere between 1 and 2. So $T = 2$ would provide a useful starting value. Now y_{\max} can be estimated as the average y_i

Continued

Box 10.2. Continued.

at $P_i \leq T$, being 10.4. The first estimate of m would be the average y at P_i values between 128 and 512, which is 6.15. The relative value of m is then $6.15/10.4 = 0.5913$. Since there are three values to estimate m , this estimate is likely to be accurate. Excel can now be used to calculate values of y_i according to the models. These calculations are presented in Table 10.2, columns C and D. The equation for the relative yield y_{rel} (in column C with $y_{\text{max}} = 1$) is then, in Excel language: $\$H\$2+(1-\$H\$2)*0.95^((A3-\$H\$3)/\$H\$3)$ for $P_i > T$ and $y_{\text{rel}} = 1$ for $P_i \leq T$. Note that A3 is a variable that only relates to C3.

Figure 10.6A shows how the model fits to the data if the starting values of the parameters are $T = 2$ and $m = 0.592$. An SS value of 0.4299 is obtained. By decreasing T stepwise: at $T = 1.9$, SS will be 0.3243, which is less than 0.41 indicating that $T = 1.9$ is a better estimate than $T = 2$. At $T = 1.8$ we find $SS = 0.25$ and at $T = 1.7$, $SS = 0.23$. The decrease in SS becomes smaller at each step, so the minimum is almost reached. A further decrease of T to 1.6 increases the SS, so $T = 1.7$ is the best estimate at the given estimate for m . Now a further decrease of Eqn 10.7 can be obtained by making

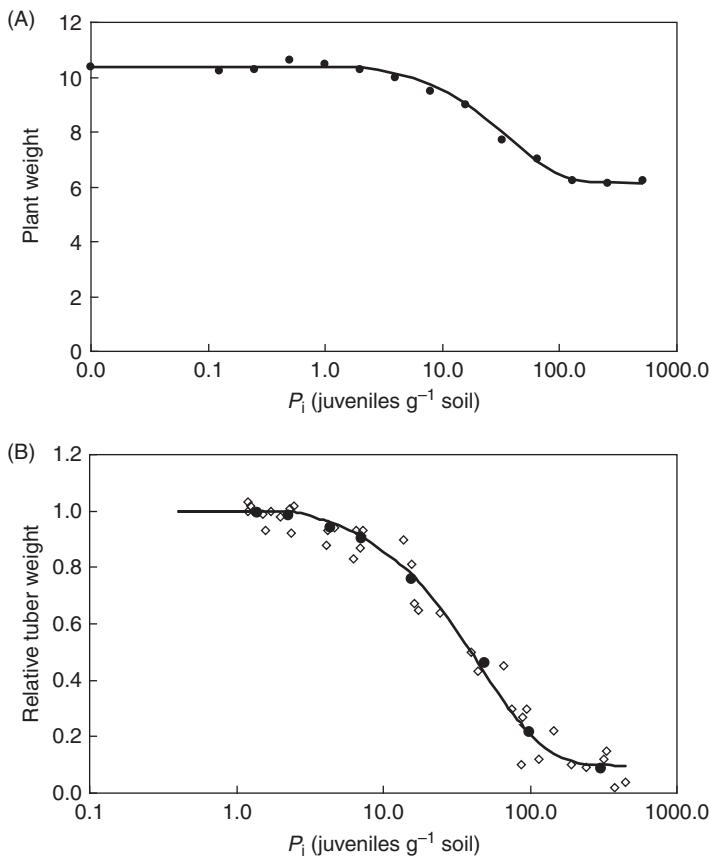


Fig. 10.6. A graphical presentation of the goodness of fit of Eqn 10.9 to glasshouse (A) and field (B) observations on the effect of potato cyst nematodes on potato tuber dry weight.

Continued

Box 10.2. Continued.

alternately small changes to m and T . At the point where no additional minimization of SS is possible, the best estimators for T and m are obtained. In this dataset SS reaches its minimum, 0.2256, when $m = 0.592$ and $T = 1.66$. The residual variance s^2 equals SS/df, where df represents the degrees of freedom. The degrees of freedom are the number of observations (14) minus 2 (the number of parameters to be estimated). So, $s^2 = 0.22/12 = 0.0188$. Finally, s^2 can be compared with the total variance of y_i , by calculating R^2 as follows:

$$R^2 = 1 - \frac{s^2}{s_{\text{tot}}^2} \quad (10.8)$$

In this experiment $s_{\text{tot}}^2 = 3.22$ and $R^2 = 1 - (0.0188/3.22) = 0.994$. Table 10.2 gives an overview of all calculations.

There are two pitfalls when using R^2 . First, values of R^2 from different datasets cannot be used to compare goodness of fit. If we have $R^2 = 0.99$ in dataset 1 and $R^2 = 0.5$ in dataset 2, it cannot be concluded that the goodness of fit in dataset 1 is better than that of dataset 2. The reason is that R^2 also depends on the value of the parameters T and m . For example, in very tolerant cultivars, with m close to y_{max} , s^2 is not small compared to s_{tot}^2 , so R^2 will never get close to 1. Nevertheless, the goodness of fit in both datasets may be perfect. A second pitfall is to use values of R^2 to compare mathematical equations to find the 'best' model. It is important to realize that R^2 is only a statistical navigation tool. The R^2 has nothing to do with biological theory building. A good model is a mathematical analogue of a consistent biological theory and can predict outcomes of future observations. The parameters of a good model have a clear biological meaning that makes possible 'translations' from mathematics into biology and vice versa.

Field experiments

Table 10.3 gives a dataset from a field trial. The trial was done for the same purpose as above: to estimate T and m . The P_i values and their log transformations are displayed in columns A and B, respectively. In column C, averages of the $\log(P_i)$ are calculated per density class, of which the antilog is taken in column D. Columns E and F represent the observed data on plant weight and the averages per density class, while plant weights according to the model are displayed in column G (for all P_i) and H (for P_i classes). From this point we follow the same procedure as in the pot experiments above. The only difference is that we now calculate the differences between observation and model per class. After trial of parameter values, calculations of the sum of squared differences between model and actual observations and error, we conclude, after a number of iterations, that $T = 2.38$ and $m = 0.098$ are the best estimates. Plant dry weight at $P_i = 0$, also referred to as y_{max} , is 12.2 t ha^{-1} . The goodness of fit, estimated by R^2 , is 0.9941 and is visualized in Fig. 10.6B. Note that the number of observations per class is not the same. If there are large differences in class sizes it is advisable to weight the squared differences per class according to their class size. This regression method is known as **weighted least squares**.

Table 10.2. Example of an Excel worksheet using ordinary least squares to estimate parameters T and m in Eqn 10.9 from a glasshouse experiment.

	A	B	C	D	E	F	G	H
1	P_i	y_i	$y_{\text{rel. model}}$	y_{model}	Deviations	Squares	Parameters	Estimates
2	0	10.36	1.00	10.38	0.0190	0.0004	m	0.592
3	0.125	10.20	1.00	10.38	0.1790	0.0320	T	1.66
4	0.25	10.26	1.00	10.38	0.1190	0.0142	y_{max}	10.379
5	0.5	10.60	1.00	10.38	-0.2210	0.0488	df	12
6	1	10.48	1.00	10.38	-0.0960	0.0092	R^2	0.989
7	2	10.25	1.00	10.38	0.1290	0.0166		
8	4	9.98	0.98	10.17	0.1873	0.0351		
9	8	9.50	0.94	9.79	0.2750	0.0756		
10	16	9.00	0.88	9.10	0.1016	0.0103		
11	32	7.70	0.78	8.11	0.4062	0.1650		
12	64	7.00	0.68	7.01	0.0078	0.0001		
13	128	6.20	0.61	6.31	0.1116	0.0125		
14	256	6.10	0.59	6.15	0.0506	0.0026		
15	512	6.20	0.59	6.14	-0.0556	0.0031		
16	$s^2_{\text{tot}} = 3.22$					SUM = 0.4255		
17						$s^2 = 0.0355$		

intervals. To produce a clear pattern, a simple system with external conditions as constant as possible must be studied first. Later, more complex systems can be studied and their patterns compared with the patterns from the simple system.

10.7. Effect of Nematicides

Nematicides can be divided into two groups: contact fumigants that kill nematodes directly and nematistatics (or nematistats) that make nematodes immobile for a period of time (see Chapter 16). With respect to application, we can divide nematicides into fumigants and non-fumigants. All nematistatics are non-fumigants, but not all non-fumigants are nematistatics. Fumigants can be brought into the soil at a certain depth. Afterwards, they move actively upwards and downwards in the gaseous form.

10.7.1. Nematistatics (= nematistats)

The best-known nematistatics are aldicarb, carbofuran and ethopropofos. The first two chemicals belong to the carbamates, the third to the organophosphates. Soil is treated immediately before planting and the chemicals make plants unattractive for nematodes.

Table 10.3. Example of an Excel worksheet dividing P_i values from field experiments into density classes in order to minimize the variance.

	A	B	C	D	E	F	G	H	I
1	P_i	Log P_i	Log P_i class	P_i class	Weight	Weight class	Model Model	Model class	Squares
2	1.2	0.08			12.20		12.48		
3	1.2	0.08			12.57		12.48		
4	1.3	0.10			12.44		12.47		
5	1.3	0.12			12.20		12.46		
6	1.5	0.18			12.08		12.41		
7	1.6	0.20			11.35		12.39		
8	1.7	0.23	0.14	1.38	12.20	12.15	12.36	12.44	0.08
9	2.0	0.30			11.96		12.29		
10	2.3	0.36			12.32		12.22		
11	2.4	0.37			11.22		12.20		
12	2.4	0.39	0.36	2.27	12.44	11.99	12.19	12.23	0.06
13	4.1	0.61			10.74		11.79		
14	4.2	0.62			11.35		11.78		
15	4.7	0.67	0.64	4.33	11.47	11.18	11.67	11.75	0.32
16	6.2	0.79			10.13		11.33		
17	6.5	0.81			11.35		11.27		
18	6.9	0.84			10.61		11.18		
19	7.2	0.86			11.10		11.12		
20	7.3	0.86	0.85	7.12	11.35	11.02	11.10	11.13	0.01
21	13.8	1.14			10.98		9.80		
22	15.5	1.19			9.88		9.49		
23	16.3	1.21			8.17		9.34		
24	17.5	1.24	1.20	15.71	7.93	9.24	9.15	9.44	0.04
25	24.6	1.39			7.81		8.02		
26	39.7	1.60			6.10		6.12		
27	44.6	1.65			5.25		5.63		
28	66.6	1.82	1.69	49.04	5.49	5.61	3.95	5.23	0.14
29	74.3	1.87			3.66		3.53		
30	88.7	1.95			3.29		2.91		
31	87.5	1.94			1.22		2.95		
32	94.3	1.97			3.66		2.71		
33	115.7	2.06			1.46		2.15		
34	144.2	2.16	1.99	98.39	2.68	2.66	1.71	2.66	0.00
35	189.9	2.28			1.22		1.39		
36	243.0	2.39			1.10		1.26		
37	320.8	2.51			1.46		1.21		
38	330.9	2.52			1.83		1.20		
39	376.5	2.58			0.24		1.20		
40	443.1	2.65	2.49	305.77	0.49	1.06	1.20	1.24	0.03
41					$s^2_{\text{tot}} = 19.45$		$s^2 = 0.1147$		

Note: ordinary least squares is used to fit Eqn 10.7 to the data.

It has been observed that nematostatics cause nematodes (*Globodera* spp. and *Ditylenchus dipsaci*) to leave plant roots but this is not an effective control measure. Good distribution through the soil is of utmost importance to the effectiveness of the nematicide as root systems are only protected when all roots are in close contact with the pesticide. Treated plants do not lose their ability to induce nematode hatch from eggs (e.g. some cyst nematodes and *Meloidogyne* spp.) but juveniles become either immobilized or disoriented and cannot find their food source, the plant roots. If this effect lasts long enough, the nematodes will eventually starve (see Chapter 7). Therefore, mortality of the nematodes depends on the time that plants remain unattractive and nematodes immobilized. Treatment of plants with nematostatics delays nematode penetration into the roots and results in a certain fraction of the root system escaping nematode attack and thus remaining healthy. As a result, the minimum yield m is increased by the fraction of the root system untouched by nematodes. Nematode penetration is postponed until the chemical is no longer effective or when the roots grow into soil layers where the nematicide is not present. Usually, nematostatics are distributed through the soil to a depth of 15–20 cm. Experiments on root-feeding nematodes demonstrate that nematostatics increase m and hardly affect T . The parameter T is only affected (increases) when nematodes die because of a long-lasting effect of nematostatics. The increase of m by nematostatics may be negatively correlated with tolerance (L. Molendijk, personal communication). This seems logical: an intolerant cultivar with a relative minimum yield m of 0.1 leaves more room for improvement ($1 - 0.1$) than a tolerant cultivar with $m = 0.9$, where m can only increase by 0.1. Therefore, relative effectiveness, expressed as $m'/(1 - m)$, where m' is the difference in minimum yield between treated and untreated plants and m is the minimum yield of untreated plants, might be a stable variable to measure the effectiveness of nematostatics.

10.7.2. Contact nematicides

Contact nematicides, which can be fumigant and non-fumigant (see Chapter 16) decrease the P_i . For cyst nematodes and *Meloidogyne*, hatching tests are often used to estimate percentage mortality. The higher the nematicide dose the longer these hatching tests must be continued as nematicides delay the hatching process (Schomaker and Been, 1999a). If the effect of nematostatics and contact nematicides is compared in one field experiment where P_i is estimated immediately before application, we estimate the effect of the contact nematicide as an increase in T and the inviolability of the root system by the nematostatics as an increase in m (Fig. 10.7). Increase of T is also observed in a glasshouse experiment if some of the nematodes die during the inoculation process or do not respond to root diffusate and do not invade the plant.

10.8. Validation of the Model

Seinhorst (1998) collected all available data in the literature about the relationship between P_i of 14 tylench nematode species and the relative dry plant weight (y) of 27 plant species/cultivars several months after sowing or planting; in total 36 plant/nematode combinations out of 29 experiments. An overview is given in Table 10.1. As T and m varied it was necessary to standardize the variables P and y . To do this, for each

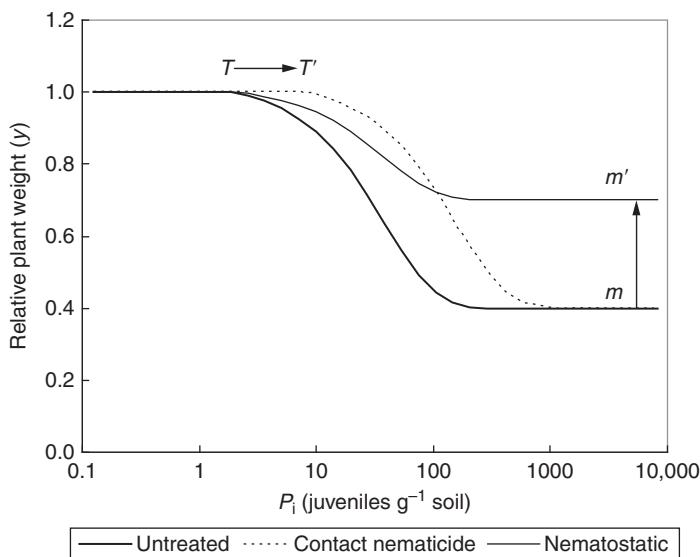


Fig. 10.7. Effect of nematicide treatments on plant yield. Treatment with a nematostatic increases plant tolerance. The parameter m increases from 0.4 to 0.7. Nematostatics can also cause mortality if their effectiveness lasts long enough. Mortality is seen as an increase in the tolerance limit T . Treatment with a contact nematicide causes a reduction of the initial population density. Compared to the original (pre-nematicide, pre-plant) population densities, this effect is observed as an increase in the tolerance limit T . In the graph a 75% mortality was presumed.

separate experiment T and m were estimated and $y' = (y - m)/(1 - m)$ and P/T were calculated. The relationship between average y' and P/T appeared to be in close accordance with $y' = z^{P-T}$, where $z^T = 0.95$ for all nematode–plant combinations. Therefore, for most combinations of tylench nematodes and plant weight Eqn 10.4 can be rewritten as:

$$y = m + (1 - m) \cdot 0.95^{\left(\frac{P}{T}-1\right)} \quad (10.9)$$

This relationship is obtained by log transformation of Eqn 10.4 and by substituting $\log(z)$ for $(1/T) \times \log(0.95)$. This common relationship for nematode–plant relationships implies, firstly, that nematodes that feed and multiply in very different ways still have the same effect on plant weight, despite external conditions, the host status of plants or the visible symptoms. A second implication is that host plants of any species of tylench nematodes are able to prevent growth reduction by these nematodes to the same degree, namely 0.95.

10.9. Population Dynamics

A population can be defined as a group of organisms that resemble each other genetically, morphologically and behaviourally, and are living in the same area or region. Population dynamics describes the general biological laws or processes that govern

increase or decrease of organisms. As nematode densities are often good predictors of plant damage, the study of population dynamics is an important discipline in nematology. The increase and decrease of nematode numbers are relatively slow processes. Therefore, control measures to prevent nematode increase or to stimulate nematode decrease must be taken at an early stage. The time of planting a susceptible crop is often too late for control measures, even for most nematicide applications. In the population dynamics of plant-parasitic nematodes that live on crop plants two phases can be distinguished: (i) the growing period of plants on which nematodes can multiply; and (ii) the period when nematodes do not have access to plants, so no food source is available. Nematode populations can only increase during the first phase. For nematodes on annual plants there are several population dynamic models available that relate P_i to P_f . Here we have chosen a model that is suitable to predict future nematode densities. Such a model must answer three conditions: (i) the model must be a mathematical translation of a biological theory and its parameters must have a clear biological meaning; (ii) the model must be as simple as possible but as extensive as necessary; and (iii) the model must allow estimation of at least starting values of the parameters directly from datasets and not only by regression.

The models of Seinhorst comply with these conditions. In these models, at small nematode densities, nematode multiplication is restricted only by the amount of food that the nematodes can capture and utilize under the given conditions. As plants at small densities provide sufficient space for all nematodes, competition does not play an important role. At high densities, nematode multiplication is limited by competition and the total amount of food that the host can supply. If plants remain smaller as a result of nematode infestation, then the total amount of available food and P_i are negatively correlated. In the following sections, nematodes with one and nematodes with more than one generation per season are discussed separately.

10.9.1. Nematodes with one generation per season

For nematodes species with one generation per year, which become sedentary after invasion, the population dynamic model is:

$$P_f = M \cdot \left(1 - e^{-a \cdot \frac{P_i}{M}} \right) \quad (10.10)$$

in which:

P_i initial nematode densities (before planting) as juveniles g⁻¹ soil

P_f final nematode densities (after harvest) as juveniles g⁻¹ soil

a the maximum rate of reproduction

M maximum population density as juveniles g⁻¹ soil

We assume that the offspring is proportional to the part of the root system that is exploited for food and that $P_f = a \times P_i$ if $P_i \rightarrow 0$ and $P_f = M$ if $P_i \rightarrow \infty$. This model is based on the same principles as Eqn 10.3. The occurrence of discrete, random events in space and/or time, such as the random encounters between nematodes and plant roots, are described by the Poisson distribution. The first term in the Poisson distribution, the

likelihood for a plant root to escape nematode attack (zero encounters), is given by $\text{EXP}(-c \times P_i)$ where c is a constant. The probability of one or more encounters is then given by $1 - \text{EXP}(-c \times P_i)$. We can understand this by imagining plant roots as cylindrical surfaces divided into equal compartments that, per cross-section, are penetrated randomly by second-stage juveniles (J2). As time and root growth go on, the cross-sections are moving up along the cylinder. If the J2 can settle in more than one compartment at the same time, this would result in overlap in territories and a decrease in the number of eggs per female. If there is no overlap, only one J2 per compartment can survive and number of eggs per female is not decreased. The size of the compartments depends on the place of the root in the root system and the growing conditions of the plant, but not on the density of the surviving juveniles. This simple model has some constraints. It makes no difference between the population dynamics in rooted and non-rooted soil and ignores reduction of plant roots by nematode infestation. Therefore, Eqn 10.10 applies only to small and medium densities where $P_i < M$.

As the values of a and M are determined not only by the qualities of plant roots as a food source, but also by external conditions, the final population density at one initial population density can vary markedly between fields and years. Therefore, it is impossible to predict the development of population densities in individual fields using only averages of a and M . To calculate the probability of all possible values of P_f , the frequency distributions of a and M are needed. For *G. rostochiensis* and *G. pallida*, a and M are log normal distributed. For *G. rostochiensis* and *G. pallida*, a and M are log normal distributed. The estimates for a and M are 25 and 300 eggs g⁻¹ soil for *G. rostochiensis* and 20 and 150 eggs g⁻¹ soil for *G. pallida*.

10.9.2. Nematodes with more than one generation

For the population dynamics of migratory nematodes with more than one generation per year, the same basic principles apply as for sedentary nematodes with one generation that are discussed in Section 10.9.1. The only difference is that nematodes with more than one generation redistribute continuously while their numbers increase. The population increase per unit of time is proportional to the difference between P_i and the so-called equilibrium density. It is comparable with the ‘law of diminishing returns’. This leads to the following relation between P_i and P_f :

$$P_f = \frac{a \cdot E \cdot P_i}{(a - 1) \cdot P_i + E} \quad (10.11)$$

E	equilibrium density (where $P_i = P_f$)
a	the maximum multiplication rate
$M = a \cdot E / (a - 1)$	the maximum population density

For very small values of P_i the final density $P_f = a \cdot P_i$ and at very large values of P_i the final density $P_f = M$. At the equilibrium density, E , the amount of food supplied by the plant is just enough to maintain the population density at planting. Among other things, it enables us to compare and quantify the host suitability of different species or cultivars for certain nematodes. Figure 10.8 presents eight examples of the relationship between nematode populations at the beginning (P_i) and the end (P_f) of a growing

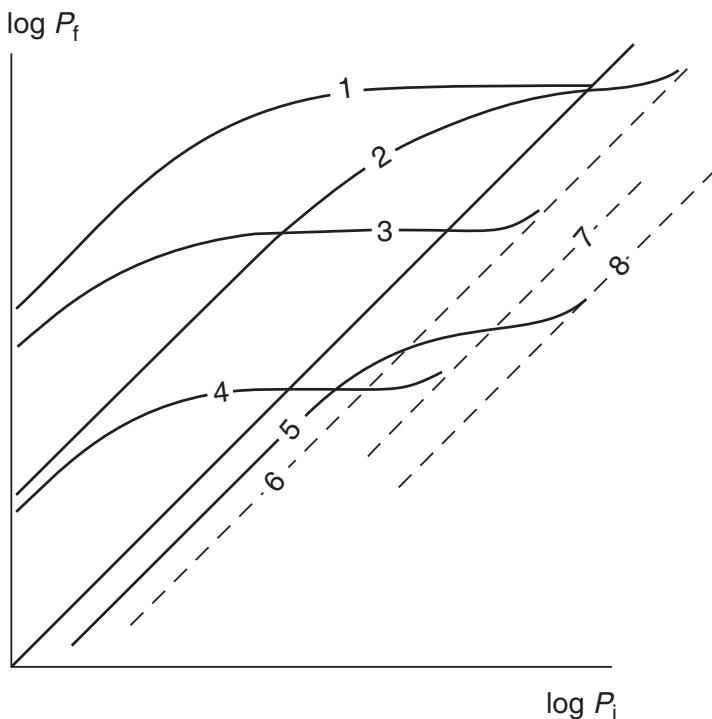


Fig. 10.8. Schematic presentation of the relation between initial and final population densities on a host. Solid lines: good (1) intermediate (2, 3) and poor (4, 5) hosts or good host grown under favourable (1), less favourable (2, 3) and unfavourable (4, 5) circumstances. Dotted lines (6, 7, 8) show the reduction of population densities in the absence of hosts dependent on the mortality rate of the nematode species. (From Seinhorst, 1981.)

season. Number 1 represents the population dynamics on a good host, 2 and 3 on a less good host and 4 and 5 on a poor host. These relationships can also be explained as the population dynamics of a good host under favourable (1), less favourable (2) and (3), or unfavourable (4) and (5) conditions. The relationships numbered 6, 7 and 8 represent the decrease in population density in the absence of hosts.

10.9.3. The effect of nematode damage and rooted area on population dynamics

Nematodes reduce plant growth and the size of plants. Stem nematodes in onions also decrease the number of plants. As a result, there is less food available for the nematodes when the P_i is larger. This is especially the case when host plants are intolerant. To account for this reduction in plant size we can expand Eqns 10.10 and 10.11 as follows for nematodes with one generation:

$$P_f = y \cdot M \cdot \left(1 - e^{-\alpha \cdot P_i} \right) \quad (10.12)$$

and for nematodes with more than one generation:

$$P_f = y \cdot a \cdot E \cdot \frac{P_i}{(a - 1) \cdot P_i + y \cdot E} \quad (10.13)$$

The variable y , described by Eqn 10.9, estimates the relative size of the root system affected by the nematodes. With root-invading nematode species, it can happen that part of the plant is not infested with nematodes if, for example, the food source is reduced and/or the nematodes are not in the vicinity of the roots. The larger the growth reduction of the plants, the smaller is the rooted area and the smaller the food source for the nematodes. Therefore, Eqns 10.2 and 10.3 only apply to the soil area containing roots. In the soil area without roots, the nematodes slowly decrease independently of P_i . To describe the population dynamics of the nematodes in the whole tillage (rooted and non-rooted) we can further expand Eqn 10.12:

$$P_f = r \cdot y \cdot M \cdot (1 - e^{-a \cdot P_i / r \cdot y \cdot M}) + (1 - r \cdot y) \cdot \alpha \cdot P_i \quad (10.14)$$

and Eqn (10.13):

$$P_f = r \cdot y \cdot a \cdot E \cdot \frac{P_i}{(a - 1) \cdot P_i + r \cdot y \cdot E} + (1 - r \cdot y) \cdot \alpha \cdot P_i \quad (10.15)$$

where:

r the proportion of rooted soil at $P_i = 0$

y the relative size of the root system, estimated from relative dry haulm weight

α the multiplication rate (<1) of nematodes in the absence of hosts

The proportion of rooted soil depends on plant anatomy and cropping systems. At large values of P_i in sensitive crops (m is small), the product $r \cdot y$ comes close to zero and $P_f \rightarrow \alpha \cdot P_i$. The resulting population dynamic models are visualized in Fig. 10.9.

10.9.3.1. Resistance

Fewer females will mature on resistant cultivars compared to susceptible ones. The number maturing depends on the degree of resistance or its complement, susceptibility. Also the number of offspring per female may be reduced, but this is not always the case. *Globodera pallida* females multiplying on resistant cultivars produce a smaller number of eggs per cyst than on susceptible cultivars but *G. rostochiensis* females have more eggs per cyst on resistant cultivars. In general, nematodes multiply less strongly on these cultivars than on susceptible ones and also sustain smaller maximum population densities.

If predictions about the population dynamics of nematodes on resistant cultivars are required, a stable measure of resistance is needed. The often used P_f/P_i ratio is unsuitable because of its density dependence; remember that P_f/P_i is larger at small densities than at larger ones. The parameters a , M (and E) vary strongly between fields and years under the influence of different external conditions, which makes them also unsuitable as stable measures for resistance. Therefore, the concept of 'relative susceptibility' (r.s.) was introduced. Relative susceptibility was first described for

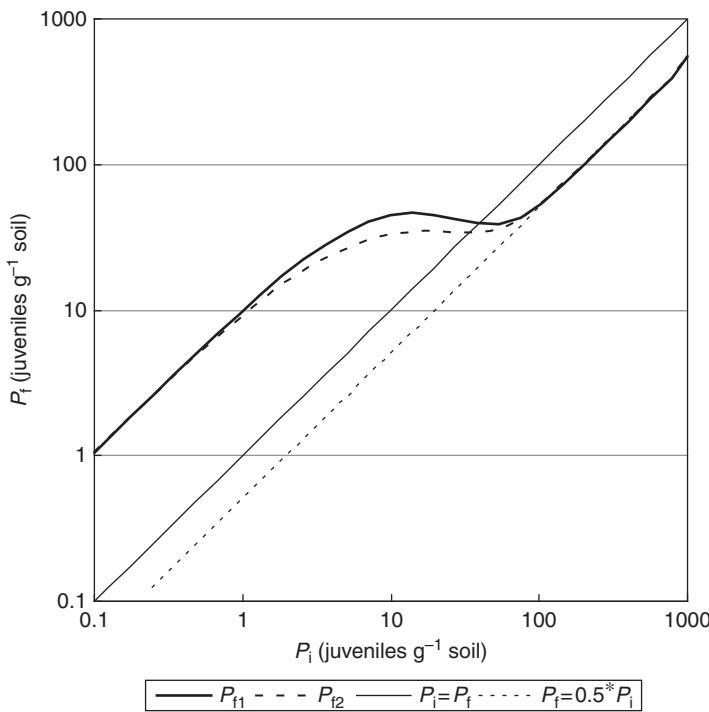


Fig. 10.9. Population dynamic models for nematodes with one generation, P_{f1} , and nematodes that multiply continuously, P_{f2} . Both models incorporate decrease of total mass of plant root by the nematodes, the rooted fraction of the soil and nematode mortality in the absence of food. In this figure, nematode mortality in the absence of food is presumed to be 50%; minimum yield (m) is set to 0.

G. pallida on partially resistant potato cultivars and was defined as the ratio of the maximum multiplication rate a of a nematode population on the resistant cultivar and on the susceptible reference cultivar ($a_{\text{resistant}}/a_{\text{susceptible}}$) or the equivalent ratio of the maximum population density M on these cultivars ($M_{\text{resistant}}/M_{\text{susceptible}}$). These ratios present two equal measures of partial resistance or relative susceptibility, provided that the tested cultivar and the susceptible reference are grown under the same conditions in the same experiment. Figure 10.10 visualizes the relation between P_i and P_f of pathotype Pa3 of *G. pallida* on the partially-resistant potato cultivar Darwina and on the susceptible cultivar Irene according to Eqn 10.14. The r.s. has been put into practice in The Netherlands and has proved to be independent of external conditions. There is one exception: during a hot summer the r.s. increased in two pot experiments when the temperature in the glasshouse exceeded 28°C. There are also reports that resistance for *Meloidogyne* spp. in tomato decreased (and susceptibility increased) under the influence of high temperatures. In temperate zones, this effect may be of little consequence, as temperatures in the soil are probably buffered sufficiently, but in tropical zones high temperatures may counteract resistance more frequently.

To make predictions about the population dynamics of a nematode population on resistant cultivars two more tools are needed. First, estimates must be made of the expected values and the variances of a and M on the susceptible reference cultivar in

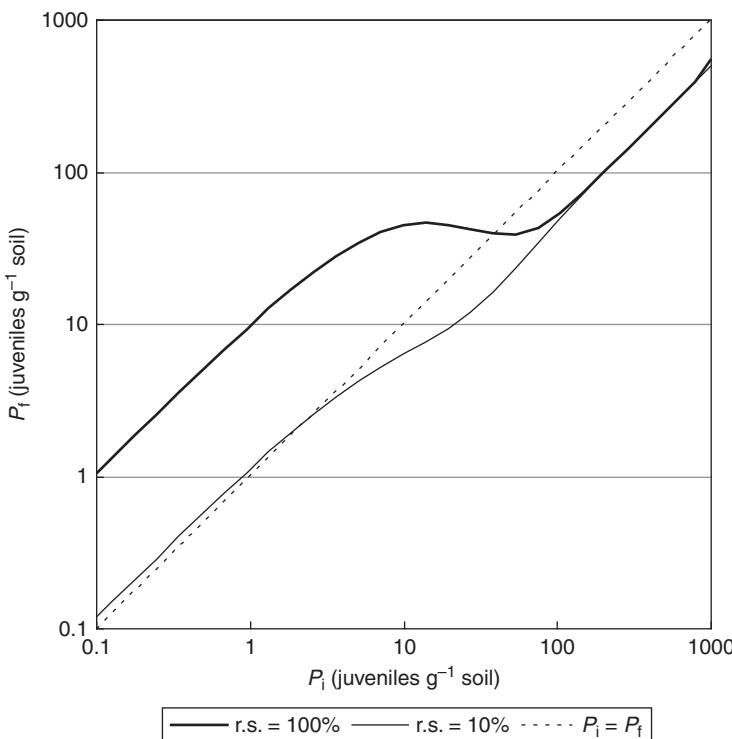


Fig. 10.10. Comparison of the population dynamics of potato cyst nematodes on a resistant and a susceptible variety. The relative susceptibility of the resistant variety is 10%; minimum yield (m) is set to 0. The tolerance of the susceptible and the resistant variety are the same. Note that tests at medium or high densities underestimate resistance.

different fields and different years. From this, frequency distributions for a and M can be made. Second, the r.s. of all cultivars resistant to a nematode species must be estimated under controlled conditions. For each nematode species, one or more carefully chosen populations are screened, depending on the variability in virulence. Some examples of partially resistant potato cultivars in The Netherlands are given in Table 10.4, together with the expected yield reductions if cultivars with these resistance qualities are grown in a 1:3 rotation.

Sufficient observations are available only for potato cyst nematodes to predict the population dynamics on resistant cultivars. Therefore, and because the same basic principles apply to all tylench nematode/plant relations, potato cyst nematodes are often used as model nematodes for other tylench species. Resistant cultivars give farmers an excellent tool to manage their nematode populations by keeping them at low densities that are not harmful. Farmers who have to deal with quarantine nematodes are often put in a difficult position, because governments demand that they should eradicate these nematodes. Fifty years of experience with some quarantine nematodes, such as potato cyst nematodes, has shown that these nematodes cannot be eradicated, not even by chemicals or fully resistant cultivars, on fields where hosts are grown frequently.

In general, nematodes inflict the same degree of damage in resistant and in susceptible cultivars but it is important to remember that both resistant and susceptible

Table 10.4. A selection of 16 Dutch potato cultivars, their relative susceptibilities (a/a_s) for two pathotypes (Pa2 and Pa3) of *Globodera pallida* and the percentage average yield reduction in a 1:3 rotation.

Cultivar	% Relative susceptibility		% Average yield reduction when exposed to eduction	
	Pa2	Pa3	Pa2	Pa3
Irene/Bintje	100	100	40.7	40.7
Aveka	0.14	0.4	0	0
Aviala	0.2	0.1	0	0
Darwina	0.3	12	0	0.6
Kantara	0.8	5	0	0
Nomade	1	4	0	0
Productent	6	56	0	25.6
Seresta	0.2	2	0	0
Agria	60	94	27.3	39.1
Hommage	9	53	0.1	24.1
Innovator	4.5	1	0	0
Elles	—	17	—	2.5
Marijke	45	51	20.1	23.1
Maritiema	2	46	0	20
Santé	5	30	0	11.8
Sinora	19	56.5	3.6	25.8
Vechtster	2.4	26	0	8.2

Note: the average relative minimum yield was taken to be 0.4. Relative susceptibilities are expressed as a percentage of the susceptible standards Irene and Bintje. For Pa3 the highly virulent 'Rookmaker' population was used.

cultivars may vary in tolerance. For example, in various field trials with *G. rostochiensis*, the minimum yield m of the resistant cultivar Agria was greater than m of the susceptible cultivar Bintje, which makes cultivar Agria more tolerant than Bintje. In most cases, tolerance and resistance to nematodes are independent characteristics in plants. That means that nematode multiplication is not related to plant damage. There are exceptions to this rule; tomato cultivars resistant to *Meloidogyne* spp. are more tolerant than susceptible tomato cultivars.

10.9.3.2. Population decline in the absence of hosts

Population decline in the absence of food is considered to be independent of nematode population density. The mortality rate of *G. rostochiensis* and *G. pallida* is greater in the first year after a potato crop (69%) than in subsequent years (20–30%). Information about the mortality rate of *H. schachtii* is less exact but a decrease of 50% per year is probably the best estimate. At least 70% of the eggs of *H. avenae* hatch the year after they developed, provided that they were exposed long enough to low temperatures. The same rate of decline applies for *M. naasi*. J2 of other *Meloidogyne* species hatch in large numbers shortly after the moult to J2. Estimates of percentage hatch vary from 70% to 90%. These reductions are largely due to spontaneous hatching of an apparently fixed proportion of the eggs. Population

densities of *Pratylenchus* spp., *Rotylenchus* spp. and *D. dipsaci* also decrease in the absence of food. Field observations are complicated as these nematodes have large host ranges, can maintain high densities on weed and even multiply on cut roots. Pudasaini *et al.* (2006) found average mortality rates in *P. penetrans* populations of 64% with very little variance after maize, carrot and potato on sandy loam. Organic matter in these fields varied from 2.7% to 3.7%. The population decrease of *D. dipsaci* depends on soil type and can reach 90% on light, humus sandy soils; on light to heavy clay soils and loamy, sandy soils with poor humus, population densities of between 1 and 20 nematodes kg⁻¹ are maintained, irrespective of whether hosts or non-hosts are grown.

11 Distribution Patterns and Sampling*

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11.1. Introduction

The spatial pattern of plant-parasitic nematode populations in an agricultural or natural ecosystem has two major components: (i) the **horizontal distribution**; and (ii) the **vertical distribution** of the organism throughout the soil or tillage. Both components will change in time because of different aspects of population dynamics, active and passive redistribution and spread.

The horizontal distribution can be divided, arbitrarily, into a micro-distributional component (within a field) and a macro-distributional component (growing regions, countries and parts of continents). The micro-distributional attributes of a nematode population are strongly linked to the population's life history, its feeding strategy and

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the availability of host plants. Sedentary endoparasitic nematodes deposit all their eggs at the same location, frequently in egg masses, generating an initially highly aggregated spatial pattern. Ectoparasitic nematodes invest a proportion of their assimilated energy into movement and selection of feeding sites. As they deposit their eggs individually, a somewhat less aggregated pattern may result. Nematode micro-distribution is primarily mediated by the distribution of food sources. For plant-parasitic nematodes, spacing and morphology of the plant root system, the frequency of cropping hosts and redistribution by machinery are dominant determinants. The integral effect of biological and edaphic influences results in varying degrees of aggregation in the spatial pattern of nematode populations within fields. Macro-distribution is mediated by such factors as the length of time the nematode population has been present in the (agro) system. If the organism has been introduced from abroad, like *Globodera rostochiensis* and *G. pallida* in Europe, a gradual spread will occur from the initial infestation site(s) to fields in the same area, different growing areas and countries importing seed potatoes.

The vertical distribution of a nematode species is constrained by two main factors. First, the depth of the soil layer that, in theory, would be accessible to the roots of a host; this can be limited by bedrock or other impenetrable layers leaving a tilth of limited vertical dimensions. Second, the rooting pattern of the host, in particular the depth of the root system of the host, which will limit the depth a species can reach. As the morphology of the root differs between different plant species, e.g. compare carrots and maize, so will the nematodes' penetration of the tilth differ. Although the vertical distribution of plant-parasitic nematodes is largely dependent on the root distribution of host crops, some variation in the abundance of different nematode species with depth has been related to soil type and texture, temperature and biotic factors.

11.2. Practical Application

The spatial distribution of population densities is interesting from a scientific point of view but also has some practical implications. As will be demonstrated, spatial patterns of nematodes (and also other pests, pathogens and diseases) vary not only among fields and regions, but also within fields and between plant and soil units. The variation within fields is of major importance in determining how samples have to be collected and what size of sample will be required to achieve a desired level of accuracy. It determines the methods to collect and process soil samples when surveys are carried out, or population densities in fields are estimated. How much soil has to be collected, how many cores are needed, which sampling pattern should be used and how much of the bulk sample has to be processed are questions that can only be answered when we formulate the purpose of the sampling method precisely and possess knowledge concerning the distribution patterns of the nematode under investigation.

Therefore, both the horizontal and vertical distribution patterns of nematode species will be discussed with emphasis on their origination and, most importantly, how this knowledge can be applied for practical use in the science of nematology and in the control of those plant-parasitic nematodes regarded as pests. Both components are of major importance for the following purposes.

- To estimate population densities of the target nematode in small plots used in field experiments. Normally, the initial population density needs to be established before the actual application of, for example, a certain crop or control method and the final population density after some time in order to obtain information on the effect of the treatment. The quality, for example the coefficient of variation (cv) of both population measurements, has to be adapted to the required distinctive power of the experiment.
- To determine the presence or absence of a certain nematode species. This implies we want to detect a nematode, for example a quarantine organism, with a certain probability. We do not want to know the actual numbers present, it is just a question of detection: yes or no. As absolute certainty is impossible (we would have to dig up the whole field), we have to define what has to be detected (e.g. the size of the infestation) and what degree of certainty will satisfy our needs.
- To estimate population densities in a farmer's field of a certain size (0.33 ha, 1 ha or 2 ha). We are now interested in the number of the target species per unit of soil, for example because we want to predict probable yield loss and have to decide whether or not a control measure has to be considered. The number of nematodes the sampling method yields should be as precise as required for this task, meaning that the variability of the estimate should be in an acceptable range in order to prevent gross over- or underestimation of the expected yield reduction.

11.3. Horizontal Distribution

Within fields, plant-parasitic nematodes are usually clustered. Depending how deep one wants to venture, three to four ‘scales of distribution’ should be distinguished. Starting from very small to the largest, the following distribution patterns can generally be identified within a farmer's field.

11.3.1. Very small-scale distribution

The very small-scale distribution pattern of all nematodes, but especially sedentary nematodes, is the result of the presence and distribution in time of roots throughout the tillage. Only where a root is present will nematodes aggregate. A so-called clumped or aggregated distribution develops, which can be considered as a population of subpopulations occupying small areas in the near vicinity of, or on, the root. It implies that each core taken will probe a different subpopulation and will show another population density, assuming that each subpopulation has an area larger than a single core but that cores are separated by distances larger than the diameter of each subpopulation. Although the resulting distribution is the origin of all other patterns that will emerge in the field, it is also the most difficult and laborious one to establish. One would need to collect systematically small volumes of soil and determine the presence of both roots and the target species until the selected volume is charted in both dimensions. Although this could be done for natural habitats, it will not be of any use in most agricultural systems where this pattern is destroyed when below-ground parts of agricultural crops are harvested. Apart from pure scientific interest, this distribution pattern has no practical importance when the aim is safeguarding agricultural produce.

Far more interesting are the patterns emerging from this distribution. However, it tells us that a single core samples only a subpopulation and will not be a very useful density estimator for any area larger than that covered by the diameter of the auger used.

11.3.2. Small-scale distribution

The small-scale distribution describes the distribution pattern over small areas in the field. It is the result of growing the host plant in a grid pattern, defined by the distance in the row between individual plants and the distance between rows. In general, the area defined by the small-scale distribution is the largest area without a defined shape or gradient of population densities or the largest area with an acceptable small variance of the population density estimator. In Fig. 11.1A and B, the small-scale distribution of *G. pallida* and *Paratrichodorus teres* is shown in a 1 m² plot with population densities presented per dm². The aggregated distribution of cysts in even such a small area is apparent and applies to all nematode species. As a result, the estimation of population densities with a limited margin of error is difficult even in such a small area. In order to estimate these errors, one first needs to describe the small-scale distribution of nematodes mathematically. This will enable the calculation of several interesting aspects for that area, e.g. the probability of finding 0, 1, 2 or more nematodes or cysts when taking one sample with an auger of a certain size. Similarly one can calculate how much soil is required to detect a single nematode or cyst with a certain probability, or how much soil is required to get a reliable estimation of the population density in that area.

11.3.2.1. The negative binomial distribution

In the majority of the nematological literature the spatial distribution of population densities is described by the negative binomial distribution, irrespective of the area under investigation. The distribution also applies for the small-scale distribution and is as follows:

$$\Pr[p(x,y) = \alpha] = \binom{\alpha + k - 1}{k - 1} \times \left(\frac{E[p(x,y)]}{E[p(x,y)] + k} \right)^\alpha \times \left(\frac{k}{E[p(x,y)] + k} \right)^k \quad (11.1)$$

where:

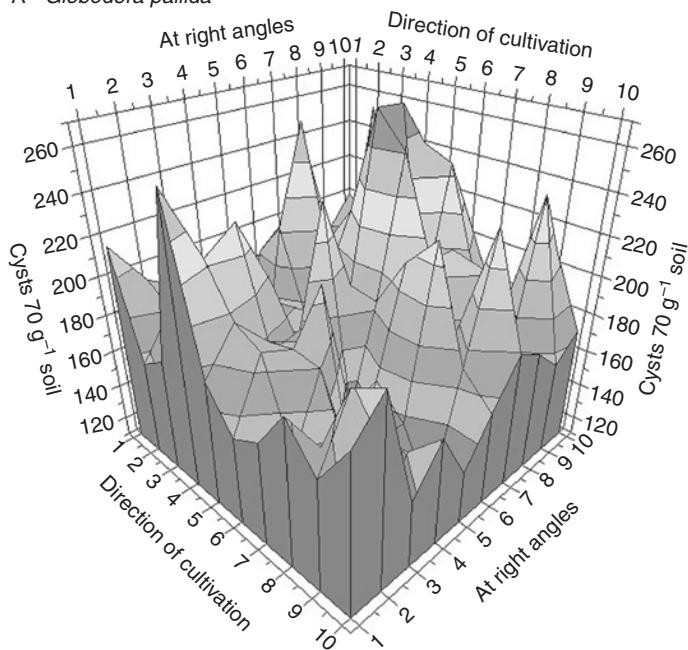
- $p(x, y)$ population density at location (x, y)
- $\Pr[p(x, y) = \alpha]$ the probability of finding a certain number of nematodes or cysts (α) at location (x, y)
- $E[p(x, y)]$ expected population density at location (x, y)
- α integer ≥ 0 : number of cysts or juveniles
- k coefficient of aggregation

Equation 11.1 can be simplified to the following equation when $\alpha = 0$:

$$\Pr[p(x,y) = 0] = \left(\frac{k}{E[p(x,y)] + k} \right)^k \quad (11.2)$$

and used to calculate the detection probability (see Box 11.5).

A *Globodera pallida*



B *Paratrichodorus teres*

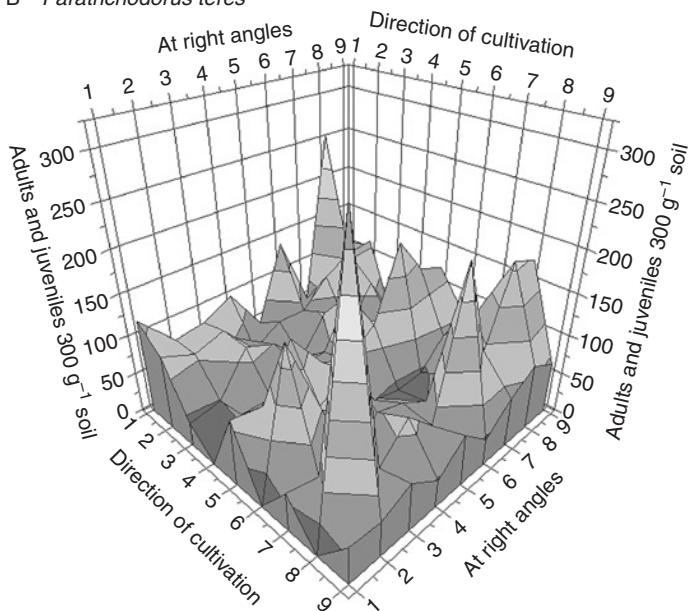


Fig. 11.1. Visualization of the mapped small-scale distribution in 1 m² of: (A) *Globodera pallida* representing the number of cysts per 70 g of dried soil; (B) *Paratrichodorus teres* representing the number of adults and juveniles per 300 g fresh weight. Each data point represents an area of 1 dm².

The negative binomial distribution is one of a series of distributions describing an aggregated or clumped distribution of population densities. The aggregation factor, or coefficient of aggregation k , describes the degree of clumping of the population, with low numbers indicating high aggregation and high numbers less aggregation. When aggregation or clumping occurs in an area, the probability of finding another individual close to the one already located is greater when the distance between the two locations decreases. The distribution becomes identical to the Poisson distribution, showing a random distribution as k increases to infinity. Fractional k -values (<1.0) indicate that the distribution is approaching the logarithmic series which occurs when $k = 0$. As k -values differ from location to location, sometimes a ‘common k ’ (Bliss and Owen, 1958) is used as an operational value for general use.

In Table 11.1, adapted from Seinhorst (1988) and updated with recent information, the aggregation factor k is presented for several relevant plant-parasitic nematode species. In some of these examples, e.g. for *Meloidogyne* spp., the aggregation factor listed is smaller (more aggregated) than it is in the field. Any error made in the estimation of

Table 11.1. Aggregation coefficient k of the negative binomial distribution for some nematode species as reported or derived from the literature and partly summarized by Seinhorst (1988).

Nematode species	Plot size (m ²)	Number of plots	Repeats per plot	k' (1.5 kg soil)
<i>Globodera rostochiensis</i>	1	4	10	72–88
<i>G. rostochiensis</i>	1	364	2	76
<i>Pratylenchus crenatus</i>	16	2	10	32–38
<i>Rotylenchus uniformis</i>	16	2	10	40–45
<i>R. uniformis</i>	1	58	2	60
<i>Globodera pallida</i>	30	29	2	361
<i>G. pallida</i>	10–20	78	2	55
<i>G. pallida</i>	20	1	40	81
<i>G. pallida</i>	30	1	40	166
<i>G. pallida</i>	45	1	40	12
<i>Heterodera schachtii</i>	30	30	2	70
<i>H. schachtii</i>	20	1	40	44
<i>H. schachtii</i>	30	1	40	64
<i>H. schachtii</i>	45	1	40	16
<i>Meloidogyne arenaria</i>	1	1	41	14–23
<i>P. minyus</i>	1	1	41	14–34
<i>Paratrichodorus minor</i>	1	1	41	4–5
<i>Pratylenchus penetrans</i>	1	3	75–80	37–46
<i>H. schachtii</i>	1	456	1	35–550
<i>M. chitwoodi</i>	1	20	10	15 ^a
<i>P. penetrans</i>	1	36	10	80 ^a
<i>Trichodorids</i>	1	15	10	50 ^a
<i>G. pallida</i> (starch area)	1	41	10	135 ^a
<i>G. pallida</i> (seed potatoes)	1	28	10	70 ^a

^aCommon k' .

Note: k' is an estimate of k , $k' = k$ if variation due to errors in laboratory procedures is negligible. At present, k for *Meloidogyne* spp. is seriously underestimated (more clustered) by the extraction methods in use.

the population density in the laboratory is added to the variability found in the soil. Large laboratory errors will yield smaller values of k . Analysing subsamples from well mixed bulk samples, elutriated either by using the Oostenbrink elutriator or the zonal centrifuge, revealed a laboratory error of more than 50% for *Meloidogyne* spp., partly due to variation contributed by the organic fraction and probably caused by the presence or absence of egg masses in the root debris. For other free-living stages of plant-parasitic nematode species like *Pratylenchus* spp., laboratory error is lower; for cyst nematodes, because of sound methodology available, it is practically negligible. Therefore, the k -value in Table 11.1 is designated as k' , indicating that it is an estimator of the real value.

Been and Schomaker (2000) used all data available for *Globodera* spp. to calculate a ‘common k ’ and found a value of 70 for seed and consumption potatoes and 135 for starch potatoes for a 1.5 kg soil sample originating from 1 m². The latter are cropped in a 1:2 cropping frequency in completely infested fields, which obviously resulted in a lower aggregation on the small scale. Although the high k value indicates that one could also use the Poisson distribution instead of the negative binomial distribution, this assumption is one of the many pitfalls of soil sampling (see Box 11.1).

Box 11.1. Pitfalls of soil sampling.

The aggregation factor k is dependent on the size of the sample collected. If k is expressed per 1.5 kg of soil as in Table 11.1, it can reach a high value. However, when a bulk sample is taken, a number of small cores will be collected using a certain sampling grid. For example, the old European and Mediterranean Plant Protection Organization (EPPO) sampling method required a bulk sample of 200 cm³ of soil obtained by collecting 60 separate cores. Therefore, each core has a volume of approximately 3.33 cm³ or 4–6 g of soil, dependent on the soil type. When one core is taken, the aggregation coefficient for that core will be proportional to the core size:

$$k = 135 \times \frac{4}{1500} \text{ up to } k = 135 \times \frac{6}{1500}$$

which yields a k -value between 0.36 and 0.53 for the core sample, assuming $k = 135$ for the 1.5 kg sample. This is a very small value indicating high aggregation and applicability of the negative binomial distribution. The method of establishing the aggregation factor k of the small-scale distribution is discussed in Box 11.2.

The negative binomial distribution fits to data of counts of any clumped biological entity. The small-scale distribution is well described by this frequency distribution. Even on the largest scale, the field, we can consider the distribution of hotspots as clumped and the negative binomial will apply, or will yield a value for the parameter k . In fact, it will almost always apply to nematode counts collected, irrespective of the area used to collect these data. However, every area will yield a different parameter value of k . Aggregation will, in most cases, increase with area as more distribution patterns will be covered. They cannot be compared. Neither is there a mechanism to correct the parameter for an adapted area. Therefore, before starting out to set parameters for this, or any other, distribution, the size of area relevant for the purpose has to be determined carefully.

Box 11.2. Estimating k and a ‘common k ’ of *Pratylenchus penetrans*.

Let us presume that there is a need to develop a sampling method for scientific research, for example to sample plots in field experiments, for *P. penetrans* in order to obtain reliable estimations of the population density in these plots. The area used as plot size has to be chosen in such a way that within that area no measurable effect of the redistribution vectors of machines, tillage, etc. can be found, or that the effect is an acceptably small one. In the latter case, we choose the largest area that will yield an acceptable variance. If no prior information is available, one could use 1 m² plots, which have proved to be feasible in most research strategies. As most host crops will not cover the complete volume of soil, the population dynamics of the rooted and non-rooted part of the soil will differ. In the rooted part multiplication will occur, while in the non-rooted part densities will decrease. To avoid any bias, the length and width of the square meter has to be selected in such a way that a proportional part of the sample will be collected from the soil in the row and between the rows regardless of the situation of the plot in the field (and the visibility of the rows after cultivation).

The aggregation factor k of the negative binomial distribution can be estimated in several ways. (Actually, we will estimate k' , an estimate of k ; k' equals k if variation due to errors in laboratory procedures are negligible.) One possible way is to sample that area repeatedly, for example ten times. We now have ten estimations of the population density of this area. The easiest method is to use the moments (mean and variance) to estimate k . First one calculates the mean:

$$\bar{x} = \frac{1}{10} \times \sum_{j=1}^{10} x_j \quad (11.3)$$

and the variance:

$$s^2 = \frac{1}{10-1} \times \sum_{j=1}^{10} (x_j - \bar{x})^2 \quad (11.4)$$

and uses the following equation to estimate k :

$$k = \frac{\bar{x}^2}{s^2 - \bar{x}} \quad (11.5)$$

Although k is a parameter, we are dealing with a biological descriptive parameter and its value will vary between locations and in time as a result of different external conditions influencing the organism. Some researchers consider k as a function of the population mean and variance, which would indicate that it differs at different densities. However, this is for the most part the result of increasing laboratory and methodological error when nematode numbers are low. As a consequence, one wants to establish a k that is applicable anywhere. The above-described exercise has to be repeated on different plots in several fields and years and a so-called ‘common k ’ (Bliss and Owen, 1958) can be calculated, which will also apply to the plots in fields

Continued

Box 11.2. Continued.

that have to be sampled in the future. Another, elegant, way of estimating k is to calculate the coefficient of variation (cv) for all the plots sampled as described above. The cv is defined as:

$$cv = \frac{s}{\bar{x}} \quad (11.6)$$

By plotting the cv of each of the individual plots against their mean population density and fitting a regression line through these points, representing the cv according to the negative binomial distribution, an estimate will be obtained for the 'common k ' value (Fig. 11.2). The formula to calculate the cv according to a negative binomial distribution is:

$$cv = \sqrt{\frac{1}{k} + \frac{1}{\bar{x}}} \quad (11.7)$$

Fitting this equation to the data points resulted in a k -factor of 80 for 1.5 kg soil samples. We see in Fig. 11.2 that a certain number of nematodes have to be counted to obtain an acceptable cv. The amount of soil, from a large bulk sample, that has to be processed to obtain the desired cv must be adapted to provide these numbers. Box 11.3 and Fig. 11.3 show how this can be used to determine the required sample size.

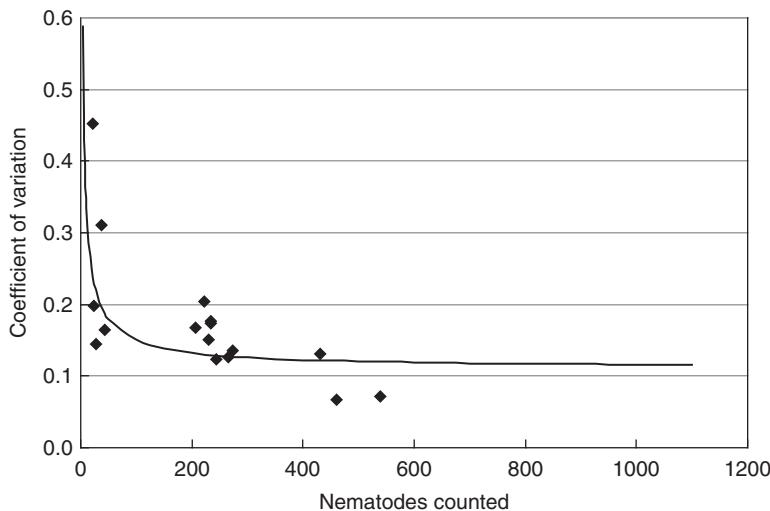


Fig. 11.2. The relationship between coefficient of variation and the number of *Pratylenchus penetrans* counted (diamonds). Coefficient of variation according to the negative binomial distribution with $k = 80$ (solid line).

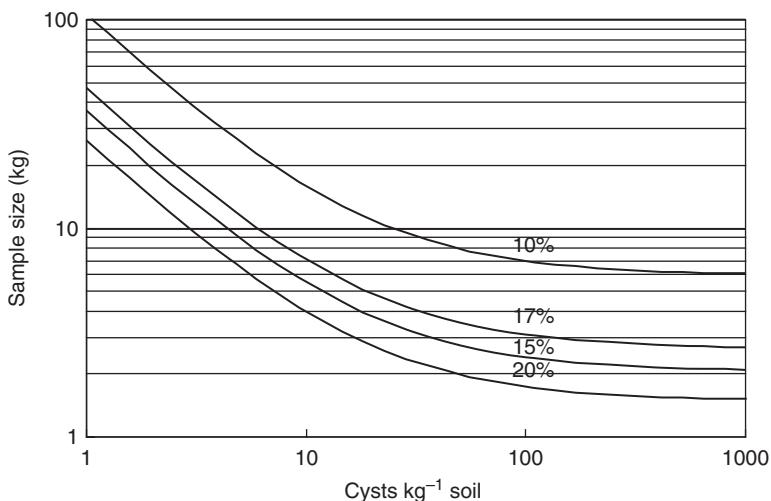


Fig. 11.3. Relationship between population density of cysts of *Globodera rostochiensis* and *G. pallida* and sample size required to obtain coefficients of variation of 10%, 15%, 17% and 20% of estimated egg densities, assuming a negative binomial distribution of cyst counts in samples with a value of k of 70 for 1.5 kg soil samples and a coefficient of variation of average numbers of eggs per cyst in 1.5 kg soil samples of 16% ($K_{\text{eggs}} = 25$). (From Seinhorst, 1988.)

11.3.2.2. How small is a small plot?

One of the problems in field experiments is the size of the plots. In field trials of resistant cultivars, pesticides or other nematode control measures, agronomists estimated crop yield at harvest by collecting the produce, e.g. tubers, from plots ranging from several square metres up to 100 m² or more per plot. If the same area is used for collecting the corresponding soil sample to estimate the nematode population density of the plot, this will result in erroneous correlations between nematode density and crop yield. Figure 11.4 shows the results of the scientific sampling method for *G. pallida*, employed on a row of square metre plots in the direction of cultivation. Log nematode densities are plotted and linear regression is applied to model the correlation. There is a clear trend of increasing population densities with increasing distance. This trend is even stronger at right angles to the direction of cultivation. When a soil sample is taken from an area covering up to 100 m², an average over all encountered population densities in that area will be acquired. As the correlation between nematode density and yield loss is not linear (see Chapter 10), the correlation will be biased when a bulk sample is collected from an area that contains a number of quite different population densities and yield responses. In fact, Fig. 11.4 visualizes a part of the second distribution pattern encountered in the field – the medium-scale distribution. For most nematodes, the area of the small-scale distribution is confined to only a couple of square metres. For example, for potato cyst nematodes it was established that the optimum size for that distribution is 1 m² (1.33 m by 0.75 m, keeping in mind the spacing of the rows and the between-row distance) and that an upper limit of 4 m² is acceptable if necessary. Haydock and Perry (1998) present a list of methods

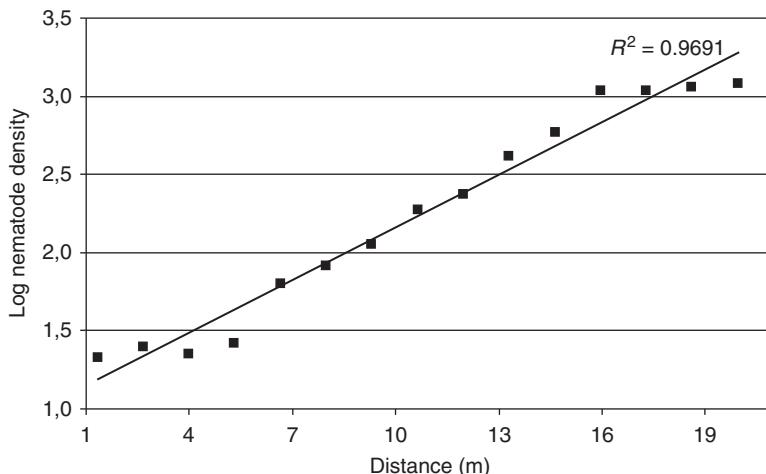


Fig. 11.4. Densities of *Globodera pallida* (log numbers per 1.5 kg soil) in a row of square metre plots along the direction of cultivation including linear regression fit.

used for scientific research on potato cyst nematodes; only one complied with the requirements stated. In conclusion, we can state that the area covered by the small-scale distribution provides the area used to estimate population densities of plots in field experiments. When the area to be sampled and its corresponding k -value is known (see Box 11.2 on how to estimate k) we can use this knowledge to calculate the size of the soil sample required for plots in field experiments (Box 11.3).

11.3.2.3. How to sample a small plot

The aggregated distribution in even such a small area as 1 m² means that one cannot just take a core sample and use that to estimate the local population density. The best way is to collect a number of samples using a rectangular grid. This will guarantee every area within the plot the same probability of contributing individuals to the population density estimation. One of the questions that arises is how many samples have to be taken – large numbers of small size cores or a few, larger cores. Two aspects are relevant:

1. The more, and smaller, cores taken to obtain a bulk sample of a certain size, the smaller the variation of the population density estimator. This is caused mainly by the fact that the area of the small-scale distribution will be divided into more parts, each contributing to the resulting density estimator. Table 11.2 presents a study of *Paratrichodorus teres* bulk samples (1.5 kg) collected with two augers of different diameter. Although no change in the average population density of *P. teres* was found, the smaller auger yielded a lower cv. Note that as a result of the improved methodology, a higher aggregation factor k is obtained.
2. By contrast, with free-living stages of plant-parasitic nematodes, a smaller auger diameter will cause more mechanical damage to a greater number of nematodes. Dead nematodes will not be recovered and underestimation of the true population density will occur. This is demonstrated abundantly by several authors and partly

Box 11.3. Calculation of sample size for plots in field experiments.

The purpose of most quantitative nematological research is finding a relationship between population densities and a variable, e.g. final plant weight or effect of a control measure. Normally the x -values should be an independent value set by the researcher. However, in nematology, population density estimation is subject to experimental error, which increases with decrease in population density. Log or square root transformations often do not reduce covariance between variance and true population density enough to make statistical analysis feasible. A possible solution to this problem is adjusting the sample size of the bulk sample to the expected population density in order to achieve the desired variation, often expressed as coefficient of variation (cv). A possible way of estimating the required sample size is detailed below. (Meaning of abbreviations used: e , eggs or nematodes unit $^{-1}$ soil; G , sample size in units of soil; c , cysts unit $^{-1}$ soil; cv, coefficient of variation (standard deviation/mean); e/c , eggs/cysts; k , coefficient of aggregation of the negative binomial distribution adapted to the unit soil; m , mean.)

For cyst nematodes:

The number of eggs is equal to the number of cysts times the cyst content:

$$e = c \times e/c \quad (11.8)$$

Therefore:

$$cv^2(e) = cv^2(c \times e/c) \quad (11.9)$$

(Applying Taylor Series) if c and e/c are independent:

$$cv^2(e) = cv^2(c) + cv^2(e/c) \quad (11.10)$$

If dependent:

$$cv^2(e) = cv^2(c) + cv^2(e/c) + 2\text{cov} \frac{(c, e/c)}{c} \quad (11.11)$$

Assuming that cysts are distributed according to the negative binomial distribution in restricted areas (small-scale distribution):

Then: per 1 unit of soil

$$cv^2(c) = \frac{1}{k} + \frac{1}{c} \quad (11.12)$$

If G units are taken

$$cv^2(c) = \frac{1}{G} \times \left(\frac{1}{k} + \frac{1}{c} \right) \quad (11.13)$$

Eliminate $cv(c)$: Eqns 11.10 and 11.13:

$$cv^2(e) = \frac{1}{G} \times \left(\frac{1}{k} + \frac{1}{c} + cv^2(e/c) \right) \quad (11.14)$$

Continued

Box 11.3. Continued.

Solve G :

$$G = \frac{\left(\frac{1}{k} + \frac{1}{c} + cv^2(e/c) \right)}{cv^2(e)} \quad (11.15)$$

Now we can choose the $cv(e)$ that is required for the experiment and calculate G . The aggregation factor k should be known from previous studies (see Box 11.2). The cv of the number of eggs/cyst, $cv(e/c)$, as well as the expected number of cysts c should be known. The expected population density, or number of cysts, at the site can be estimated by pre-sampling a couple of plots. Another way, and avoiding this problem, constitutes taking a bulk sample of such a size that extra subsamples are available if the standard elutriated sample is too small.

For k and $cv(e/c)$ a best estimate from the literature (Table 11.1) or one's own experiences with other field experiments can be used. For potato cyst nematodes, the best estimate of a 'common k ' = 70 for a sample size of 1.5 kg soil (Been and Schomaker, 2000). For $cv^2(e/c)$ values ranging from 12 to 20% (mean 16%) are often found (Seinhorst, 1988), provided that they are estimated from a sufficient number of (small) plots. However, these values may be higher if pesticides are used frequently. Graphs can be constructed giving the required sample size dependent on both the desired cv and the actual population density in the plot. In Fig. 11.3 this relationship is presented to obtain egg densities of potato cyst nematodes for four levels of variation.

For free-living stages of plant-parasitic nematodes:

Equation 11.15 can be simplified to:

$$G = \frac{\left(\frac{1}{k} + \frac{1}{e} \right)}{cv^2(e)} \quad (11.16)$$

Again, a suitable estimate of k is required (Table 11.1) or one is chosen which is comparatively low (high aggregation = worst case approach).

summarized by Seinhorst (1988) for *Rotylenchus uniformis*, *Pratylenchus crenatus*, *Tylenchorhynchus dubius* and *Paratrichodorus pachydermus*, with up to 1.6 times more individuals counted when larger cores are used and compared to a standard 1 cm diameter core.

For cyst nematodes the size of the auger is not a problem as cysts are not easily damaged. Also, the smaller the core, the easier and faster a core sample down to 25–30 cm is collected. Generally, using a 1.5 cm diameter core and collecting 60–80 cores in a grid pattern, a soil sample of approximately 1.5 kg can be collected easily and quickly for almost all relevant nematode species. It is not necessary to elutriate the whole bulk sample of 1.5 kg, provided enough individuals can be retrieved and counted from the first subsample processed and the required cv is achieved (Fig. 11.3; Box 11.3). If subsampling is employed the theory of subsampling has to be understood (Box 11.4).

Table 11.2. Results of a comparative study on the effect of auger size on the reliability of population density estimation of *Paratrichodorus teres*.

Statistics	Trichodoridae auger (3.5 cm diam.)	Potato cyst nematode auger (1.5 cm diam.)
Number of cores	8	60
Bulk sample size (g)	1500	1500
Subsample size (g)	500	500
Mean ($n = 10$)	80.0	84.6
Standard deviation	25.4	13.8
Coefficient of variation	31.7	16.3
<i>k</i> -value	11.4	67.9

Note: There were no effects on the actual population density estimator averaged over ten replications. However, the reliability of individual replication is greatly improved by collecting a larger number of small cores when using the auger for potato cyst nematode sampling as expressed by the coefficient of variation. Note that as a result of the improved methodology, the *k*-value of the negative binomial distribution increased. There was no damage to the nematodes by the smaller auger as the mean value did not decrease.

11.3.3. Medium-scale distribution

The medium-scale distribution describes the change of population densities over larger areas than those covered by the small-scale distribution. It is the pattern that results when active and mechanical redistribution and spread acts on the small-scale distribution pattern. Soil will be lifted by machinery, mixed and displaced, either in the direction of cultivation (cultivation, ploughing and harvesting) or at right angles to it (ploughing, winter ploughing). This redistribution results in the horizontal growth of the primary point infestation over adjacent areas. Quite distinct shapes can result, such as the development of the so-called infestation focus or hotspot. Within the area covered by the medium-scale distribution there are different population densities at different locations that are related to each other. As most farmers in a growing area or country use the same kind of machinery, and sometimes even the same cropping frequency, the resulting infestation foci of a nematode species tend to be of the same shape at any given location. An oval-shaped spot (lens) in the field will appear where plant growth is retarded or, in extreme cases, completely inhibited. The area of the actual infestation is larger than that covered by the lens as densities at the border of the infestation are beneath the threshold for visible damage. The longest axis of the hotspot will lie in the direction of cultivation with slowly declining population densities, and its short axis, at right angles to the direction of cultivation, with sharply declining densities. Nematode densities will rise exponentially towards the centre (Fig. 11.6). In the case of extremely high population densities, host plants in the centre of the lens might actually die and postharvest population densities in the centre of the focus will be lower than those in the perimeter as a

Box 11.4. The effect of subsampling.

In many nematological field studies it is common practice to collect a bulk sample but to investigate only a part, taken from the bulk sample, after mixing. The size of such a subsample is mostly small, but never infinitely small, relative to the bulk sample. If a soil sample is perfectly mixed, the original negative binomial distribution of cysts or nematodes per unit weight is lost and the cysts/nematodes are randomly distributed in the soil (Seinhorst, 1988). If infinitely small subsamples were taken, the distribution of the nematodes between the subsamples would follow a Poisson distribution. However, in practice, subsamples are not so small in comparison with the bulk sample and, therefore, the numbers in the subsample and in the remainder of the bulk sample do not follow a Poisson distribution but rather a binomial distribution (McCullagh and Nelder, 1992). To visualize the mathematical problem, cvs of nematode counts in subsamples were calculated in a model study for bulk samples with 100 nematodes, assuming a binomial or a Poisson distribution of nematode counts between subsamples. The investigated proportion p of the bulk sample varied from 0.1 to 1. The two functions of the cvs (Eqns 11.17 and 11.18) are compared in Fig. 11.5. Two conclusions can be drawn. First, if the investigated proportion of the bulk sample is relatively small, the cvs calculated with the binomial and Poisson distributions are almost similar. Second, the assumption that nematode numbers between subsamples are Poisson distributed is basically wrong as this distribution misses a correction for finiteness. Application to subsampling of soil samples would imply a considerable cv even when the whole bulk sample is processed.

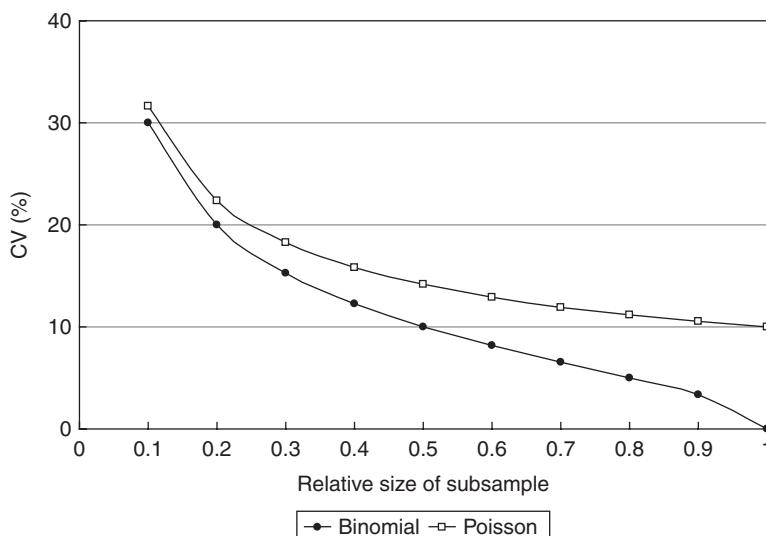


Fig. 11.5. Relation between relative size of one subsample from a bulk sample, and coefficient of variation, cv, in per cent, according to a binomial (●) or a Poisson (□) distribution of cysts or juveniles. Number of cysts or juveniles in the bulk sample is set at 100.

Continued

Box 11.4. Continued.

$$\text{Poisson distribution: } \text{cv} = \frac{\sqrt{x}}{x} \quad (11.17)$$

$$\text{Binomial distribution: } \text{cv} = \frac{\sqrt{x \times (1-p) \times (p)}}{p \times x} \quad (11.18)$$

Incidentally, one can read in nematological literature that subsampling adds an extra factor of variation and one is advised to avoid subsampling if possible. The consequence is that only small samples, say 100 ml, are collected and processed. We now know that the extra error is in accordance with a binomial distribution. As the error of the bulk sample from a small plot is in accordance with the negative binomial distribution, we can now explore how much error is added by subsampling and how the total error can be reduced. Let us presume we have an elutriator that can process 100 cm³ of soil, that $k = 10$ for a 1 kg soil sample and an expected population density of 100 nematodes kg⁻¹. We will collect 200 cm³ of soil and elutriate 100 cm³. Proportional with the size of the soil sample k will be 200/1000 × 10 = 2 for a 200 cm³ sample and the expected value is 20 nematodes.

The field sampling error expressed as cv will be 74.2% according to Eqn 11.7. According to the binomial distribution an extra error of 22.4% will be added when half of the sample is processed. The cvs can be combined using the Taylor series; in this case the new cv equals:

$$\text{cv}_{\text{new}} = \sqrt{\text{cv}_{\text{bulksample}}^2 + \text{cv}_{\text{Subsample}}^2} \quad (11.19)$$

Therefore, the numeric solution is

$$\text{cv}_{\text{new}} = \sqrt{74.2^2 + 22.4^2} = 77.5$$

Subsampling added 3.3% to the cv we already suffered by collecting a bulk sample of 200 cm³. Therefore, we can conclude that subsampling indeed adds an error, although a comparatively small one. However, let us consider the following alternative. We do not subsample and only collect and process 100 cm³ of soil. The cv of the bulk sample according to Eqn 11.7 is now 104.9% and much larger than the previous one. If we decide to collect ten times as much soil (1000 cm³), mix the soil thoroughly and extract and process a subsample of 100 cm³ the combined error will be reduced to 38.7%. The amount of soil processed is still the same and an error is added by subsampling. However, the amount of soil collected from the field is ten times as much. As the aggregation factor k is directly correlated with the amount of soil collected, k for the bulk sample has increased from 1 (cv 104.9%) to 10 (cv 32.4%) while the subsample error has increased from 0 to 21.2%. However, the net result is a lower combined error.

Conclusion:

- Increasing the bulk sample will increase the k of the bulk sample and decrease the cv of the bulk sample.
- If we subsample after mixing we maintain the large k of the bulk sample and the error we add only depends on the number of counted nematodes.
- The error of this processed sample (cv) can be smaller when the error reduction by a higher k value is larger than the added error by subsampling.

Of course optimization of this process depends on the k -value and the fraction of the bulk sample processed. If the size of the subsample is only a small fraction of the bulk sample no extra gain in error reduction will be yielded. If nematode densities are too small, extra reduction of the estimation error is only possible by counting more nematodes, for example by elutriating more subsamples.

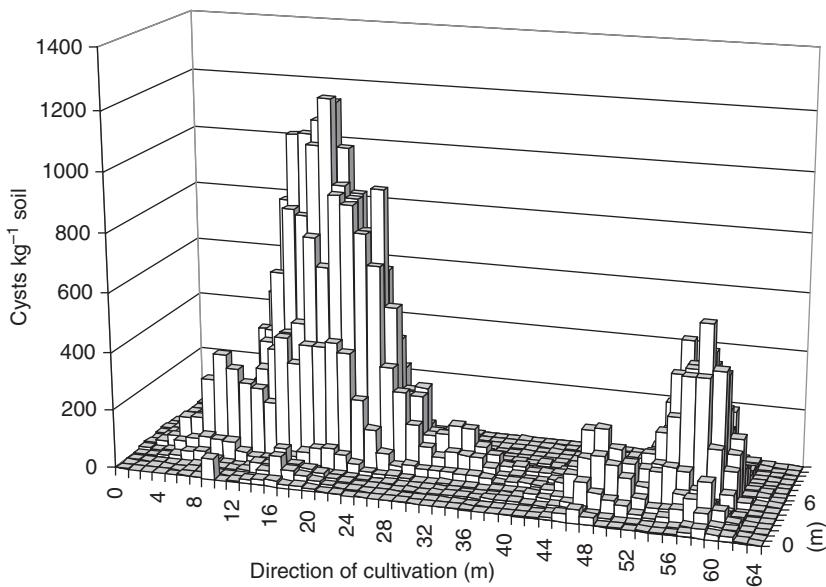


Fig. 11.6. Infestation of *Globodera pallida* on marine clay soil in The Netherlands. Each bar represents 1 m² with number of cysts per 1 kg of soil. Primary infestation is visible to the left and two secondary infestations to the right, located in the direction of cultivation.

result of natural decline in the absence of the host. When the infestation focus is subject to intensive crop rotation with a host, population densities in the centre will reach a maximum (carrying capacity of host/crop rotation) over an increasing area. The method of calculating the detection probability (presence of one or more nematodes or cysts in a soil sample) if an infestation focus is present in the field is discussed in Box 11.5.

Although almost all nematodes newly introduced in a field will produce hotspots, there are considerable differences between nematode species in size, extent and rate of spread of the hotspot. Hotspot development of the stem nematode *Ditylenchus dipsaci* is caused by conditions that affect the efficiency of the nematodes in finding and penetrating plants. In patchy infestations of stem nematodes these conditions for attack appear to be more favourable in the centre of the patch than towards the borders, resulting in a decrease of nematode numbers with increase of the distance from the centre. This results in persistence of the patchiness; the hotspots do not grow.

The dimensions of hotspots differ between nematode species. Whilst potato cyst nematodes, which are mainly distributed by mechanical redistribution of the cysts, cause the emergence of hotspots with a distinct and precisely defined shape of comparatively small dimensions, species like *Meloidogyne chitwoodi*, *M. fallax* and *P. penetrans* seem to cause extremely large infestations in a short time. As a result, a potato cyst nematode hotspot can be found in fields that are otherwise free of the nematode, whilst when a *M. chitwoodi* hotspot is discovered, almost always a huge area and sometimes the complete field is infested.

Box 11.5. Detection of potato cyst nematodes in The Netherlands.

The following scheme was used to develop sampling methods for the detection of potato cyst nematodes with a predefined probability. Fields that, as a result of statutory soil sampling, were found to be infested with the potato cyst nematodes were provided by the Dutch Plant Protection Service. The statutory soil sampling method covers an area of 0.33 ha. First, the infestation was located within that area by collecting 1.5 kg soil samples from 1 m² plots using the scientific sampling method for density estimation. The plots were situated over the field in a grid pattern. Each 1 m² plot (1.33 by 0.75 cm) was the centre point of, and representing, a larger plot of 8 m by 3 m, with the longest distance in the direction of cultivation in which the greatest dispersion is to be expected. This sampling scheme provided a coarse map covering the infested area. After location of the infestation, the infested area was sampled again, now by sampling each square metre. Again 1.5 kg of soil was collected and the number of cysts determined; the result of such an effort is displayed in Fig. 11.6. According to this scheme several fields per growing area were mapped and finally more than 40 foci were available for analysis.

All foci had approximately the same shape, with population densities increasing exponentially towards the centre of the focus, the density of which is designated as the central population density. By calculating log densities, multiple regression analysis or generalized linear model analysis (McCullagh and Nelder, 1992) can be applied. The gradient parameters can now be calculated for all four directions with the central population density as starting point. A comprehensive mathematical model describing the shape and size of a focus, depending on the central population density, could be established (Schomaker and Been, 1999b).

However, for the development of detection methods a simplified model, which is symmetrical along both axes, was used with only two parameters (Eqn 11.20). Now, using the negative binomial distribution for the small-scale distribution, the possibility of detecting no cysts at all at a certain location within the focus, where a core sample of certain size will be collected, can be calculated. By calculating 1 minus the probability of finding no cysts in every core sample collected from within the focus, when a sampling grid of certain dimensions is superimposed on the infestation, the probability of detection can be computed. An evaluation of existing sampling methods using a specially designed software program called SAMPLE (Been and Schomaker, 2000) was carried out.

$$E[p(x, y)] = p(0,0) \times l^x \times w^y \quad (11.20)$$

where: $p(0,0)$, population density in the centre of the focus; l , length gradient (direction of cultivation): average value = 0.83; w , width gradient (at right angles to l): average value = 0.64; x and y coordinates within focus.

Using the simple model, a standard focus was postulated, which should be detected with 90% reliability. The central population density was set to 50 cysts kg⁻¹ of soil for the following reasons:

- No growth reduction should occur when a potato crop was grown in order to obtain the maximum sanitary effect of a resistant cultivar; 50 cysts kg⁻¹, with an average cyst content of 250 eggs cyst⁻¹, would result in population densities of about 2 eggs g⁻¹ soil in a 1:3 rotation before the next host, close to the damage threshold.
- No visual damage should be noticeable in the potato crop at the time when visual inspections would be made.
- The degree of reliability was chosen as high as economically possible (taking into account the cost of sampling) to enable detection at one or two crop cycles before the statutory soil sampling method of the government would detect the infestation. In that

Continued

Box 11.5. Continued.

way, if potato cyst nematodes were present, the farmer would have ample time to implement control measures to prevent detection by the government in the future.

The statutory sampling method (EPPO), used before 2010, only detected the standard focus with an average detection probability of 12%. Several new methods have been developed (Been and Schomaker, 2000) that could detect this small infestation with 90% probability. Figure 11.7 visually displays the different foci that can be detected with 90% probability by the old statutory EPPO sampling method (600 ml ha^{-1}), which was applied until 2010, the new statutory sampling method of 1500 ml ha^{-1} , applied since 2010 (Seehofer, 2007), and the AMI:50 sampling method, targeted at seed potato production in a 1:3 cropping frequency. Foci detected by the AMI method have a 60 times smaller central population density and an 80 times lower number of cysts in the tilth of the infested area, than those detected by the old EPPO method. In fact, foci with a central population density of $3000 \text{ cysts kg}^{-1}$ soil have not been found; the maximum found is $1500 \text{ cysts kg}^{-1}$ in seed potatoes. At these densities all potatoes would suffer heavy damage and sampling would be unnecessary. The development and introduction in 2010 of the new 1500 ml ha^{-1} sampling method was a result of the research presented in this chapter.

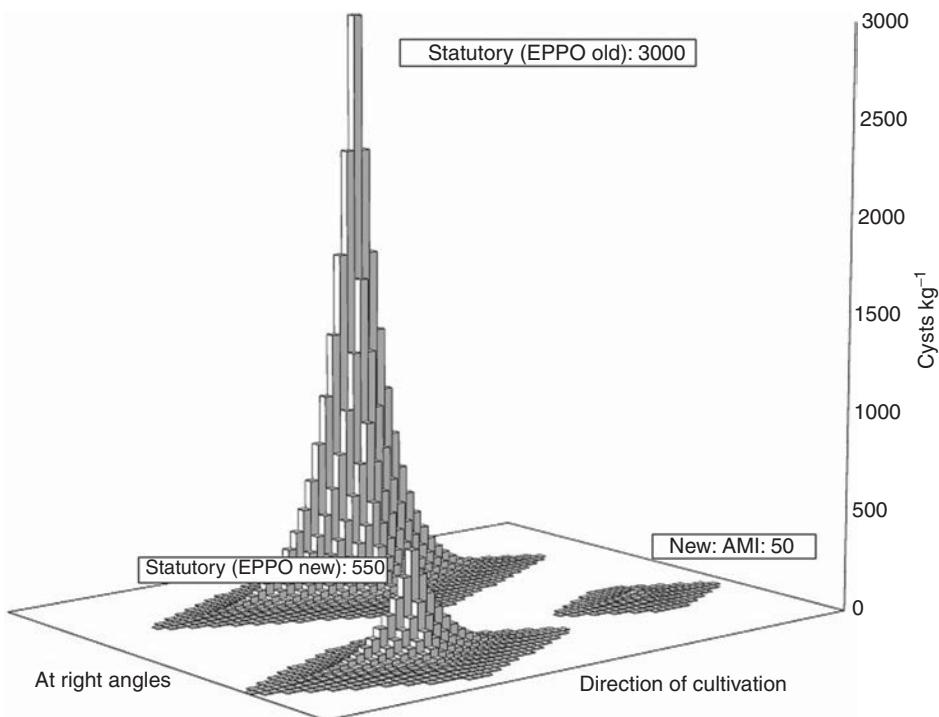


Fig. 11.7. Comparison of foci size detected with 90% average detection probability by the old statutory, the new statutory and the AMI:50 sampling method; 3000, 550 and 50 cysts kg⁻¹ of soil in the centre of the infestation, respectively.

11.3.4. Large-scale distribution

Once the first hotspot is established, passive redistribution by machinery will not only cause the focus to grow, but will also, by way of clods of infested soil adhering to the machinery, result in secondary infestations when these clods are deposited further along the row. The secondary infestations will be found primarily in the direction of cultivation and, less frequently, at right angles to that direction. As long as susceptible hosts are grown, these infestations will grow and finally merge and result in completely infested fields, with maximum population densities defined by the carrying capacity of the host (see Chapter 10). The intermediate distribution pattern can be regarded as the combination of several medium scale patterns. Figure 11.6 presents this development stage for *G. pallida*. Been and Schomaker (1998) were unable to correlate the size of the primary focus, which can be regarded as being age dependent, with the number of secondary foci in the field. Several researchers used (infrared) aerial photography or crop scanning to visualize these agglomerates of foci in farmers' fields. Figure 11.8 displays a conglomerate pattern of the sting nematode, *Belonolaimus longicaudatus*, on a peanut farm.

Figure 11.9 presents the final stage of spread within a field of *G. pallida* after the continuous growth of susceptible potato varieties in a 1:2 cropping rotation. No pattern can be distinguished. However, when investigated more closely by sampling every square metre of each 5 × 3 m block, nematode densities in consecutive square metres are not independent but are closely related to each other. This relationship can be used to describe the variability of population densities in these blocks and, if generalized,



Fig. 11.8. Aerial photograph showing a conglomerate of infestation foci of the sting nematode, *Belonolaimus longicaudatus*, on a peanut farm merging into a full field infestation. (Courtesy J.D. Eisenback, NemaPix 2, 1999.)

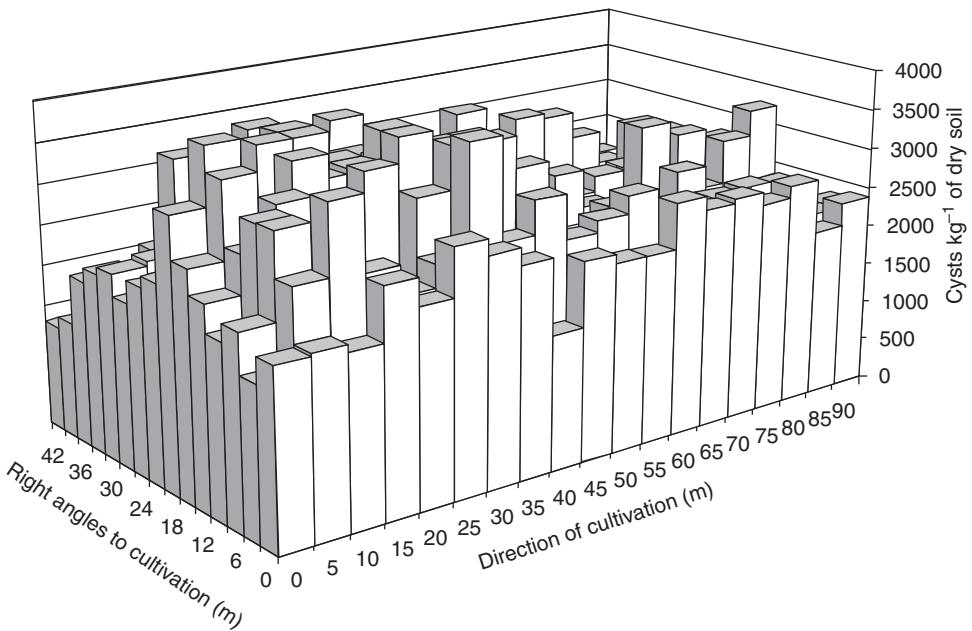


Fig. 11.9. Full field infestation (large-scale distribution at its climax) of *Globodera pallida* in the starch region of The Netherlands. Samples of 1.5 kg were collected from plots of 1 m² (1.33 × 0.75). Plots were spaced every 5 m in the direction of cultivation and every 3 m at right angles to that. The whole sample was processed.

can be used to predict population densities between the grid points of other blocks. When a cross-section of mapped fields is available, sampling methods for full field infestations can be designed, provided this cross-section represents the majority of actual full field infestations. The developed sampling method will then also apply to the majority of unknown (not mapped) infestations. Using this approach, sampling methods for full field infestations of the potato cyst nematode could be developed with a cv of 15% in The Netherlands. This uncertainty is acceptable to provide farmers with predictions about possible yield losses and population development in time.

11.3.5. Macrodistribution

The dispersion of a nematode species does not stop at the borders of a farmer's field. Any primary infestation in a growing area will ultimately spread to its neighbouring fields, which in turn will contribute to an accelerated spread throughout the area. Figure 11.10 illustrates the result of such a progression of the soybean cyst nematode, *Heterodera glycines*, in the USA. Some of the major factors involved in macro distribution of nematodes are given in Box 11.6. Obviously, hygiene is of utmost importance to prevent the spread of species of plant-parasitic nematodes. However, in the majority of cases it will only be possible to slow down the spread; once a nematode population

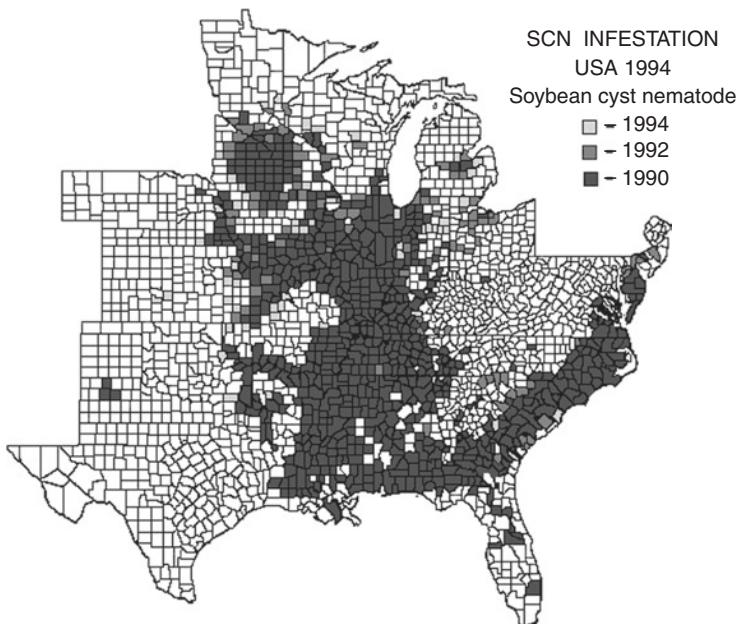


Fig. 11.10. Distribution of soybean cyst nematode, *Heterodera glycines*, in the USA provided by R.A. Riggs of the University of Arkansas. (Courtesy Eisenback and Zunke, NemaPix 1, 2000.)

is present in an area, its gradual expansion throughout that area is inevitable even when strict phytosanitary precautions are taken. The most effective strategy up to now has been the employment of the whole range of possible control and phytosanitary measures to maintain the pest at low population densities at which only small yield losses will occur, or to prevent the unlimited and excessive presence of quarantine pests in seed and export lots.

11.3.6. Other sampling approaches

There is a wide variety of approaches in the development of sampling methods for agriculture that have to yield population density estimates suitable for advisory services; some of those most commonly encountered in the literature will be summarized briefly here.

11.3.6.1. Mapping fields using core samples

One of the commonly used approaches is the mapping of a farmer's field by collecting single cores using a grid of predetermined dimensions and counting the nematodes in each of these cores. These data are then used to calculate sample statistics (mean, standard deviation, variation and cv) or are fitted to different distribution functions.

The first error of this approach is that a single core will not give us a reliable local density estimate when the underlying spatial density distribution is not random – as we now know it never is. Therefore, a single core will only tell us something about the number of nematodes found in the volume of soil collected with that core. It gives us no information about the population densities outside the core.

Box 11.6. Factors involved in macrodistribution.

1. Dispersion vectors

Propagation material. Nematodes can be retrieved from almost all material used for propagation. *Ditylenchus dipsaci* can be retrieved from seeds of different hosts, *Meloidogyne* spp. and *Globodera* spp. cysts can be found in and on potato tubers, *Pratylenchus* spp. and trichodorids (including tobacco rattle virus) in bulbs, and *P. penetrans* from rootstock of roses. To ensure that propagation material is free from nematodes, several methods, e.g. hot water treatment for bulbs, shallots, etc., are available (see Chapter 13). Legislation and statutory control measures are employed to prevent their presence in export lots (see Chapter 12).

Machinery. Clods of infested soil adhering to machinery will not only cause secondary foci in the same field but also initiate primary foci in previously non-infested fields tilled by the contaminated machinery. Soil fumigation equipment used to fumigate infested soils has been the primary vector dispersing potato cyst nematodes in The Netherlands.

Soil. Adhering soil, collected when produce is amassed for transport or packed for shipping, can import infections when it is used to fill up low spots in a farmer's fields or to change the soil structure.

Wind. Wind can cause the spread of nematodes, especially cyst nematodes. For example, when part of the surface of the topsoil is frozen, winter storms lift up both soil and cysts, causing 'sandstorms' that cover large areas and result in the spread from one field to many others over large distances. This not only causes new infestations, but also enhances the dispersion of more virulent populations, emerging after the use of resistant hosts. To prevent sandstorms, green manure crops have been used successfully to contain the topsoil after harvest of the host. However, the green manure crop has to be carefully selected as almost all of these crops enhance the survival and even, being hosts, the multiplication of other plant-parasitic nematodes.

Water. Rain transports soil and plant-parasitic nematodes to trenches, which will spread the nematodes throughout an area. Using the water from these trenches for irrigation will further spread these nematodes. Drainage systems will finally deliver the nematodes to rivulets, streams and rivers.

2. Habitat suitability factors

Once a nematode species is established, variations in habitat suitability factors, such as soil type and texture, selection pressure from host distributions, cropping history, temperature and moisture will determine the extent of its spread over regions, countries and parts of continents. For example, both *G. rostochiensis* and *G. pallida* can be found in all temperate climatic regions and soil types where potatoes are cropped but these species are of minor importance in Mediterranean countries as a result of unfavourable high soil temperatures. Soil type can be a barrier to nematode species; trichodorids or *Meloidogyne* spp. are not found in heavy clay soils.

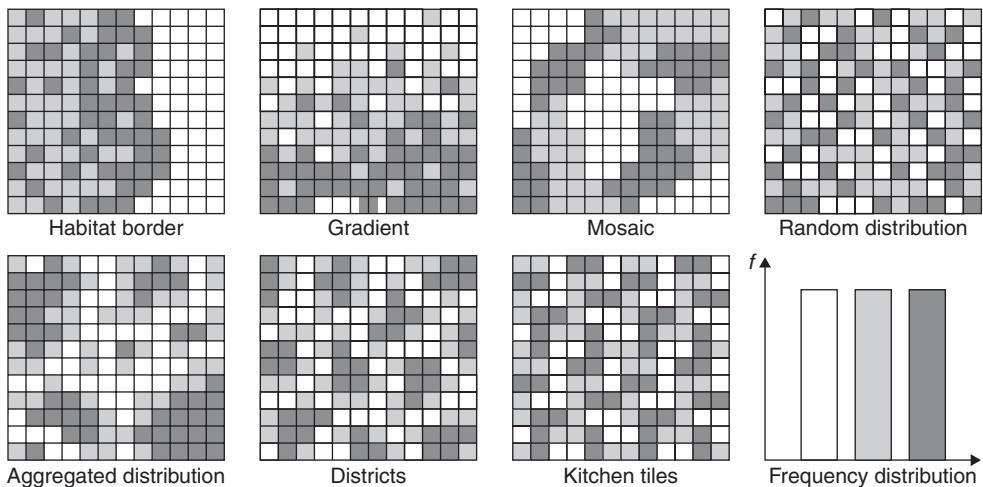


Fig. 11.11. Different spatial distribution patterns having the same frequency distribution (f). (After Ekschmitt, 1993.)

Fitting spatial sampling data to frequency distributions that are used as an offset for further calculations implies a reduction of information because all spatial information is omitted. In Fig. 11.11 a series of well-known spatial distribution patterns is displayed ranging from distinct patterns such as habitat borders, gradients and shapes, to random and aggregated patterns (Ekschmitt, 1993). The frequency distribution of the actual densities is uniform; each has the same probabilities. Sampling of these distributions in the field would result in bulk samples with nematode densities all having the same frequency distribution. For the development of sampling methods, especially for whole fields, this reduction of information is unfavourable.

11.3.6.2. *Repeated collection of bulk samples from a field*

Another approach for investigating the reliability of a population density estimate of a field by applying a particular sampling method is the repeated application of that method on the same field and calculating basic statistics. The mean, standard deviation and cv of the population densities of repeated bulk samples are calculated and used to evaluate the current sampling scheme.

This approach has some serious drawbacks. It will yield some information to optimize the current sampling method in order to obtain the desired accuracy of the density estimation. However, the resulting optimized method will only be reliable for the investigated field at that moment in time. It will not apply in the next year when population densities of the target nematode have changed or to another field in the same year where population densities are different from the start. It does not provide any insight into the underlying spatial distribution and, therefore, no information is available to calculate the effect on the reliability of any change to the original method, e.g. a different sampling size or a different sampling pattern or grid.

11.3.6.3. Sampling pattern/sampling grid

When a soil sample is collected from a field, usually separate core samples are collected from different locations within the field and combined into a bulk sample. The grid pattern used to collect this bulk sample has been an item for discussion and theorization in a number of investigations. Figure 11.12 shows some of the proposed and applied sampling patterns to traverse a square field, alongside which the separate cores have to be collected, starting with a diagonal pattern (A), a cross-diagonal pattern (B), a parallel-diagonal pattern (C), a W-shaped pattern (D), a zigzag pattern (E), a tilted square pattern (F), a perimeter pattern (G), an M-shaped pattern (H), a random pattern (I) and a rectangular grid pattern (J), as used in the case study detailed in Box 11.5. Of course, one wants to know the rationale behind these patterns. In general, the assumption was made that the distribution of nematodes throughout a field is random. A random pattern like pattern (I) then would be suitable but also inconvenient to execute as a standard procedure for statutory or advisory soil sampling. In fact, if randomness applies, it would not matter from which location in the field the separate cores samples are collected. However, as there were sufficient indications that the random distribution did not really apply and that

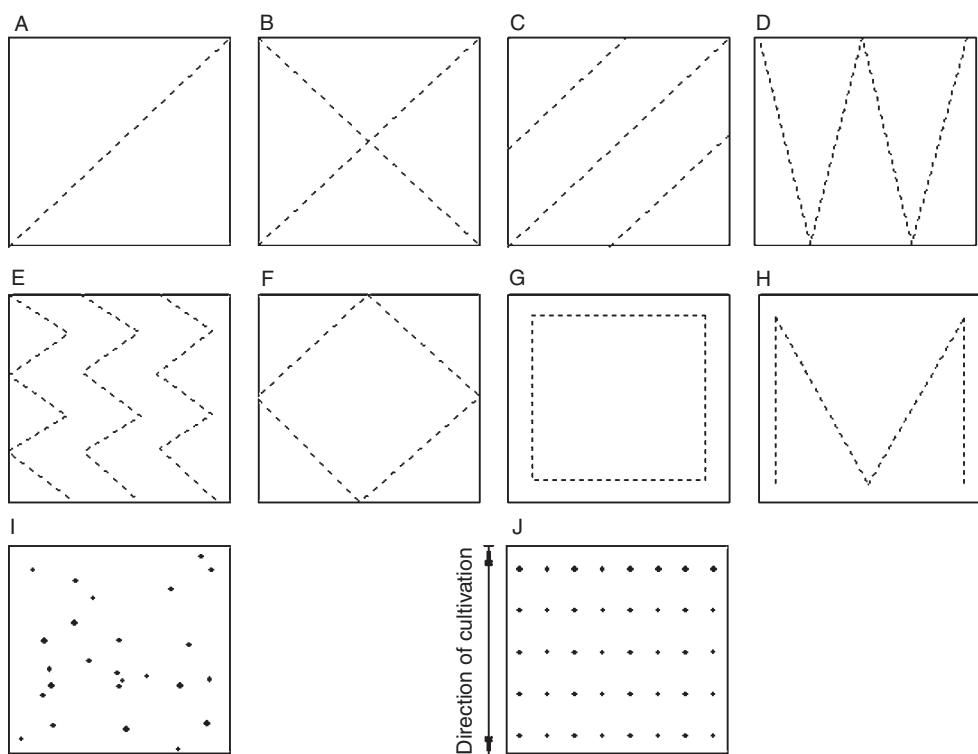


Fig. 11.12. Different patterns used to collect separate core samples from a field, which will form the bulk sample: A: diagonal pattern, B: cross-diagonal pattern, C: parallel-diagonal pattern, D: W-shaped pattern, E: zigzag pattern, F: tilted square pattern, G: perimeter pattern, H: M-shaped pattern, I: random pattern, J: rectangular grid pattern.

differences in population densities throughout the field occur, most sampling patterns try to incorporate some of these different population densities by collecting cores while traversing the field in some way. The question arises as to whether there is a way of doing this that accords with current knowledge. Let us consider the infestation foci of the potato cyst nematode; its shape is described by Eqn 11.20, and especially by the length and width gradient, 0.83 and 0.64, respectively, describing the rate of decline of population densities from the centre of the focus in both directions. An optimized grid would be adapted to the fact that the change in population densities in the direction of cultivation is smaller than the change at right angles to that direction, by taking more cores in the width and fewer in the length per unit distance (Been and Schomaker, 2000). The population density in the centre defines the size of the focus. The sampling grid should now be configured in such a way that at least one sample, but preferable more, will actually hit the infestation regardless of its location in the sampled field. This implies that the area of the field, divided by the area of the focus, will yield the minimum number of cores that have to be taken, each core representing an area as large as one infestation focus. This implies that all sampling patterns described in Fig. 11.12, except the zigzag pattern (E) and the stratified grid pattern (J), are disqualified. The use of stratified sampling using a rectangular grid adapted to the shape of the infestation as described by Eqn 11.20 and visualized schematically in Fig. 11.12 (J) presents the optimum solution. The zigzag pattern (E) can be considered as a small modification of the rectangular grid, with each second row of the grid shifted for half of the grid length, to facilitate sampling of two rows in one passage over the field. However, this approach yields only a marginal difference in walking distance and, in practical sampling schemes, is disregarded.

The defined sampling pattern, the rectangular grid, now yields one core sample hitting the infestation. This can be in the centre where densities are highest or in the border where densities decline to zero. Huge core sample sizes are required to obtain a high probability of detection when the latter occurs. Better results (smaller bulk sample; higher detection probability) will be obtained when smaller core samples are taken in a denser grid with the same length/width ratio. More core samples will actually hit the focus and the probability to hit higher densities in the central area will increase. As a result detection probabilities will increase.

The same reasoning applies when the whole field is infested. Although detection will be no problem, more, but smaller, cores will increase the reliability of the population density estimator (up to a certain level) as more areas of the field will contribute to the estimator.

11.4. Vertical Distribution

The possible extent of the vertical distribution of a plant-parasitic nematode species is important information in any sampling strategy. How deep has one to sample to make sure that the estimation of the population density over the sampled horizon will cover the actual population density the host will be exposed to during growth? Both scientists and extension workers need this information. Commercial sampling agencies, applying both statutory and advisory soil sampling methods for governments or for integrated pest management, respectively, want to know the necessary (in practice, the minimum) depth required to sample as the speed of collecting samples and,

therefore, the costs involved depend on the depth at which cores have to be taken. Further, one wants to know whether the vertical extension of a nematode population will be reached by control measures because layers that are not accessed will serve as a source of new inoculum.

A standard depth of 25 cm is currently used in the majority of countries when collecting single cores composing bulk samples for advisory purposes for cyst nematodes and the free-living stages of plant-parasitic nematodes. For trichodorids, occasionally samples are taken at a depth of 50 cm. A standard depth of 25 cm might suffice for sedentary nematodes like cyst nematodes but can be insufficient for other nematode species. Free-living stages of plant-parasitic nematodes are reported to adapt to microclimatic changes in the soil layer and move to more favourable locations in the soil column (see Chapter 8). The most extreme reported is the active movement of trichodorids to deeper soil levels to avoid the effect of granular nematicides applied to the soil surface. More common is the effect of drought causing most non-cyst nematodes to be less abundant in the topsoil layers and more numerous in deeper soil layers. Depending on the aim of sampling and the biology of the target nematode, different depths of sampling have to be chosen. Obviously, first of all, knowledge of the vertical distribution has to be acquired.

The literature available on the vertical distribution is limited. Extensive research to determine the adequate depth for soil sampling procedures has been carried out for potato cyst nematodes in The Netherlands. Soil samples were taken up to a depth of 80 cm immediately after harvest and after the soil was substantially cultivated (e.g. winter ploughing) to reveal any changes in the vertical distribution. Although potato cyst nematodes were occasionally present at depths of 80 cm, and probably sometimes even deeper, cysts at this depth were only present in a minority of the 32 fields investigated. Generally, 90% of the cyst population was found in the upper 35 cm of soil. It was concluded that, immediately after harvest of the potato crop and before any cultivation, the vertical distribution of potato cyst nematodes in the upper 25 cm of soil was uniform and, therefore, sets no demands on the depths of sampling. Soil cultivation had no additional effect on the vertical distribution of cysts and had no consequences for the required depth of soil sampling. In Fig. 11.13, for five of the sampled fields the number of cysts per 500 g of dried soil from soil samples taken down to depths of 25 cm both in the ridges and between ridges are presented. Samples of the first 1 cm of the tilth were also taken and compared with the first 5 cm horizon but no accumulation of cysts after harvest could be detected in the upper centimetres. Whitehead (1977) investigated the vertical distribution of potato, beet and pea cyst nematodes in heavily infested soils (layers: 0–20, 20–40, 40–60 cm) and reported similar results for the number of eggs g⁻¹ soil. Cysts of *Heterodera goettingiana*, the pea cyst nematode, were rarely found below 20 cm.

Seinhorst (1988) monitored densities of *R. uniformis* and trichodorids per 3 cm layers down to a depth of 21 cm and showed that nematode numbers were significantly lower in the three upper layers compared to the others. This seems to be generally the case for all free-living stages of plant-parasitic nematodes. Pudasaini *et al.* (2006b) modelled the vertical distribution of *P. penetrans* under four different hosts throughout the year. The vertical distribution of the nematode was related to the presence of roots of the four different crops, black salsify, carrot, maize and potato, indicating that even for a single nematode species the vertical distribution can differ and is predominantly determined by the host (Table 11.3).

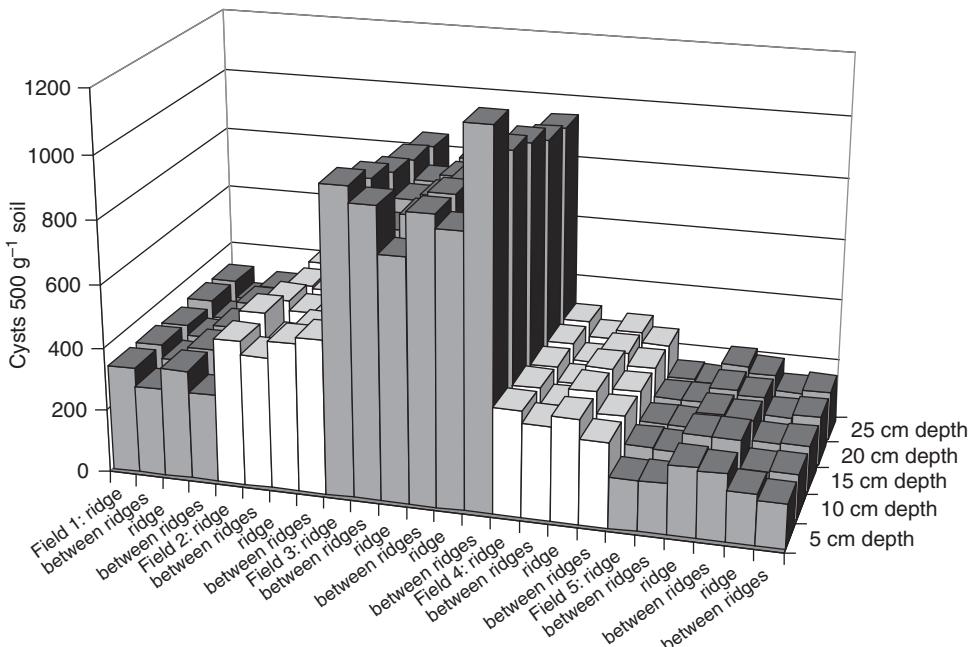


Fig. 11.13. The vertical distribution of *Globodera pallida* in five different fields at harvest and after soil cultivation (winter ploughing, harvesting) in five different layers down to 25 cm, in both the ridges and between ridges.

Table 11.3. The density of *Pratylenchus penetrans*, expressed as a percentage, in different soil layers in four crops. Values in bold indicate the presence of roots. (From Pudasaini *et al.*, 2006b.)

Crops	Percentage distribution of nematodes in different soil layers (in cm)						
	0–10	11–20	21–30	31–40	41–50	51–60	61–70
Maize	17	26	27	15	9	4	2
Black salsify	17	27	31	20	5	0	0
Carrot	27	37	27	7	1	0	0
Potato	23	34	27	14	2	0	0

Clearly, the root system of the host is the most important factor influencing the vertical distribution of plant-parasitic nematodes. In temperate zones, drought and temperature only play a role in the upper few centimetres of the tilth. In tropical zones these two factors will have more impact on the vertical distribution.

12 International Plant Health – Putting Legislation into Practice*

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12.1. Introduction and Terminology

For thousands of years, as people have migrated to new areas of the world they have taken plants and plant products with them. However, in doing so people have also inadvertently moved associated pests. In addition to many biotic and abiotic factors (animal migration, water flow, winds, etc.) nematodes have been particularly dependent on international trade and the migration of people between countries for their present distribution. The difficulty associated with detecting nematodes and the lack of information about their biology and the damage they cause has contributed to the increased risk of them being moved unnoticed in trade on associated hosts or in soil residues. In the last 20 years or so international trade patterns have changed markedly, with an increase in the volume, frequency and speed of trade in ornamental plants and root crops such as potato tubers. Such consignments inevitably increase the risk of plant-parasitic nematodes being introduced into new areas and affecting plant health.

As more has become known about their biology, plant-parasitic nematodes have increasingly been included in plant health legislation around the world and phytosanitary measures are being used to try to prevent their spread. Regulatory agencies implement phytosanitary measures to minimize the transport and spread of organisms harmful to plants, i.e. plant pests, by human activities. Plant pests comprise any species, strain or biotype of plant, animal or pathogenic agent injurious to plants and plant products, including, of course, plant-parasitic nematodes. The regulatory systems used are referred to by different terms in different countries, including ‘plant health’, ‘plant quarantine’ and ‘plant protection’ (Khan, 1989; Ebbels, 2003), and more recently ‘plant biosecurity’ in some countries such as New Zealand (<http://www.biosecurity.govt.nz>). In this chapter, we will use the term ‘plant health’ as it is widely used internationally. We will discuss international plant health with an emphasis on plant-parasitic nematodes and with reference to key nematode species.

A multitude of terms have been used in different countries to describe not only the field of ‘plant health’ but also the phytosanitary measures for the exclusion, eradication, containment and suppression of plant pests, as well as surveys, risk assessments and closely related topics. Here we refer to the terms and definitions from the Glossary of Phytosanitary Terms, one of the International Standards for Phytosanitary Measures (ISPMs) produced by the International Plant Protection Convention (IPPC) (see Section 12.3.1) as part of the global programme for harmonization of policy and technical assistance administered by the Food and Agriculture Organization (FAO) of the United Nations (<http://www.fao.org>) (a summary of abbreviations is given in Box 12.1). The Glossary is the ISPM No. 5 and is available from <http://www.ippc.int>. This and other standards adopted until 1997 by the FAO Conference and now by the Commission on Phytosanitary Measures (CPM), the governing body of the IPPC, are recognized by the World Trade Organization (WTO) as global references for the harmonization of phytosanitary measures under the Agreement on the Application of Sanitary and Phytosanitary Measures (known as the WTO-SPS Agreement).

Box 12.1. Abbreviations used in this chapter.

CPM	Commission on Phytosanitary Measures
EPPO	European and Mediterranean Plant Protection Organization
FAO	Food and Agriculture Organization
GATT	General Agreement on Tariffs and Trade
IPPC	International Plant Protection Convention
ISPM	International Standard for Phytosanitary Measures
NPPO	National Plant Protection Organization
PRA	Pest Risk Analysis
RNQP	Regulated Non-Quarantine Pest
RPPO	Regional Plant Protection Organization
WPM	Wood Packaging Material
WTO	World Trade Organization
WTO-SPS	World Trade Organization Agreement on the Application of Sanitary and Phytosanitary Measures

According to the IPPC, phytosanitary measures include any legislation, regulation or official procedure having the purpose of preventing the introduction and/or spread of plant pests and may normally only be applied to regulated pests, although the IPPC also provides for emergency measures to be taken against any pest that is a potential threat to a member country's territory. The term 'quarantine pest' is defined by the Glossary as 'a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled'. Organisms are categorized as quarantine pests based on these defining criteria, which are evaluated through pest risk analysis (PRA) (see Section 12.7). Examples of quarantine nematode pests for the European Union (EU) are the pine wood nematode, *Bursaphelenchus xylophilus*, and the Columbian root-knot nematode, *Meloidogyne chitwoodi*. However, these nematode species are not quarantine pests at the national level for the continental USA because both *B. xylophilus* and *M. chitwoodi* occur there and are not limited in distribution in the country.

Many nematodes that may be of phytosanitary importance are intercepted in international trade by plant health inspectors at points of entry. Often these are unknown species that have the potential to become significant pests if allowed to enter and establish. Nematode pests that were previously unknown or are not well known may be subject to emergency quarantine actions to avoid possible introduction and spread before risks are better understood. The general lack of accurate country or regional lists can make it difficult to ensure that a given nematode species, especially a new species, is not already native and therefore should not be subject to quarantine regulations. The recent recognition of the existence and significance of *M. minor* in Europe is such an example. National Plant Protection Organizations (NPPOs) are the official services established by governments to discharge the functions specified by the IPPC, and are almost universally provided with broad powers to take such actions in the light of new and unknown threats. However, emergency measures cannot continue indefinitely without evaluation to determine their appropriateness and technical justification. Measures should be modified as appropriate based on experience and the acquisition of new information. Cannon *et al.* (1999)

describe how the UK adopted a systematic protocol to determine appropriate measures for nematode pests intercepted in the UK on Chinese penjing (dwarfed trees); other examples include certain species of root-knot nematodes (*Meloidogyne* species) intercepted by member states of the EU on imported rooted cuttings.

Against this background, the ISPM Glossary defines another specific category of regulated pests known as regulated non-quarantine pests (RNQPs). They may be distinguished from quarantine pests as they may be present (even widespread) in the importing country, and their impact is generally known. Their presence in plants for planting would affect the intended use of those plants with an economically unacceptable impact and which is therefore regulated within the territory of the importing contracting party with the aim of suppression, rather than the eradication or containment strategy applied to quarantine pests. Regulatory restrictions for RNQPs can only be applied to specified plants for planting and do not consider the probability of establishment as a factor in evaluating risk. Actual RNQPs will differ from region to region, according to the relative importance of a particular pest, and whether it is native to the region or not. In Europe, *M. enterolobii* is an example of an RNQP. Sometimes a pest is a RNQP only until a pest risk analysis proves there is no case to answer. More information on the concept and application of measures for RNQPs can be found in ISPM 16 on the IPPC website.

12.2. Historical Considerations

Nematodes that are moved on plants or plant parts are said to be following a pathway. A pathway, as defined by the IPPC, is any means that allows the entry or spread of a pest. Soil adhering to agricultural vehicles is an example of a pathway for nematodes. Indeed, historical records suggest that *G. rostochiensis* may have arrived in the USA in this way and interception records held by The Food and Environment Research Agency (Fera) in the UK show that several nematode species, such as *Heterodera* and *Pratylenchus* species, can be spread by such means. Movement has also been facilitated by a lack of knowledge about the habits and pathogenicity of nematodes. This problem continues even to the present day.

The exchange of crops and their nematode pests between continents increased after the European discovery of the Americas. Typically, nematode species were first described from a country that was not their centre of origin. *Tylenchulus semipenetrans* was introduced and first recorded in California on citrus and subsequently described from there and Florida by Cobb (1913), but its origins were in the Far East, from where it also spread to the Mediterranean region. Here a new biotype was recorded, which was able to infest and reproduce on olive (*Olea europaea*), a native tree of agricultural importance for the Mediterranean region. The potato cyst nematodes (PCNs), *Globodera pallida* and *G. rostochiensis*, were first classified as *Heterodera* species; *Heterodera rostochiensis* was first described in Germany (Wollenweber, 1923), and *Heterodera pallida* was described in the UK (Stone, 1972). The genus name *Heterodera* was changed to *Globodera* for all round-shape cyst nematodes, including PCNs, by Behrens (1975).

Later, a comparative study of ribosomal DNA of native *Globodera* species on wild solanaceous hosts in Mexico and PCNs on cultivated potato supported a

thesis that Mexico was the centre of origin for *G. pallida* and *G. rostochiensis* (Ferris *et al.*, 1995). However, research to date suggests they have evolved to their greatest extent on native Solanaceae in the Andean regions of South America, where they were first recorded in 1952 (Wille and Bazán de Segura, 1952); from here they have subsequently spread to become economically important pests of cultivated potatoes (*Solanum tuberosum*) worldwide, illustrated by recent molecular research (Grenier *et al.*, 2010; Hockland *et al.*, 2012). Other major crop and nematode movements included sugar beet (*Beta vulgaris*) with the beet cyst nematode (*H. schachtii*) and soybean (*Glycine max*) with the soybean cyst nematode (*H. glycines*), which were introduced into North America from Europe and Asia, respectively.

The involvement of particular plant-parasitic nematode species with crop loss was often not recognized until many years after they had been described. A plant-parasitic nematode of wheat, *Anguina tritici*, was first discovered by Needham in 1743 (Needham, 1743; Box 5.2) but the role of nematode pests in suppressing plant yield was not realized until the mid-1800s. *Heterodera schachtii* was discovered in 1859, but it was not described and accepted as a causal agent of the crop decline until 1871. At this time, PCNs had not been distinguished from the beet cyst nematode, thus masking the importance of these species.

In the last 20 years the nature of trade in agricultural products has changed considerably. For example, in 1998, the major suppliers of agricultural commodities to the USA were, in order of importance, Canada, Caribbean Basin, Mexico, EU, South America, Australia and New Zealand, and Far East countries (Brown, 1999). By 2004, South-east Asia had considerably increased the export of agricultural products to the USA, thus increasing the risks of introducing harmful nematodes from one geographical region to another. In Florida, the volume of maritime cargo has increased from less than 2 million t in 1990 to almost 5 million t in 2000 (Klassen *et al.*, 2002); a comparable increase has been reported for air cargo (Dobbs and Brodel, 2004). In Europe, similar trends have been recorded and can be viewed on the Eurostat website (<http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home>).

All horticultural imports (bulbs, cut flowers and other ornamentals, such as hardy nursery stock, cuttings and other young plants) have the potential to spread nematode pests, including newly identified strains or races of recognized species such as the stem and bulb nematode (*Ditylenchus dipsaci*), leaf and bulb nematodes (*Aphelenchoides* spp.) and root-knot nematodes (*Meloidogyne* spp.). Trade in staple foods and seeds such as potatoes has increased into the UK, with imports of seed potatoes increasing from 15,000 t in 1993 to 34,000 t in 2003, with a consequent risk of importing soil containing plant-parasitic nematodes.

Despite the impact of an increasing volume of cargo around the world, there remain many serious pest species, or species that have the potential to become pests, that are not yet widespread in all areas where they could survive. In such cases, the prevention of further spread, control and eradication of such pests are major objectives for NPPOs around the world. Such objectives are closely supported by national and international schemes to produce healthy and vigorous planting material, and so plant health legislation has a place in the development of sustainable crop health systems by controlling the pests at their source. The challenges involved in putting such legislation into practice will be described here.

12.3. International Phytosanitary Frameworks

12.3.1. Development of legislative powers

The spread of plant pests in trade and their increasing association with crop losses in the mid-19th century stimulated international concern, leading to the first internationally agreed plant health measures in trade being taken against the American vine phylloxera, now known as *Viteus vitifoliae*, in 1878. This insect pest had spread rapidly throughout the vineyards of Europe, causing losses in France alone of approximately the equivalent of €72 million (US\$96 million). The ‘International Convention on Measures to be Taken against *Phylloxera vastatrix*’ (the former name of the grape phylloxera *Dactulosphaira vitifoliae*) embodied many of the principles recognized today in international plant health. The most important of these, as listed by Ebbels (2003), are given in Box 12.2.

Use of this international convention against the American vine phylloxera highlighted various deficiencies, especially in relation to the lack of a clear definition of concepts and terms. Today, the CPM agrees to promote a common understanding of key phytosanitary concepts and terms, and to encourage the harmonization of phytosanitary measures under the WTO-SPS Agreement. This Agreement, one of the outcomes of the Uruguay Round of negotiations of the General Agreement on Tariffs and Trade (GATT) from 1986 to 1994, entered into force for most countries in 1995. It states that the purpose of an SPS measure should be limited to the protection of human, animal or plant life or health; it applies to all sanitary (food safety for humans; animal life and health) and phytosanitary (plant life and health) measures that can affect international trade. At the time the WTO-SPS Agreement was negotiated, countries were concerned that reduced tariffs and subsidies could lead to the use of non-tariff barriers to trade, including SPS measures, in order to protect domestic production. Thus, the WTO-SPS Agreement resulted out of a need to have rules in place to prevent, or at least reduce, the unjustifiable use of measures to block trade in agricultural commodities between countries. More details on this Agreement can be found on the website <http://www.wto.org>. Other free trade agreements exist, such as the North American Free Trade Agreement (NAFTA), which came into force in 1994 and is an agreement signed by the governments of Canada, Mexico and the USA, creating a trilateral trade bloc in North America.

Box 12.2. Main principles of international plant health.

1. The responsibility to give an official written assurance on the pest-free provenance of host material being traded internationally.
2. The prohibition of international trade in certain kinds of material that might spread the pest.
3. The designation of official bureaux responsible for administering such trade.
4. Powers to inspect traded material and to take remedial action on items not complying with the requirements of international phytosanitary measures.
5. The prompt exchange of relevant information, particularly on new outbreaks.
6. That all these measures were to be embodied in national law by the participating countries.

Whilst the WTO-SPS Agreement recognizes the IPPC as the standard-setting body for developing international standards, guidelines and recommendations in respect of phytosanitary measures affecting trade, the IPPC has a longer history. Earlier efforts to expand international cooperation in the management of plant pest threats resulted in the creation of the IPPC in 1951. This multilateral treaty superseded all previous agreements and quickly became the premier international instrument upon which most national systems were based. The IPPC was revised in 1979 and again in 1997 and is now an international agreement on plant health with 177 member countries. It aims to protect cultivated and wild plants by preventing the introduction and spread of pests. The Secretariat of the IPPC is provided by the Food and Agriculture Organization of the United Nations, and the work programme is administered through the IPPC Secretariat and the CPM in cooperation with NPPOs and Regional Plant Protection Organizations (RPPOs). The last revision was a substantial one, setting out the phytosanitary concepts to be applied by contracting parties to be consistent with SPS obligations and also expanding and clarifying fundamental guiding principles associated with limiting the spread and impact of pests.

In line with the principles of the IPPC, national governments around the world develop their own plant health and quarantine regulations, which are usually enforced by an integral NPPO. A list of all NPPOs is provided by the IPPC (<http://www.ippc.int>). State or provincial governments within a country may also establish plant health agencies that function within these political subdivisions and often work in cooperation with the NPPO; usually, however, the national regulations for protecting the country from exotic pests take precedence over those at the state or provincial level (Khan, 1989). Additionally, for some countries, legislation passed by intergovernmental organizations is binding for member countries; examples of such organizations are the EU (Khan, 1989; Ebbels, 2003) and the Junta del Acuerdo de Cartagena (JUNAC) in South America (Khan, 1989).

Information on the latest EU plant health legislation can be found on http://ec.europa.eu/food/plant/organisms/index_en.htm. This website also gives links to many other sites detailing work relating to plant health in Europe. Similar information for the US federal government can be found on <http://www.aphis.usda.gov>. In the USA there is federal legislation but each state may have its own legislation to deal with particular issues. A list of important websites relating to plant health is given in Box 12.3.

12.3.2. Putting legislation into practice

Regional and inter-regional cooperation and harmonization in all areas of plant health is encouraged under Article IX of the IPPC and is performed by recognized RPPOs, including the European and Mediterranean Plant Protection Organization (EPPO), established in 1951 as the first RPPO (<http://www.eppo.int>). Another example is the North American Plant Protection Organization (NAPPO), which represents the USA, Canada and Mexico. A directory of other RPPOs is available on the IPPC website (<http://www.ippc.int>).

RPPOs perform their role in various ways. They produce non-binding (i.e. without legal status) recommendations to member countries for harmonizing regulations among their members. Some hold working parties, panels and expert groups to

Box 12.3. International plant health websites. Legislation in plant health and related issues is constantly changing. Websites of relevant organizations are an important way of obtaining up-to-date information.

Website	Comments/website for:
http://www.aphis.usda.gov	US Department of Agriculture, Animal and Plant Health Inspection Service. Contains information about APHIS's role in safeguarding US agriculture from exotic animal and plant pests.
http://www.eppo.int	European and Mediterranean Plant Protection Organization. Regional site for EPPO standards and information for European National Plant Protection Organizations.
http://epp.eurostat.ec.europa.eu/portal/page/portal/eurostat/home	Home page of Eurostat, the source of EU statistics.
http://ec.europa.eu/food/plant/organisms/index_en.htm	EC legislation relating to plant health: Council Directive 2000/29/EC.
http://www.ippc.int	International Plant Protection Convention. Contains details of Regional Plant Protection Organizations and lists current ISPMs.
http://www.fao.org	Food and Agriculture Organization of the United Nations.
http://www.wto.org	World Trade Organization.

address ongoing concerns such as phytosanitary regulations, PRA and diagnostics. They may also deal with specific pests and measures of concern to the region or other regions. One of the major roles of RPPOs is to raise awareness and provide guidance on newly identified and emerging pests that might pose a threat to agriculture or the natural environment in their region. This may be done through the production of lists of pests considered to be of quarantine concern, as well as so-called 'alert lists' containing those organisms that may become important. Nematologists around the region may be consulted in the production of such lists. More recently, such decision making has been facilitated by the development of PRA, such as the listing and analysis for *B. xylophilus*, as discussed by McNamara and Smith (1993).

12.4. Early Legislation Enacted against Plant-parasitic Nematodes

Before 1900, quarantine actions initiated in several countries against exotic damaging organisms mainly concerned insects and fungi because damage by nematodes was not recognized. At that time, only sporadic information was available, such as for

the cyst-forming nematodes and root-knot nematodes on crops such as cucumber, cereals, sainfoin and coffee.

Phytosanitary legislation specifically addressing plant-parasitic nematodes was introduced after 1900 and initially consisted mainly of isolated quarantine actions implemented against specific nematodes by a few countries concerned with preventing the spread of these nematodes to areas in which they had not been found. In the USA, the first regulatory action against any exotic nematode plant pest was implemented in 1909 by the US Department of Agriculture against a root-knot nematode infesting flowering cherry trees imported from Japan. The destruction of these trees almost caused a diplomatic crisis because the nematode-infested cherry trees were a gift from the Japanese government to the wife of the US President. This action, however, prompted approval of the first Plant Quarantine Act by the US Congress in 1912.

One species of PCN, *G. rostochiensis* (also known as the golden nematode), was detected in the USA for the first time in 1942 in Long Island, New York. It probably arrived with contaminated military equipment returning from Europe after the First World War. A golden nematode official control programme was enacted by the state of New York in 1944 to contain the spread of the nematode, action subsequently followed by the Golden Nematode Act in 1948, passed by the US Congress to protect the country's potato industry.

The first European legislation against nematodes was probably the Beet Eelworm Order in 1943 and following years, which was promulgated in Britain to manage the spread and increase in population levels of *H. schachtii* by adopting specific cultural practices. The Potato Root Eelworm Order, promulgated in Northern Ireland in 1945, was intended to suppress population levels of PCNs by enforcing the implementation of crop rotations and the use of certified seed potatoes.

12.5. International Phytosanitary Initiatives against Plant-parasitic Nematodes

There is a core of nematode species that are targeted by legislation around the world. Of course, not all species of plant-parasitic nematodes appear on every country's list of regulated pests because they may be endemic in some countries or regions and exotic in others. A list of plant-parasitic nematodes most frequently regulated in international trade over three decades is given in Table 12.1. Plant-parasitic nematodes, with their endoparasitic, sedentary and migratory habits, are a major target of phytosanitary measures because they can be easily transported unseen via roots, soil and above-ground parts of plants. The pinewood nematode *B. xylophilus*, a major forestry pest in some parts of the world that has also been introduced into two EU countries, Portugal (Mota *et al.*, 1999) and Spain (Robertson *et al.*, 2011), can be either mycetophagous or ectoparasitic and is spread by infested timber and timber products or by beetle vectors on a local scale. As this species has spread, it has been regulated by an increasing number of countries.

Reasons for the spread of other species over the years are a matter of speculation. In some instances, the significance of spread by uncertified potato tubers, bare-rooted plants for planting and excessive soil residues has been recognized, leading to additional regulation. However, the new appearance of *Longidorus diadecturus* in 2011 data, included in Table 12.1, appears to be a result of the expanding membership of the

Table 12.1. Number of countries regulating species of plant-parasitic nematode in 1982, 2000 and 2011.¹

Nematode species	1982	2000	2011
<i>Globodera rostochiensis</i>	51	106	119
<i>Bursaphelenchus xylophilus</i>	—*	46	82
<i>Globodera pallida</i>	—*	55	80
<i>Ditylenchus dipsaci</i>	23	58	72
<i>Ditylenchus destructor</i>	12	53	65
<i>Radopholus similis</i> ²	11	55	58
<i>Heterodera glycines</i>	—*	52	55
<i>Aphelenchooides besseyi</i>	9†	70	54
<i>Meloidogyne chitwoodi</i>	—*	—*	52
<i>Nacobbus aberrans</i>	—*	38	52
<i>Xiphinema americanum</i>	—*	30	43
<i>Xiphinema californicum</i>	—*	—*	39
<i>Meloidogyne fallax</i>	—*	—*	39
<i>Longidorus diadecturus</i>	—*	—*	33
<i>Pratylenchus penetrans</i>	—*	—*	25
<i>Aphelenchooides fragariae</i>	13	47	21
<i>Anguina tritici</i>	—*	24	21
<i>Bursaphelenchus cocophilus</i>	—*	21	13
<i>Heterodera schachtii</i>	16	22	14
<i>Xiphinema index</i>	—*	42	6†

¹Data for species regulated in more than ten countries as recorded by Kahn (1982), Lehman (2004), Hockland *et al.* (2006) and the APHIS/USDA Phytosanitary Certificate Issuance and Tracking System (PCIT) (2011). Countries belonging to the EU were regarded as individual countries.

²2011 data for *Radopholus similis* includes countries regulating *R. citrophilus*, which has been proposed as a species in its own right but is now variously accepted as a subspecies or race of *R. similis*. In 2011, 11 countries listed only *R. similis* in their regulations, two countries listed only *R. citrophilus* and 45 countries listed both.

* Insufficient or no records at this time.

†Species regulated by fewer than ten countries but included here to show changes between 1982 and 2011. Since 2004, *L. diadecturus*, *M. chitwoodi* and *M. fallax* were regulated by more than ten countries. In 2011, the combination of *B. cocophilus* and its weevil vector, *Rhynchophorus palmarum*, was regulated by more than 35 countries.

EU and other countries adopting the official EU list, rather than an increased risk in the spread or significance of this pest. At the time of the first EU list of quarantine nematodes, this species was thought to transmit viruses in its type country, USA, and was thus included. Recent data in Table 12.1 showing a significant decrease in importance of some species may be due to increased survey work showing the previously unknown distribution of some species, or establishment of some species in new areas. *Xiphinema index* remains an important regulated pest in California and Florida; *H. schachtii*, widespread in Europe, which contributes almost 50% to world sugar beet production, has been the subject of intensive, rigorous control measures that include new resistant cultivars, but it remains a significant regulated pest in South America and parts of Asia.

Nematode pests of potatoes are amongst the most highly regulated because they are readily spread in infested tubers or associated soil residues and because potatoes destined for consumption may instead be propagated. PCN species are in the top three nematodes listed in Table 12.1. Other than PCNs, *H. glycines* is the only

cyst-forming nematode commonly regulated by countries. It is of major concern for several countries in North America (Canada), South America (e.g. Argentina, Brazil, Chile, Uruguay) and African countries such as South Africa. This nematode is of less concern in the Far East where it is native. The false root-knot nematode, *Nacobbus aberrans*, another major potato pest, is included on the list of regulated pests for many countries including Brazil, Japan, Korea, Paraguay and the EU. This nematode has not yet received the same level of concern as PCN species, although it has the potential to infest a wider range of crops (Manzanilla-López *et al.*, 2002).

In general, root-knot nematodes are not regulated as a group because the major economically important species are already widely distributed. However, several species emerging from Africa and the Americas are now causing concern. Table 12.1 includes two root-knot nematodes for the first time in this edition. *Meloidogyne chitwoodi* is included primarily because it is a serious pest of potato and other economically important crops such as carrot. It is on the lists of prohibited pests of many countries (Canada, the EU, Mexico, and other countries in Latin America and the Far East). *Meloidogyne fallax*, another pest of potato, is also now recognized as an important pest by many countries. Other root-knot nematodes are being included on ‘alert’ lists worldwide: *M. citri*, a pest of citrus in China which was thought to have a restricted host range but has now been shown to infest and reproduce on hosts such as tomato (Vovlas and Inserra, 2000); *M. ethiopica*, a very damaging pest of kiwi and grape in Brazil and Chile, respectively, recently introduced into Europe (Širca *et al.*, 2004); *M. enterolobii* (= *M. mayaguensis*), which has been shown to overcome root-knot nematode resistant genes in some economically important crops (Brito *et al.*, 2007; Kiewnick *et al.*, 2009) and has been added to the EPPO Alert List A2 as recommended for regulation as a quarantine pest.

Because endoparasitic nematodes are found within plant parts, they can easily be moved in infested plant material in trade. Migratory endoparasitic nematodes, such as the burrowing nematode, *Radopholus similis*, are common in lists of quarantine pests. The occurrence of a *R. similis* race able to infest and damage citrus in Florida and the lack of reliable and rapid morphological and molecular analyses to identify this race has prompted a worldwide ban against this nematode, especially in citrus-growing countries. More than 50 countries regulate this nematode, which is considered the most damaging nematode pest of citrus. The risk from introducing other damaging citrus nematode pests is believed to be sufficiently reduced by measures in place for *R. similis*, such that further restrictions are not deemed necessary by many countries. Specific restrictions against other endoparasitic migratory root feeders are imposed by Argentina, Brazil, Chile, Egypt, Mexico, Morocco, South Africa, Syria and Uruguay but the risk associated with these nematodes is considered less serious than that posed by *R. similis*. Listed root-lesion nematode pests include mainly tropical and subtropical species such as *Pratylenchus coffeae*, *P. loosi*, *P. scribneri* and *P. zeae* and the temperate species *P. penetrans*; the latter is a new entrant in Table 12.1 for 2011.

Migratory endoparasitic species of foliar parts of plants are regulated by many countries; species belonging to the genera *Aphelenchoides* and *Ditylenchus* are most common in quarantine lists. Their frequent inclusion in the majority of the lists is due to the fact that these nematodes are easily transported inside plant tissues. These nematodes may cause symptoms that can be detected by visual inspection by well-trained inspectors, but at low levels such symptoms may not be apparent (Kohl *et al.*,

2010). Often the appearance of plants may also suggest nutritional disorders and so laboratory confirmation is necessary. The insect-vectored species such as *B. xylophilus* and *B. cocophilus*, which also live inside foliar parts of plants, are also of major concern for many countries.

Ectoparasitic nematodes are found in the soil surrounding plant roots, rather than within plant material, so restrictions on the amount of soil associated with plants and plant products will reduce the risk of their introduction (nil tolerance or a few derogations internationally), so that these nematodes are less likely to follow trade pathways and hence they are rarely included in regulated pest lists. However, ectoparasitic nematodes that vector nepoviruses (dagger and needle nematodes) are of major concern for European and a few South American countries (e.g. Brazil and Uruguay). Nematodes in the *Xiphinema americanum* *sensu lato* species complex are among the most commonly regulated ectoparasitic species, as the overall risk of introduction of virus vectors in this group is considered higher than that of many other damaging ectoparasitic nematodes such as lance (*Hoplolaimus* spp.) and sting nematodes (*Belonolaimus* spp.), even though the pathway of introduction of all these species poses the same potential risk. Further details of nematodes listed by countries around the world can be obtained from the EPPO Plant Quarantine Data Retrieval (PQR) System, details of which can be found on the EPPO website (<http://www.eppo.int>), or the IPPC website (<http://www.ippc.int>).

12.6. Phytosanitary Problems Posed by Plant-parasitic Nematodes

Plant-parasitic nematodes pose particular problems to phytosanitary authorities because they rarely produce obvious symptoms on plants or plant products that can be detected by routine visual inspection. Likewise, nematological analysis of plant material or soil is often not feasible at points of entry because such analyses are time-consuming and require specific equipment, expertise and quarantine conditions for efficient nematode extraction. Types of nematode that can be distinguished by symptoms they cause, such as the root-knot nematodes (*Meloidogyne* spp.), may not produce distinguishing symptoms at low levels of infestation. Thus, the most practical approach is to impose exclusion measures that prevent the importation of the substrate and any plant they inhabit (see Section 12.8).

12.7. Determining the Risk Posed by Plant-parasitic Nematodes Using Pest Risk Analysis

12.7.1. The development of formal procedures

Harmonization is a key principle of the WTO-SPS Agreement. This means that when NPPOs implement phytosanitary measures in trade to mitigate immediate or potential risk of pests to plant health within their jurisdictions, the measures must be based on available international standards (i.e. the ISPMs developed and adopted under the IPPC described earlier). If no international standard is available, or in cases where measures deviate from standards, measures need to be technically justified by

a scientific risk assessment as described under the WTO-SPS Agreement. Under the IPPC, a scientific risk assessment is referred to by the term ‘pest risk analysis’ (PRA). The IPPC definition of PRA includes evaluating biological or other scientific and economic evidence to determine whether a pest should be regulated (pest risk assessment) and the strength of any phytosanitary measures to be taken against it (pest risk management).

The IPPC has published a range of reference and concept standards and has begun developing pest and commodity-specific standards. Therefore, most current decisions on phytosanitary requirements, such as allowing or prohibiting the entry of a commodity or requiring a quarantine treatment of a commodity, must be justified by a PRA as described in ISPM No. 2 *Framework for pest risk analysis* and ISPM No. 11 *Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms*. These two standards provide significant guidance for the international harmonization of PRA. All current ISPMs are published on the IPPC website, the International Phytosanitary Portal (IPP) at <http://www.ippc.int>.

One of the crucial steps in a PRA is the determination of whether or not the pest or pests of concern meet the definition of a ‘quarantine pest’ because, except in rare cases where ‘regulated non-quarantine pests’ are involved, only quarantine pests can be regulated in international trade. Only quarantine pests need to undergo complete risk assessment (i.e. evaluation of the likelihood of pest entry, establishment and spread, and of the potential economic and environmental consequences should the pest become established) and risk management (i.e. the identification and evaluation of pest risk management options to reduce the risk of introduction and spread of the regulated pest, followed by the selection of the most appropriate management option(s)).

As discussed earlier, an organism can only be considered a quarantine pest for a particular country if it is not present in that country or of limited distribution and under official control. Official control of regulated pests must be directed towards eradication or containment within the country and not merely a reduction or suppression of the population, and the official control programme must be implemented nationally. In the USA, for example, if a pest is present in more than one state but a state quarantine programme is implemented by only one of those states, then this would not meet the definition of ‘official control’ for the purpose of declaring a pest a regulated pest at the national level. Therefore, the quarantine status of a pest can change over time, not only as populations become established or eliminated, but also as regulatory action is implemented at various levels.

PRA can be initiated for a variety of reasons, such as if a particular pest is intercepted at points of entry, a new pest risk is identified by scientific research, or a pathway other than a commodity import (e.g. natural spread, international mail, garbage) is identified. A PRA can also be initiated because a country makes a request to the NPPO of a second country for authorization to allow the importation of an agricultural commodity (e.g. fruits and vegetables for consumption or plant products for propagation) into that second country. The importation of an agricultural commodity represents a pathway that pests can potentially follow to enter and establish in a new country.

In the preparation of a PRA initiated by a commodity import request, only quarantine pests likely to be carried on the particular commodity require complete risk analysis (i.e. risk assessment followed by risk management). The pathway of most

fruits and vegetables for consumption is unlikely to carry plant-parasitic nematodes because these organisms usually only attack underground parts of plants. The exceptions to this include plant-parasitic nematodes that attack foliage (e.g. *Aphelenchoides* spp.) or endoparasitic nematodes that may be carried in edible roots and tubers such as beets and potatoes (e.g. *N. aberrans* and *M. chitwoodi*, respectively). There is little evidence that nematode populations can become established if the host commodities are consumed after import as intended. Disposal of such commodities in compost, cull piles or garbage would need to be followed by dispersal onto susceptible hosts or their growing media for the nematodes to become established in the importing country.

By contrast, without the implementation of specific phytosanitary measures, the pathway of plants for propagation, such as potato tubers for seed, bulbs and nursery stock, is much more likely to carry plant-parasitic nematodes (e.g. PCNs, *Ditylenchus dipsaci*) and result in the establishment of these nematodes in the importing country. Thus, compared to commodities for consumption, seeds and plants for planting pose a greater risk for the introduction and/or spread of nematode pests.

In addition to the type of plant commodity being imported, other factors typically used in assessing pest risk include, among others: (i) the biology of the pest (e.g. host range, feeding habits, life cycle, habitat, symptoms produced, overwintering/dormancy ability, dispersal ability, interaction with plant pathogens); (ii) the economic and environmental impacts of the pest in other parts of the world; and (iii) the host and environmental conditions in its geographical distribution, as well as those conditions in the importing country. The more knowledge of these factors one has, the more accurate the estimate of risk. Unfortunately, biological information is often lacking for many new pests intercepted in international trade, including most new nematodes. Furthermore, soil temperature data, so necessary for nematode assessments, is often non-existent and has to be extrapolated from air temperatures, a technique that has disadvantages, as discussed by Baker and Dickens (1993). Not only is soil temperature influenced by air temperature, but also by ground cover, soil texture, wetness, sun angle and day length. In addition, rainfall data often cannot be used because of the added complication of irrigation at monitoring sites.

During the final stage of the PRA, i.e. pest risk management, the estimated pest risk helps determine the most appropriate phytosanitary measures needed to reduce risks. There are several important WTO-SPS/IPPC principles that should be followed when deciding whether or not to allow importation of a commodity and, if so, what phytosanitary measures should be implemented to manage the risks associated with that commodity (many of these principles are embodied in the WTO-SPS Agreement).

The first of these principles is that of '**minimal impact**', also known as 'least restrictive measures', which states that measures must not restrict trade more than that required to achieve the appropriate level of protection for the importing country. In other words, the selected risk management measure(s) should be 'proportional to the risk identified in the pest risk assessment' (ISPM No. 2).

The principle of '**equivalence**' means that an importing country must recognize that different phytosanitary measures can potentially be used to achieve their appropriate level of protection. For example, if an irradiation or hot water treatment is equally effective in eradicating a particular plant-parasitic nematode pest on a particular commodity compared with a chemical management treatment, then exporting countries should be allowed to use one of the alternatives.

The WTO-SPS principle of ‘non-discrimination’ states that importing countries should not discriminate between countries that have the same phytosanitary status; that is, countries that have the same pests for a certain commodity should not be treated differently in terms of what phytosanitary measures are required. For example, all countries with *G. rostochiensis* should be required to meet the same phytosanitary requirements for commodities (that may carry the nematode) to enter a given country.

Finally, the principle of ‘transparency’ requires countries to provide information regarding their risk analysis procedures, including information justifying why certain phytosanitary measures were selected.

By following the key principles outlined above, and ensuring that decisions are based on scientific evidence through risk analysis, countries benefit from a more predictable, transparent, and fair trading system while at the same time enhancing their ability to protect their own agriculture and natural environments.

12.7.2. Practical problems

The risks of *B. xylophilus* and *M. chitwoodi* presented by different trade pathways have been assessed (Tiiakkala *et al.*, 1995; Braasch *et al.*, 1996; Evans *et al.*, 1996). Initially, the trade patterns involving *M. chitwoodi* concentrated on the most important economic host, potato, but subsequent research has highlighted other pathways involving other root vegetables, such as carrots, and ornamentals including flower bulbs. The knowledge that *M. chitwoodi* can be transported inside potato tubers forms the basis for most inspection procedures, but in the field it is often difficult to detect low populations which produce few symptoms on potato skins, and immature females, most often found in fresh tubers, are opaque and difficult to see during inspections, necessitating laboratory testing in suspect cases.

Many species, such as *M. chitwoodi*, are not host-specific and pose real problems for quarantine specialists developing phytosanitary control measures. Such measures work best where the species in question has a narrow host range, a slow rate of population increase and a rapid rate of decline under non-host crops. PCNs and *T. semipenetrans* are good examples of such species.

12.8. Phytosanitary Measures for Plant-parasitic Nematodes

As indicated in the previous section on PRA, selection of the most appropriate phytosanitary measures for managing plant-parasitic nematodes (or any pest) requires a detailed study of their biology and associated trade pathways, including the trade of crop hosts between countries and environmental factors favouring their establishment. Both direct and indirect measures are adopted to prevent the spread of nematodes and other organisms listed for exclusion (Box 12.4).

Disinfestation measures target nematodes in imported plants or plant products. For example, wood packing material (WPM) is a pathway for the pinewood nematode, *B. xylophilus*. ISPM No. 15 approves heat and methyl bromide treatments for WPM in international trade to control *B. xylophilus*. Usually, measures against the beetle vectors of this species are not practical. Nematodes are, however, posing

Box 12.4. Examples of typical phytosanitary measures used to prevent the spread of plant-parasitic nematodes include:

- a specific quarantine treatment of an import commodity, or
- prohibiting the entry of a host commodity likely to carry a particular nematode, or
- prohibiting the entry of untreated media, or
- that plants be imported with bare roots (i.e. free of media), or
- that plants should have been grown in approved growing media according to an officially agreed protocol, or
- that the commodities should originate from an area determined to be free of a particular nematode (i.e. a 'pest-free area').

increasing problems for phytosanitary inspectorates when enforcement of control is required. There are fewer chemical treatments that can be employed, which raises the importance of preventative or exclusion measures to reduce likely losses.

Exclusion measures include prohibiting the entry of a nematode's plant hosts and associated plant material that have a high risk of being contaminated, regardless of whether the nematode has been detected. However, the most important measure for nematode exclusion is the prohibition of the import of soil, growing media and packing materials that may harbour nematodes. Virtually every country bans the import of untreated soil because it may carry not only unknown populations of nematodes, but also pathogenic bacteria, actinomycetes, fungi, mites, certain life stages of insects and weeds. Consequently, countries generally require that plants be imported free of media, i.e. with bare roots. Unfortunately, even bare-rooted plant material is often contaminated with some soil particles; therefore there is a lowered, but still partial, pest risk.

The bare root requirement is also designed to allow easy inspection for galls or lesions on the roots. Any drying effects during transport may reduce adult nematode populations on plant surfaces but the extent to which this occurs cannot be relied on for quarantine purposes; indeed, nematologists in quarantine laboratories regularly find live nematodes in samples of soil residues from consignments. In particular, drying of roots does not kill or reduce the viability of nematode cysts, nematodes living in roots or eggs in gelatinous matrices from the root tissue. Many nematodes, such as *Pratylenchus* species, develop and reproduce inside the root tissues and are protected from any drying effects that may occur on root surfaces.

Requiring that plants be grown in approved growing media according to an official protocol is another form of exclusion measure. These growing media vary by country; furthermore, many countries require such media (e.g. peat, rock wool or perlite) to be sterile or previously unused, as nematodes are able to colonize artificial media if roots are present.

12.8.1. Certification and marketing schemes

These schemes are administrative systems for phytosanitary and quality control of commercially produced plant material, including plants for propagation and plant products for consumption. They can be applied to production of both true seed and

of vegetative propagation, and planting material normally propagated in this way are described in detail by Ebbels (2003). Whilst some schemes are more concerned with genetic purity and related aspects such as germination potential, most include some aspect of freedom from pests such as plant-parasitic nematodes (e.g. *D. dipsaci*).

Official pre-cropping and growing season inspections may be required and soil or suspect material may be sampled for laboratory analysis. Cost:benefit ratios have been shown to be very favourable for diseases (Ebbels, 1988) but, although similar assessments have not been done for plant-parasitic nematodes, such schemes are credited with maintaining high-quality nematode-free stocks in several crops such as narcissus and strawberry. If the pests are regulated in such schemes, with stated tolerances, perhaps for different qualities of plants for planting, they may be candidates for RNQP status, permitting the imposition of equivalent import measures on similar planting material.

12.8.2. Phytosanitary certificates

Countries wishing to export plants, plant products or other regulated articles that could present a risk to plant health have to satisfy the phytosanitary requirements of the importing country before it will accept such consignments. This is documented by providing a Phytosanitary Certificate, which states that the consignment meets the phytosanitary requirements of the importing country and may include additional declarations regarding specific pests or procedures. Inspections of the regulated articles can be carried out under the authority of inspectors; the actual issue of the Phytosanitary Certificate before movement must be by an inspector who is a government official.

12.9. Phytosanitary Measures and their Associated Cost: Benefits

In spite of the impact of multilateral trade agreements and phytosanitary regulations, interceptions of regulated nematodes do occur, such as on infested container-grown plants or in potato tubers. On such occasions the consignment may be destroyed, returned to origin, diverted to a different end use (such as consumption), or diverted to another country to prevent the spread of pests, as invariably no effective treatment or other control exists. A few cultural methods may be employed where the nematode species have been identified as low risk. For example, root washing may be an option in the case of ectoparasitic species, but this may damage the plants.

It is difficult to eradicate a nematode species of particular economic importance when an outbreak has occurred on a crop or range of crops. Suppression (i.e. the application of phytosanitary measures to reduce the pest population) or containment (i.e. the application of phytosanitary measures in and around an infested area to prevent spread of a pest) may be more feasible and such campaigns may be cost-effective. In particular they set an example to the agricultural and horticultural industries of the principles of pest control. On occasions, the benefits may be difficult to justify in monetary terms but there may be other environmental concerns or advantages to the trade in general. Cost:benefit analyses rely heavily on the availability and

quality of data but the problem with most nematode species is that little is known about their economic effects.

It remains in national and international interests to restrict the spread of harmful organisms and so minimize their impact on trade, industries and the environment. The following examples explain how certain nematode species are being suppressed and contained by three different phytosanitary programmes and the associated cost:benefits.

12.9.1. Phytosanitary suppression programmes for *Radopholus similis* in the USA

The programme to suppress and prevent further spread of the citrus race of *R. similis* in Florida is very important because of the phytosanitary legislation involving this nematode in many countries worldwide (see Section 12.5). In the early 1950s, a serious decline of citrus orchards appeared, primarily in central Florida. In 1953, it was discovered that *R. similis* was the causal agent of the problem, known as spreading decline. Nematode-infested propagative material from Florida nurseries infested with both *R. similis* and *T. semipenetrans* had been disseminated into Florida's new and old citrus-growing areas where these nematodes were not native.

The management strategies implemented in the affected areas included surveys to delimit these infested areas, tree removal followed by soil chemical treatment (nicknamed 'push and treat') and isolation of orchards with chemical-treated buffer zones (barriers) around the infested areas. Concomitantly with the 'push and treat' programme, a citrus nursery certification programme was established, which required nursery stock to be grown under rigorous sanitation programmes to ensure that commercial citrus seedlings were free of *R. similis* and *T. semipenetrans*.

The combination of the initial suppression programme (push and treat), the barrier programme to isolate the infested orchards and the citrus nursery certification resulted in a reduction of nematode-infested hectares from 3798 ha in the 1950s to 1538 ha in 1984. The number of citrus nurseries infested by *R. similis* declined drastically from 278 in the 1950s to none from 1970 until now. Recent studies indicate that *R. similis* would have spread at least to an additional 18,000 ha without implementation of the phytosanitary programmes. The benefit of preventing the spread of *R. similis* to such an area of land potentially susceptible to the spreading decline was estimated at about US\$1.4 billion (€1.05 billion) for a 35-year period. The cost of the suppression programmes was about US\$100 million (€75.2 million) or 7% of the benefit value (Lehman, 2004).

The eradication approach of 'push and treat' was discontinued in the 1980s because of the serious environmental consequences resulting from the excessive use of fumigant nematicides. The citrus nursery nematode programme, which was initiated 50 years ago, has continued and the benefits growers received in 2000 was estimated to be US\$17 million (€12.8 million) for *R. similis* and US\$33 million (€24.8 million) for *T. semipenetrans*. The cost to the citrus industry of implementing the citrus certification programme was US\$75,000 (€56,390) (Lehman, 2004).

The direct damage that *R. similis* causes to the citrus industry in Florida has had a rippling indirect and adverse effect on the ornamental industry of Florida; unfortunately, both the banana and citrus races of *R. similis* (see Chapter 5) are able to infest

and reproduce on many ornamentals in many different families including plants in the Araceae, Lauraceae, Marantaceae, Musaceae (which includes banana), Palmae and Strelitziceae, although the banana race does not attack citrus plants (Rutaceae). The absence of reliable morphological and molecular identification tools to distinguish between the races has resulted in a ban on all infected plant shipments from Florida by all the citrus-producing states in the USA and by many countries in order to protect their banana, citrus and ornamental interests. For the same reason this ban has also applied to other tropical states such as Hawaii and other countries (regardless of the race they have).

A financial disaster for the ornamental industry in Florida has been averted by the adoption of a nematode certification programme in the ornamental industry similar to that described earlier for citrus nurseries. Florida ornamental nurseries implement nematode eradication programmes using strict phytosanitary practices in order to export ornamentals to national and international markets. These programmes include the use of approved growing media such as sterile peat, clean sand, sawdust or wood shavings, or biologically inert fillers such as perlite or vermiculite. Clean propagative material is grown in these media and kept in clean containers not in contact with the ground, which may be inhabited by prohibited pests and pathogens. Other common sanitation requirements include clean irrigation water, weed control, appropriate sloping of the ground to avoid flooding of the nurseries, and construction of cement slabs or benches to protect the containers and growing media. Nurseries are periodically (usually annually) inspected and sampled by officers of the Florida Department of Agriculture and Consumer Services, and if prohibited nematodes are found the nursery is suspended from shipping plants until the production line is clean. Such phytosanitary programmes are expensive and are justified only by the high market values of ornamentals (more than US\$1 billion (€0.75 billion) in Florida).

12.9.2. Nematode phytosanitary exclusion programme for cotton and vegetables

As in some other states of the USA, plant health officials in California conduct inspections at the state's border stations to verify that agricultural commodities are free from its state-regulated pests, including nematode pests. Here the cost of excluding non-indigenous nematode pests has been estimated to be 3% of the potential crop loss value these nematodes would cause if accidentally introduced into the state. For the year 2000, this potential crop loss value was estimated to be US\$600 million (€450 million).

In particular, the benefits of excluding the reniform nematode, *R. reniformis*, from the California cotton industry (valued at US\$1 billion (€0.75 billion)) are estimated at US\$7.2 million (€5.4 million). These benefits were calculated by assuming similar crop losses (7%) to those that have occurred in other US states. Similar benefits are achieved for the California melon and vegetable industry, valued at US\$4 billion (€3 billion), because this pest causes 10% crop losses to cantaloupe and snap bean elsewhere. The annual cost of the certification programmes implemented in Florida to exclude California's state-regulated nematodes from California is US\$100,000 (€75,190), which represents only about 1.3% of the losses that the California cotton industry would expect annually if *R. reniformis* became established in their cotton fields.

12.9.3. Eradication programmes for *Globodera rostochiensis* and *G. pallida* in the USA

The first regulatory measures against *G. rostochiensis* in New York were implemented at state level in 1944. Stringent phytosanitary programmes were established by the Golden Nematode Act, promulgated in the USA in 1948; these have prevented the spread of *G. rostochiensis* within and outside the state of New York since that time. In 2012, APHIS removed some of the regulated areas in this state. The use of seed potatoes originating from states where *G. rostochiensis* has not been found has been critical in preventing the spread of this nematode in the USA. An outbreak of *G. pallida* occurred in 2006 in Idaho, where exclusionary measures were also enacted at a cost of US\$58.8 million (€44.2 million) from 2006 to 2012 (Skantar *et al.*, 2007; Anon, 2011; and data kindly provided by Jonathan M. Jones, National Program Manager, USDA-APHIS-PPQ, 2012). Considering that PCN species can suppress potato yields by more than 10%, the benefits of excluding these nematodes from potato-growing areas in the USA are estimated to be US\$300 million (€225 million) annually at 1995 values. These benefits are far greater than the US\$445,000 costs (€334,595) (1996 data) required for preventing the spread of *G. rostochiensis* in and outside New York (Dwinell and Lehman, 2004).

12.10. Future Challenges for the Control of Regulated Nematodes

The volume, frequency and speed of transport of plants and plant products around the world mean that it is impossible to inspect adequately every consignment that might contain nematodes. Therefore, one of the main challenges facing NPPOs is to target inspections so those consignments that pose the most risk to the agricultural industry in their countries are examined to ensure that commodities that cannot be adequately inspected are otherwise restricted. Accurate statistics help to provide the basis for good planning of phytosanitary services (Ebbels, 2003). Besides the volume and distribution of work, which will indicate the numbers of staff needed, statistics will provide information on the volume and fluctuation of various types of trade, the problems encountered and the association of problems with particular sources, areas or suppliers. This, in turn, helps to determine priorities for targeting inspections or monitoring and provides information for PRA.

The trend towards sustainable farming systems and the development of sophisticated integrated pest management programmes, combined with the loss of many chemical products used to control nematodes, means that another future challenge is to develop a better understanding of the biology of plant-parasitic nematodes so that as many cultural measures as possible can be used to suppress them at their source. Such measures might include, for example, increasing the interval between susceptible crops to reduce the rate of multiplication of pest species. One of the stipulations of seed potato growing regimes in Europe is specified intervals between cropping, so that the majority of PCN second-stage juveniles would have hatched by the time of planting of the next host crop. In addition, the growing desire to use plant waste for composting presents an additional risk unless appropriate measures are taken to kill any nematodes therein. Many phytosanitary programmes serve as model programmes

for crop health management in agriculture in general, but their implementation and success requires the support and cooperation of the agriculture industry.

12.11. Challenges Facing Scientific Advisers and Researchers

Scientific service support is essential for the plant health services in any country or region, and for a phytosanitary programme to work well there needs to be good cooperation between the regulatory agencies, crop consultants, and farmers and growers, as illustrated in this chapter by the success of programmes aimed at citrus and ornamentals in Florida.

The ever-decreasing skills in identification and diagnosis are in demand as an increasing number of national and international standards for plant health services are established, not only for the production of clean propagation material, inspection and sampling procedures, but also to provide the basis for PRA and eradication and containment programmes. At the same time there are increasing demands to formalize quality procedures in laboratories, leading to the production of identification protocols that provide guidance for international agreement. This section discusses some of the challenges in putting science into practice to comply with phytosanitary legislation.

12.11.1. Morphological identification and the role of collections

The identification of plant-parasitic nematodes, at the time of writing, is still largely dependent on recording morphological features and subsequent judgements by nematode identification specialists or taxonomists. Such judgements may, of course, differ at any one time, but are important in a sector where perhaps only one or a few specimens may be isolated from a sample and where an international consensus over the organism causing problems is vitally important. Original descriptions are important tools, as are authenticated reference slide collections. However, the international decline in taxonomic skills and the lack of resources for curation and conservation of collections have led to concerns over the whole basis of identification of regulated nematodes (Hockland, 2005). A recent example of the importance of such issues was the international controversy over the use of names *M. mayaguensis* and *M. enterolobii* for international phytosanitary measures. This has been resolved by morphometric and morphological comparisons by experts so that *M. mayaguensis* is now regarded as a junior synonym of *M. enterolobii* (Karssen *et al.*, 2012).

12.11.2. Molecular tools and their role in detection

An array of different technologies has been developed over the years to help specialists in morphological identification, including electrophoresis and PCR (see Chapter 2). They are especially important where morphological identification is particularly difficult or where only immature specimens have been intercepted. However, it is often not realized that the development of such techniques as reliable, routine methods for use as quarantine identification tools requires additional intensive research. Resulting

protocols are inevitably only developed for a restricted range of species, thus still necessitating a preliminary, provisional identification by a morphological specialist to detect an unusual finding.

Analytical methods examining the genetic make-up of organisms are being continually refined and adapted to develop new phylogenetic models that are becoming an integral part of nematode systematics (De Ley and Blaxter, 2002), and the associated technological equipment, though expensive, is becoming a familiar part of most diagnostic laboratories. Despite great advances in the use of molecular methods for the identification of diseases, especially viruses, the pace of development of reliable, accredited diagnostic protocols in nematology remains slow for species listed in legislation. Recently, EPPO has taken a leading role in both development and accreditation practices, and collaborated with the European Co-operation for Accreditation (EA) to achieve greater cooperation in raising standards (<http://www.eppo.int/EPPOStandards/diagnostics>). The emergence of a range of *Meloidogyne* species with the potential to cause economic damage (*M. ethiopica*, *M. floridensis*, *M. graminicola*, *M. enterolobii* and *M. minor*) has prompted research in biochemical and molecular tools, but personnel in plant health services should have a full understanding of the limits of molecular technology; their real value for the future probably lies in the provision of screening tools, which would indicate any requirement to check identities further. This is because most current protocols for distinguishing regulated species may not include unregulated, native species of the genera that occur in the countries where interceptions or outbreaks occur, or new species that might be imported from elsewhere. Thus, plant health identification and detection services need to encompass a range of scientific skills if unnecessary statutory action is to be avoided, e.g. for other unregulated species of *Bursaphelenchus*, *Globodera* or *Meloidogyne*. Furthermore, research into the use of molecular tools for detection of plant-parasitic nematodes in soil needs to be developed with care and the implications fully understood, if the status of infestations is to be truly represented. Thus, the integrated role of experienced diagnosticians, taxonomists and molecular scientists in nematode identification and detection for phytosanitary services remains a vital one.

12.11.3. Science versus legislation

Phytosanitary legislation requires clarity and consistency to avoid misinterpretation. The names of regulated plant-parasitic nematodes need to be established; however, as with other plant pests, this invariably poses a problem in taxonomy where the taxonomic details of some nematodes are frequently changing in line with new species concepts. Consequently, this demands an awareness that some species might be subject to many taxonomic changes and there may exist many synonyms in the legislation of some countries; this needs to be recognized if confusion is to be avoided and if correct phytosanitary action, including control, is to be taken.

An example of this is the controversy surrounding *R. citrophilus* and *R. similis*, which are both listed in European legislation. *Radopholus similis* was thought to consist of different pathotypes but Huettel *et al.* (1984) concluded that the banana race and the citrus race were distinct species; the name *R. similis* was restricted to the banana race and the citrus race was described as *R. citrophilus*. Subsequently, Kaplan *et al.* (1997) synonymized *R. citrophilus* with *R. similis*; Valette *et al.* (1998) proposed

R. citrophilus as a junior synonym of *R. similis*, although in 2000 Siddiqi proposed it as a sub-species of *R. similis* and Elbadri *et al.* (2002), using molecular techniques, demonstrated marked intraspecific variation in various isolates of *R. similis*. This continuing taxonomic uncertainty has caused more confusion for quarantine specialists involved in PRA work, as the host lists previously attributed to *R. similis* have to be used with care. Similarly, the controversy over the names *M. enterolobii* and *M. mayaguensis* illustrates that even though identification issues may have been resolved (see Section 12.11.1), studies of assessments of risk need to consider literature for both species. Such difficulties require the expertise of taxonomists, whose numbers are sadly in decline but who provide the essential framework for taxonomy and identification by developing species concepts and theories for the classification and identification of organisms, and hence determine correct names, set standards for descriptions, determine key morphological characters, develop identification keys and catalogue data such as those for distribution.

The integration of morphological and molecular advances in identification can also result in scenarios that test phytosanitary legislation; it needs to remain directed at the damaging genotypes (or more strictly the absent damaging genotypes), and if these are difficult to identify or in flux then perhaps it is best that legislation continues to remain cautious, with, for instance, nematodes included in *X. americanum sensu lato*. In 2008, *Globodera* spp. populations able to parasitize potato and genetically distant from PCN were reported in Oregon and Idaho, USA (Skantar *et al.*, 2011). Phytosanitary actions against these unidentified *Globodera* spp., which also occur in South America, are planned by USDA-APHIS. Similarly, the recent implementation of a new PCN Directive in Europe has raised the issue of the identity of PCN pathotypes, or rather populations which exhibit different pathogenicity in various parts of the world, especially in their hub of diversity in Central and South America. It is essential that the potato cultivars bred and used in Europe with resistance only to the PCN populations that exist there are not exposed to new types of biodiversity (Anon., 2012; Hockland *et al.*, 2012). Science has a role to play in providing evidence to this effect.

12.11.4. The future of diagnostics

Whilst the highest standards of delivery have always been the aim of diagnostic laboratories worldwide, the development of international standards has placed increasing demands on attaining prescribed levels of quality in the delivery of services and research. International standards for phytosanitary measures and also for diagnostics are becoming increasingly important, but their adaptation in some areas, such as the identification of species, which entails the use of judgment by experts rather than the output from machines, has proved a difficult philosophy for accreditation schemes to embrace. In addition, the variability of resources available in individual laboratories means a range of protocols has to be included. Nevertheless, selected protocols are slowly achieving international status.

The combination of scarce scientific resource and the cost of providing prescribed levels and speed of delivery have led some countries to negotiate contracts for science services with those countries that still have the ability to deliver. Inevitably, this will lead to centres of expertise serving a community in a particular geographical location

or region. Whilst this may have economic advantages, it should not discourage the broad development of essential identification and diagnostic expertise that is vital for the whole basis of phytosanitary work.

At the time of writing, phytosanitary services are starting to embrace the full potential offered by advances in molecular science, computer technology and the internet. The demise of taxonomic expertise at a local level is stimulating the creation of databases and networks to take advantage of scarce skills at short notice and RPPOs like EPPO have a role to play in facilitating this (see development of the EPPO Plant Quarantine Retrieval System on the EPPO website, <http://www.eppo.int/DATABASES/pqr/pqr.htm>). As the global community becomes connected and works to the new quality standards, so it is hoped that the shared experience and expertise in plant health will result in improved understanding of plant-parasitic nematodes for phytosanitary services.

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13 Biological and Cultural Management*

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13.1. Introduction

Biological control of nematodes principally concerns the exploitation of microbial agents. As cultural methods of crop protection, including cultivation and applications of organic matter (soil amendments), have profound effects on microbial abundance, diversity and activity in soil, it is appropriate that both approaches to nematode management are considered together. Several aspects of biological and cultural control have been reviewed (Stirling, 2011; Timper, 2011).

This chapter is directed towards the **management** of nematodes as opposed to their **control**. Control implies the use of a single measure to reduce or eliminate nematode pests, which in most cases is not possible, whilst management involves the manipulation of nematode densities to non-injurious or sub-economic threshold levels using several measures with consideration of the whole production system; maintenance of diversity is an objective of management but not of control (Brown and Kerry, 1987). Consequently, of increasing importance is the additional need to take into consideration the impact of the pest management strategies on biodiversity and the ecological balance in the soil.

Biological control is defined here as the management of plant diseases and pests with the aid of living organisms. This definition includes predators and parasites of organisms that kill or damage their hosts and also microbes that indirectly influence the establishment, function and survival of pathogens and pests. The plant may have significant influences on these interactions but plant resistance is not considered as a component of biological control. Neither is plant resistance discussed here as a cultural management tactic because it is the main topic of Chapter 14. It should be noted, however, that resistant rootstocks can influence the soil microbial populations around roots. Grafting of preferred, but nematode-susceptible, cultivars on to hardier, nematode-resistant rootstocks has long been an accepted practice for perennial crops, such as coffee, as well as for vegetables like tomato, and is gaining more popularity. More direct changes in the plant rhizosphere through the incorporation of organic materials, which influence microbial communities and help manage diseases and pests, are considered as **cultural control** in this chapter. The link between biological control and cultural methods becomes evident when the exploitation of natural enemies as biological control agents for the management of plant-parasitic nematodes is discussed. Both biological and cultural control methods are important for the biomanagement of pests

through the combination of measures other than chemical pesticides to improve soil quality and plant health. The application of bioactive compounds, which are derivatives of plants or metabolites derived from fungi or bacteria used as bionematicides, are discussed (see Chapter 16) but are not considered as biological control. Although many organisms derive their nutrition from nematodes, the most studied natural enemies of nematodes are bacteria and fungi.

There is considerable public and legislative pressure to reduce the use of nematicides because of their potential health and environmental risks, but few organisms have been developed as practical control agents and none is in widespread use. Apart from microbially induced suppression of nematode pests, research suggests that biological control agents will generally provide too little control to be effective alone and their successful use in sustainable management strategies will depend on their integration with other control measures. These include the use of genetic resistance, quarantine measures and various cultural control practices. Numerous reviews on the use of cultural control practices for nematode management are available (e.g. Chen *et al.*, 2004; Luc *et al.*, 2005; Perry *et al.*, 2009). A single management option rarely leads to the sustainable management of a nematode problem. A successful nematode management strategy ideally will involve the selection of a combination of complementary components, providing they are applicable, appropriate and economical. Furthermore, a successful strategy should be flexible to pest and disease changes, as cropping systems and knowledge evolve over time. Selection of a practice inevitably depends on a multitude of considerations, not least the scale of the cropping system; a smallholder farmer in Africa, for example, has wholly different criteria against which to assess a situation than does the large-scale intensive cereal farmer in North America or the highly intensive ornamental crop producer in Europe. Thus, biological and cultural nematode management options described here are not necessarily universally applicable and must be adapted to meet local needs. Such considerations are especially true for exploiting cultural control methods, such as the use of bulky soil amendments that may be only locally available. Moreover, much of the information on biological and cultural control practices emphasizes the importance of an understanding of the target plant-parasitic nematode, including its identification, hosts and environmental preferences. However small-scale farmers, especially those in developing countries, who would benefit much from these management strategies, have little knowledge or access to such information. Therefore, they need technical guidance and assistance from well-informed extension people with adequate training in nematology, which, in many circumstances, is also sorely lacking.

13.2. Suppressive Soils

Soils are considered suppressive when nematode multiplication on susceptible crops is less than that normally observed on the same cultivar in another soil, in similar abiotic conditions. Many soils that are suppressive to nematodes are often not recognized as such, because nematode problems have not occurred for several years. However, observations on abnormal declines in nematode populations or their damage in fields where plant-parasitic nematodes used to be a problem have led to the discovery of natural biological control and the organisms involved. Methods have been developed to assess suppressive soils (Box 13.1).

Box 13.1. Methods to assess the extent and nature of suppressive soils.

- Comparison of nematode multiplication in untreated soil and soil treated with a chemical or physical biocidal treatment.
- Inducing suppression by the transfer of microbial agents from a suppressive to a conducive soil.
- Addition of different numbers of infective nematodes to a conducive and a suppressive soil and comparing nematode reproduction.
- Correlation of population densities of specific organisms in soils with increased levels of suppressiveness using biological, culturing and molecular methods to measure their abundance and activity (Borneman *et al.*, 2004).

Most organisms found to be involved in nematode suppression are nematophagous fungi (e.g. *Pochonia chlamydosporia* (synonym *Verticillium chlamydosporium*), *Hirsutella rhossiliensis*, *Dactylella oviparasitica* and *Trichoderma* spp.) and bacteria (e.g. *Pasteuria penetrans*) that parasitize their nematode hosts. Microbes that compete for nutrients, produce toxins or induce host resistance, such as some rhizosphere bacteria (e.g. *Pseudomonas* spp., *Agrobacterium radiobacter*, *Bacillus subtilis*) may reduce nematode damage but may not provide the long-term control of nematode populations associated with suppressive soils.

Studies of suppressive soils have led to the identification of several important potential biological control agents, which may be abundant and occur in many fields and orchards, and even in natural environments (Stirling, 2011). Natural biological control may be widespread but, in most cases, it is inadequate to keep nematode populations below their damage threshold densities for crops grown in most agricultural systems. The challenge is to determine the specific conditions that are conducive for biological control agents to work in agricultural systems so that we can manipulate the environment, or the biological control agents, to suppress population densities of plant-parasitic nematodes adequately.

13.3. Biological Control Agents

It is not possible to include here all the natural enemies of nematodes that have been described and studied. Instead, we give an overview of the major groups with potential as biological control agents and include details of some of the most studied organisms.

13.3.1. Predators

Predatory nematodes, mites, insects and several other invertebrates, such as tardigrades, feed on nematodes. Predators are common in soil and can also feed on other organisms. The biology and feeding habits of some predatory nematodes, e.g. *Mononchoides gaugleri*, have been studied in detail. They can destroy several nematodes per day using their teeth, enzymes or toxins to kill their prey. Micro-arthropods, such as mites and

springtails, have a role in the regulation of nematode populations, especially in natural ecosystems where they may be abundant. However, their lack of specificity for plant-parasitic nematodes makes them unsuitable for use in biological control programmes aimed at specific nematode pests. Also, their mass production and delivery to the soil is considered impractical.

13.3.2. Nematophagous fungi

Some nematophagous fungi are obligate parasites (Box 13.2), which need nematodes to survive, others are facultative or opportunistic parasites (Box 13.3), which can survive saprophytically, and others have characteristics that are intermediate between these two categories. Nematophagous fungi may be most readily divided into those that have extensive hyphal growth outside their hosts, such as the nematode-trapping

Box 13.2. Obligate parasites.

Type of organism: *Nematophthora gynophila*

Mode of action: parasitism via adhesive spores.

Advantages:

- virulent against active and sedentary nematodes;
- resting spores, several species with long shelf life;
- responsive to pest densities.

Limitations:

- difficult to produce *in vitro*;
- limited spread in soil;
- narrow host range.

Box 13.3. Facultative parasites.

Type of organism: trapping fungi; parasites of nematode eggs.

Mode of action: parasitism via traps produced on modified mycelium and/or penetrative hyphae.

Advantages:

- easily produced *in vitro*;
- some species rhizosphere competent;
- wide host range.

Limitations:

- may be difficult to regulate switch from saprophytic to parasitic activity;
- efficacy dependent on plant species, nematode host and other soil conditions that affect saprophytic growth;
- several species do not form resting structures and may be difficult to formulate.

fungi, and the opportunistic parasites of nematode eggs, and those fungi that are mainly endoparasitic. The trapping fungi immobilize nematodes using non-adhesive traps or sticky structures usually produced on mycelia before they infect their host. Opportunistic fungi colonize the rhizosphere and attack the sedentary stages (females and eggs) of nematodes developing on plant roots. The endoparasitic fungi penetrate nematodes after germination of their adhesive spores, which attach to the nematode cuticle. Nematode-trapping fungi ensnare active nematodes using one or more types of mycelial trap. For example, *Arthrobotrys dactyloides* uses constricting rings, *Dactylella candida* makes non-constricting rings and adhesive knobs, *Monacrosporium cionopagum* forms adhesive branches and a two-dimensional adhesive network, whilst *M. ellipsosporum* traps nematodes with adhesive knobs or adhesive branches that may form loops. Some nematode-trapping fungi are good saprophytic competitors but trap few nematodes, while others are efficient in capturing nematodes but do not establish well in soil. Little is known about their growth and development in soil, especially the factors that cause the switch from the saprophytic to the parasitic phase. The trapping fungi apparently need a carbohydrate source to proliferate but other factors, such as those leading to mycostasis (inhibition of spore germination and/or growth caused by the residual microflora), also play a role in their abundance and trophic state in soil. Recent research exploiting genomics has begun to investigate the key molecular mechanisms involved in the interaction between trapping fungi and nematodes (Tunlid and Ahrén, 2011). Difficulties in the establishment of these fungi in the soil, their limited trapping activity and especially their non-specific capture of plant-parasitic nematodes reduce their potential in biological control. Some *Arthrobotrys* spp. have been formulated and applied under specific conditions, but with inconsistent results.

Arthrobotrys oligospora is the best known and most studied nematode-trapping fungus. It makes a three-dimensional hyphal network to trap soil-dwelling nematodes. The network is coated with an adhesive that contains lectins, which bind to specific carbohydrates present on nematode cuticles. The network attracts nematodes more than vegetative mycelium and the presence of nematodes induces trap formation. However, most studies were performed *in vitro* and it is not clear if these interactions are relevant in soil. Immobilization of the nematodes may be enhanced by a nematotoxin produced by the trap. Once the nematode is trapped, a hypha penetrates the cuticle within 1 h and forms an infection bulb. Penetration is thought to be mostly mechanical but collagenase might play a role. Assimilative hyphae develop and the fungus colonizes the nematode body. Later, conidiophores develop from the cadaver and bear conidia in a succession of clusters. *Arthrobotrys oligospora* is a good saprophytic competitor capable of using a wide range of carbohydrates. As with most trapping fungi, it captures all kinds of nematodes. The fungus is also able to colonize plant roots and cause cell wall modifications without affecting plant growth.

Many fungi infecting veriform plant-parasitic nematodes form adhesive spores that adhere to the cuticle of the nematode when it passes the fungus. These fungi are mostly obligate parasites and poor saprophytic competitors in soil but generally have a broad nematode host range. For example, *H. rhossiliensis* (Fig. 13.1) was found to reduce nematode invasion, and consequently nematode populations, of *Meloidogyne javanica*, *M. hapla*, *Heterodera glycines* and *Cricotyphus xenoplax*. Other examples of similar endoparasitic fungi that have been studied more closely are *Drechmeria coniospora*, *Nematoctonus* spp. and *Verticillium balanoides*. Not all endoparasitic

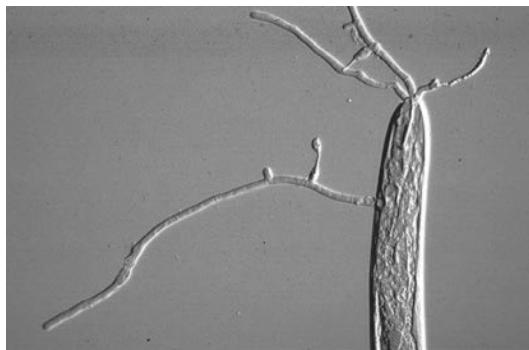


Fig. 13.1. Sporulating hyphae of *Hirsutella rhossiliensis* growing out of an infected juvenile of *Heterodera schachtii*. (Courtesy B. Jaffee, University of California, Davis.)

fungi produce adhesive spores; some form zoospores that swim to the nematode cuticle, attach and encyst, often around the natural openings of the host. These encysted zoospores form a penetration tube that enters the nematode body, which the fungus colonizes by hyphal growth. The hyphae differentiate into sporangia where zoospores are produced. Some zoosporic fungi, such as *Catenaria anguillulae*, which attack vermiform nematodes, are believed to be opportunistic but others, such as *Nematophthora gynophila* and *Catenaria auxiliaris*, infect sedentary young female cyst nematodes and are obligate parasites. Zoospores require water-filled pores to be active, and nematode infection is much affected by soil moisture levels. As many parasites of vermiform nematodes and the zoosporic fungi are difficult to culture *in vitro* and establish in soil, they are considered unsuitable for large-scale application as biological control agents.

By contrast, facultative fungal parasites of sedentary stages (eggs, developing juveniles and females) have attracted most interest because of their potential as biological control agents. Their target pest is immobile, and thus easier to infect, and these facultative parasites are able to survive saprophytically in the rhizosphere and most are relatively easy to mass culture. They are not as specialized as the fungal parasites attacking soil-dwelling nematode stages and usually infect their host by simple hyphal penetration, sometimes with the formation of an appressorium. Many kinds of fungi have been isolated from sedentary stages but few have been studied in detail. *Cylindrocarpon destructans*, for example, is found regularly, as are species of *Fusarium*, *Gliocladium*, *Pochonia* and *Trichoderma*, all opportunistic fungi whose pathogenicity to nematodes may differ considerably between isolates. *Purpureocillium* (synonym *Paecilomyces*) *lilacinus* is a well-studied parasite of a number of nematodes, including *Radopholus similis* and *Tylenchulus semipenetrans*, but most research has focused on the parasitism of *Meloidogyne* spp. and *Globodera rostochiensis* eggs. The fungus is abundant and active in subtropical and tropical regions. It is effective in reducing nematode damage to a range of crops in field trials and has been widely evaluated and developed by several small companies around the world. Soil application of the fungus has often resulted in variable levels of nematode control, although improvements through commercial development indicate that more consistent and promising results may be obtained. It is marketed in Germany (BioAct®WP) and in South Africa (Pl Plus®) for cyst and root-knot

nematode management. Both products use the same strain 251 of the fungus, which is applied as dispersible granules for application in water.

Pochonia chlamydosporia parasitizes females and eggs of cyst and root-knot nematodes (Fig. 13.2). Hyphae penetrate eggs after formation of an appressorium on the eggshell. A serine protease and chitinases, which degrade the eggshell, and a nematotoxin, phomalactone, produced by *P. chlamydosporia*, may be involved in pathogenicity. Chlamydospores, which are resilient to environmental extremes, are produced as a survival structure and are used as an inoculum to establish the fungus in the soil and rhizosphere. Isolates of the fungus differ greatly in their ability to produce chlamydospores, colonize roots and infect nematodes. A range of biological (e.g. dilution plating on a selective medium) and molecular (polymerase chain reaction (PCR), real-time PCR, restriction fragment length polymorphism (RFLP)) methods have been developed to monitor the occurrence, abundance and activity of the fungus in the soil, rhizosphere and nematode egg masses. Rhizosphere colonization differs with plant species and is improved when the plant is infected by nematodes. The fungus also has host preferences: isolates obtained from root-knot nematodes are less able to infect cyst nematode eggs than isolates originating from cyst nematodes (Kerry and Hirsch, 2011). Single applications of the fungus at rates of 5000 chlamydospores g⁻¹ soil have provided control of root-knot nematodes on vegetable crops in tropical soils but results in Europe have been less satisfactory. The fungus is more effective when applied to low, pre-cropping densities of root-knot nematodes, preferably on poor hosts for the nematode, than when applied to large nematode infestations on highly susceptible crops. The factors that control the switch from saprophytic to parasitic activity are poorly understood. Even though *P. chlamydosporia* may be more abundant in soil with large amounts of organic matter, its parasitic activity may be no greater than in a mineral soil. Formulations based on fungal hyphae and conidia have been developed but chlamydospores are the most robust form of inoculum. Toxicological tests on chlamydospore-based inoculum have been successfully completed, with products expected for more widespread evaluation.

Trichoderma spp. commonly occur in soil and have received increasing attention for nematode management; isolates have been identified that successfully antagonize

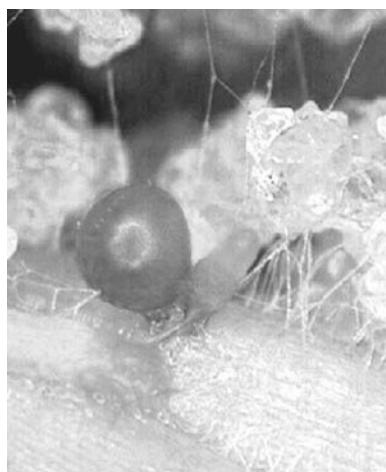


Fig. 13.2. Hyphae of *Pochonia chlamydosporia* growing into cyst of *Globodera* spp. attached to potato root.
(N. Viaene.)

and control a wide range of plant pathogens including nematodes (Sharon *et al.*, 2011). Recent research, focused on strains *T. asperellum*-203 and *T. atroviride* IMI 206040, previously defined as strains of *T. harzianum*, have shown control activity against *M. javanica*. Variability in the efficacy of different strains has been shown and the application of mixed strains has led to increased levels of nematode control with some strain combinations but not others. This result indicates differing modes of action and, indeed, studies have focused on conidial attachment and parasitism, revealing that a wide range of lytic enzymes (proteases and chitinases) are produced, the induction of which is likely to be environmentally mediated (Sharon *et al.*, 2011). When searching for strains suitable for the management of root-knot nematodes, studies indicated that isolates from egg masses are more effective than those from soil. Furthermore, assessing the genetic potential to produce chitinolytic enzymes was suggested as an additional selection criterion.

13.3.3. Endophytic fungi

Endophytic fungi (Box 13.4) grow within plant tissues without causing disease. Arbuscular mycorrhizal (AM) fungi, e.g. *Glomus* spp., are the best-known endophytes associated with plant roots; they are obligate symbiotic parasites of plants. Their role in protecting the plant from nematode damage and in reducing nematode densities in the soil has been studied in different plant–nematode interactions, but most involve *Meloidogyne* spp. (Fig. 13.3), with only few studies looking at cyst and migratory nematodes (Hallmann and Sikora, 2011). AM fungi enhance plant growth by improving plant access to nutrients, especially P, and particularly under conditions of poor nutrient availability. AM fungi also aid access to and uptake of water, alleviate heavy

Box 13.4. Endophytes.

Types of organism: arbuscular mycorrhizal (AM) fungi and other non-pathogenic root-colonizing fungi and bacteria.

Mode of action: competition for space and nutrients in roots and/or antagonism, increased (systemic) host defence/tolerance.

Advantages:

- active against wide range of nematodes including migratory endoparasites in roots;
- may suppress nematode colonization and multiplication;
- may promote plant growth and reduce nematode damage;
- easily produced *in vitro* and formulated;
- may be applied as a seed/seedling treatment.

Limitations:

- non-mycorrhizal fungi are closely related to plant pathogens and may be difficult to register for release;
- activity varies between by crop cultivars.



Fig. 13.3. Five-week-old soybean plants infected with 1000 *Meloidogyne incognita* per pot and treated with the nematicide carbofuran (left), with the control fungi *Glomus mosseae* and *Trichoderma pseudokingii* combined (right) and without treatment (centre). (D. Coyne.)

metal toxicity and suppress pest and disease damage, including that of nematodes. Colonization of roots by AM fungi before nematode invasion may reduce nematode multiplication rates to a greater extent than after nematode invasion. The specific mode(s) of action of nematode antagonism are not well understood but are thought to range from very specific mechanisms to several mechanisms working in concert. They may also interfere with the production of root diffusates or produce nematotoxic compounds. AM fungi are produced commercially as crop growth enhancers. Other endophytes, such as *Neotyphodium* spp. in the leaves of grasses and asymptomatic and non-virulent strains of *Fusarium oxysporum* in banana and tomato roots, may rely on a toxic mechanism to reduce nematode infestations in roots. Increasing interest in endophytic pest and disease management has broadened the scope of research activity, with various fungal and bacterial isolates being assessed for potential use. The levels of nematode management can be acceptable, although isolates of the same species differ markedly in their efficacy against nematodes. Few, if any, commercial products based purely on endophytic activity are currently available, although isolates of *F. oxysporum* active against *Radopholus similis* have undergone development and field testing on banana plantations in Central America and East Africa. Again the modes of action are poorly understood, but induced resistance plays an important part in the interaction.

13.3.4. Bacteria

Most bacteria that interfere with nematode behaviour, feeding or reproduction do so indirectly by producing antibiotics, enzymes or toxins (Box 13.5). Many products, such as volatile fatty acids and nitrogenous substances, are formed by bacteria during decomposition of organic materials and may influence nematode populations in the soil and rhizosphere. Screening rhizobacteria or their metabolites (extracts of their cultures)

Box 13.5. Antagonists.

Types of organism: rhizosphere bacteria.

Mode of action: toxins; modification of root exudates and/or induced resistance.

Advantages:

- easily produced *in vitro*;
- may be applied as a seed/seedling treatment;
- active against a wide range of plant-parasitic nematodes;
- may promote plant growth and reduce nematode damage.

Limitations:

- may be effective for a relatively short time;
- no direct effect on nematode multiplication;
- activity affected by crop cultivar and soil environment.

in Petri dishes has led to the discovery of bacterial strains with strong antagonistic properties. However, the production and importance of such metabolites in the rhizosphere is not clear. *Burkholderia* spp., *Pseudomonas* spp., *Bacillus* spp. and *A. radiobacter* may reduce nematode invasion of roots through effects on nematode hatching and mobility or may induce plant resistance. Several of these bacteria are also plant growth-promoting bacteria (PGPB).

Of the few bacteria known to parasitize nematodes, *Pasteuria* spp. are endospore-forming bacteria and show the greatest potential to be developed into biological control agents. *Pasteuria* endospores have been observed adhering to and parasitizing all of the economically important plant-parasitic nematodes. *Pasteuria* species and isolates show varying degrees of host specificity; for example, a *Pasteuria* population isolated from cyst nematodes appeared to be more promiscuous than those isolated from root-knot nematodes (Mohan *et al.*, 2012). Molecular studies have led to the establishment of phylogenetic relationships between *Pasteuria* spp. and have provided tools to test if plants are infected with *Pasteuria*-infected nematodes (Rao *et al.*, 2012). The life cycle of *Pasteuria* spp. is initiated when dome-shaped endospores adhere to the cuticle as the nematodes move through soil (Fig. 13.4). It is thought that collagen-like fibres on the surface of the endospore are involved in a Velcro-like attachment process with a cuticle receptor (Davies and Curtis, 2011). Depending on the nematode species, endospores germinate either immediately (e.g. in *Heterodera avenae*), or later when the nematode has entered the root and initiated a feeding site (e.g. *Meloidogyne* spp.) by producing an infection peg that penetrates the cuticle. Following germination, small rod-shaped bacteria develop exponentially to produce granular masses that eventually enter sporogenesis and form the next generation of spores (Davies *et al.*, 2011). In *Meloidogyne* spp. the infected female continues to develop, becoming infertile as up to 2 million endospores are produced by the bacterium, destroying the reproductive system. These endospores are released into the soil upon decomposition of roots and infected females, providing new inoculum of the biocontrol agent. Spores can survive in air-dried soil for several years, but successful infection of nematodes greatly depends on the distribution of the spores in the soil, which can be influenced by soil type, tillage practices, moisture

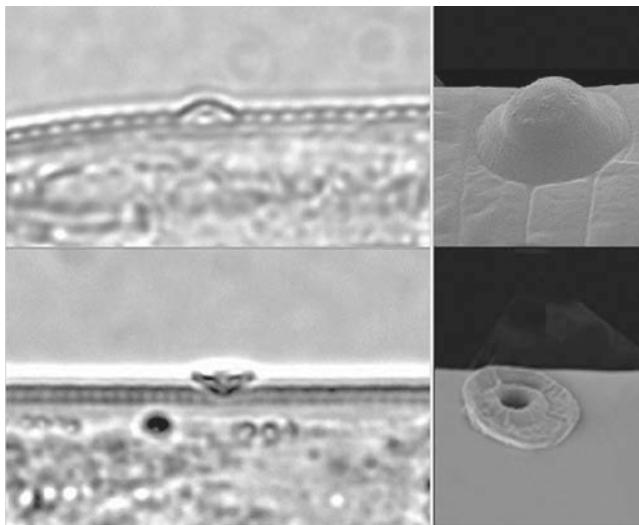


Fig. 13.4. Dome-shaped endospores from *Heterodera cajani* (top, brightfield and SEM) also attaching to *Globodera pallida* (bottom, brightfield and SEM). (Courtesy S. Mohan and K.G. Davies, Rothamsted Research, UK.)

and temperature. Juvenile nematodes in subsequent generations on the same plant only move short distances from the egg to nearby roots and are less infected than those in the first generation. A natural decline of root-knot nematodes in fields of tobacco was shown to be caused by *Pasteuria* spp. but most studies on the efficacy of the bacteria as a biological control agent have been performed in pots because of difficulties in producing sufficient spores for large-scale trials. Declines in nematode numbers and root galling have been reported in small plots. Application is complicated because some isolates of the bacterium are very host specific and spore burden is not always correlated with virulence. The need for thorough distribution of spores around the roots requires inundative release of the bacteria at rates of $>10^4$ spores g⁻¹ soil. A main disadvantage of *Pasteuria* spp. for use in biological control was its obligate parasitism and hence difficulty for mass production, but breakthroughs at Pasteuria Bioscience Inc. (USA) have led to *in vitro* culture of *Pasteuria* spp. Already *Pasteuria* spp. parasitic on sting nematodes (*Belonolaimus longicaudatus*) are being produced *in vitro* and marketed as Econem® for nematode control on golf greens. The availability of mass production for niche markets paves the way for field-scale applications. Once more is known about the specificity of the populations towards different nematode species, *Pasteuria* spp. have increasing potential to be a most successful biological control agent.

13.4. Interactions with Rhizosphere Microflora

The soil microflora in the rhizosphere is influenced not only by soil treatments, such as application of organic matter or soil disinfestation, but also by the presence of nematodes and by the plant species and cultivar; these factors may influence the activities of biological control agents in the rhizosphere. Increases in nematode-trapping

fungi, for example, did not always result in a greater reduction in the numbers of plant-parasitic nematodes, as these fungi also feed on other types of nematodes and can survive saprophytically. Complex interactions between microorganisms and the environment occur in the rhizosphere but are poorly understood. For example, part of the microflora associated with the egg masses of root-knot nematodes is antagonistic to *P. chlamydosporia* *in vitro* and may have the potential to influence its efficacy as a biological control agent. By contrast, rhizosphere bacteria may enhance the trapping activity of *A. oligospora*. The interactions between rhizosphere microorganisms and microbial biological control agents are complex and may involve competition for space and nutrients as well as antibiosis. Direct trophic interactions may also be important; fungivorous nematodes, collembola and mites are able to reduce the activity of fungal biological control agents applied to soil.

Molecular techniques offer great possibilities to study changes in the microbial community in the rhizosphere, as species and isolates of microorganisms can be more easily monitored than with classic tools such as selective media. Changes in the structure of bacterial and fungal communities in the soil can now be studied using advanced techniques such as phospholipid fatty acid analysis (PLFA), denaturing gradient gel electrophoresis (DGGE), bar coding and high-throughput sequencing of DNA or RNA. It is expected that with these new techniques, increasing insight will be gained into the role of soil biodiversity and the mechanisms of ecosystems resilience, i.e. the capacity of an ecosystem to respond to disturbance and recover quickly. This knowledge could possibly be used to improve mechanisms for introduction or further enhancement of biological control agents in agricultural soils where plant-parasitic nematodes cause unacceptable yield reductions.

13.5. Application of Biological Control Agents

Successful biological control greatly depends on a thorough understanding of the population dynamics of the natural enemies and target pest, and of their interactions. For many organisms that have potential as biological control agents for nematodes such information is lacking, except for the pioneering work of Jaffee *et al.* (1992) in model systems. Parasitism by some microbial biological control agents is density dependent, i.e. the probability of a specific host being parasitized increases with the density of the host. Although the activity of facultative parasites, including some nematode-trapping fungi, is density dependent, their ability to survive saprophytically and their wide host range should enable them to be more effective at low pest densities than obligate parasites, such as *P. penetrans*, or those with poor saprophytic survival capabilities, such as *H. rhossiliensis*. The size of the inoculum reservoir required for significant infection rates and the relatively low transmission rates in soil tend to dampen fluctuations in the dynamics of the interactions between nematodes and their microbial parasites and pathogens. This theoretical framework is of major importance for the development of rational biological control strategies.

Application of biological control agents for the management of plant-parasitic nematodes is notoriously difficult, and most empirical studies have provided inconsistent results. However, some agents may be practically exploited in nematode management and some, such as *P. lilacinus*, *Trichoderma* spp. and *Pasteuria* spp., have been commercialized. The steps towards commercialization include selection of the most

effective isolates, production of inoculum and formulation of the microbial agents. As biological control is not a replacement for chemical control, application of biological control agents should be considered within the framework of other management strategies, especially their interaction with cultural control methods (Stirling, 2011; Timper, 2011).

13.5.1. Selection of biological control agents and isolates

The choice of a biological control agent will depend on the nematode species and nematode stage to be controlled, as it is clear that some organisms are host specific or attack only certain developmental stages and others feed on many kinds of nematodes. A combination of biological control agents with different modes of action or range of environmental tolerance limits may result in improved nematode control. However, no benefits and even reduced effects can occur and so careful selection of organisms for combination is required to avoid competition or antagonistic effects (Meyer and Roberts, 2002). In practice, combinations of agents may be too expensive to produce and register.

Comparisons of different isolates of nematophagous fungi and bacteria have revealed that there are enormous intraspecific differences in the performance of the microorganisms as potential biological control agents. Performance refers to the ability to parasitize the nematode and also to the survival capacity in soil under different environmental conditions (e.g. the production of survival structures, saprophytic growth), establishment in the rhizosphere (root-colonizing capacity) and soil (dependence on nematode hosts and production of resting structures). With improved knowledge of their biology, it is possible to focus selection for biological control agents. For example, when assessing *Trichoderma* spp. as biological control agents of *Meloidogyne* spp., those isolated from egg masses of *Meloidogyne* spp. appeared more effective than isolates recovered from soil (Affokpon *et al.*, 2011). In general, screening programmes to select isolates with biological control potential have resulted in <10% of the isolates being selected in simple bioassays and more being rejected in tests in soil. Hence, focus on selection of appropriate isolates is a key stage in the development of a biological control agent.

Development of molecular markers for different isolates of an individual species has made it possible to monitor their development in soil, rhizosphere and egg masses, and determine whether differences observed in bioassays in the laboratory are relevant in the field. In suppressive soils there is much diversity between isolates of *P. penetrans* and *P. chlamydosporia* within a single site; such diversity may enable the community to function in different environmental conditions, on different parts of the root system and against a variable host. Therefore, selection of a single isolate of a specific agent for use in management strategies may prove inadequate.

13.5.2. Inoculum production and formulation

Most fungi and bacteria with activity against nematodes are not obligate parasites and can be cultured on artificial media or grain products (e.g. wheat bran). It is

important that the efficacy of the biological control agent is maintained, even after frequent subculturing. Although regular passage of the isolate through the nematode host to maintain its pathogenic capability has been recommended (Crump, 2004), it is not clear if this is important for facultative parasites that establish in soil through saprophytic growth.

Once inoculum has been produced, it needs to be formulated for storage. An ideal formulation has a long shelf life, allows for easy transportation and application, is readily distributed in soil and provides optimum conditions for growth and survival of the introduced agent. The formulation must also be compatible with other crop protection measures. For example, a fungal control agent such as *P. chlamydosporia*, if applied as a seed treatment, must be compatible with other seed treatments or seed coatings that may well contain a fungicide.

Alginate pellet formulations of spores and mycelial fragments of several fungi have been tested and were shown to be an efficient way to store, distribute and apply the fungi while maintaining biological control activity. However, the pellets and their contents may be destroyed by collembola and mites and at high concentrations alginates may be phytotoxic. Together with the biological control agents, amendments, such as chitin, can be added as a food source for the microflora. However, this practice does not always result in increased parasitic activity of the biological control agent. A cost-effective method of applying a biological control agent, especially for small scale systems, is to combine it with its culture medium. This has the disadvantage of bulkiness and increased transport costs while the microorganism may be distributed unevenly unless carefully mixed. The biological control agents can also be added directly to compost or soil used for seedling transplants (transplant mixes), where they are able to colonize the rhizosphere at an early stage or endophytically infect roots of young plantlets before these are transplanted. Alternatively, young plants could be protected by biological control agents using seed coatings or bare-root dips, but these methods depend on the organism spreading extensively in the rhizosphere and must be compatible with other crop protection treatments, e.g. fungicidal applications. Drenching of the rooting medium with a liquid suspension of the biological control agent can help overcome this, or the microorganism can be dispersed in the water of floating trays for seedling production. The frequency of application and dosage need to be determined for each organism, and may dictate the mode of application. *Pochonia chlamydosporia* was able to reduce root-knot nematode populations on vegetables throughout one year, whilst potato cyst populations were reduced 2 years after fungal application (Crump, 2004). Unless a long-lasting resting stage is produced, density-dependent biological control agents may have to be applied in large quantities (inundative releases) because their numbers will decrease concomitantly with numbers of their nematode host.

13.6. Integration of Biological Control with Other Control Measures

The development of biological control strategies may involve the application of a biological control agent, but also the use of methods to increase the impact of the residual soil microflora on pest nematode population dynamics.

13.6.1. Crop rotation

As biological control agents alone rarely provide adequate control, they should be integrated with other methods such as crop rotation, resistant cultivars or antagonistic plants, either to reduce the nematode populations in soil or to promote the establishment of the biological control agent (Kerry and Hominick, 2002). Different crops produce different root diffusates and these affect microbial activity in the soil and rhizosphere but little is known of their effects on the performance of biological control agents. The rhizosphere-colonizing capacity of *P. chlamydosporia* differs greatly among host plants and nematode control is greatest on roots that support extensive fungal growth but are poor hosts of the nematode. Hence, most nematode control is achieved by combining the fungus with a poor host for the plant-parasitic nematode to reduce the infestation in soil before the next susceptible host.

13.6.2. Soil disinfection

Several control measures, such as soil disinfection through soil solarization, biofumigation, steaming (see Section 13.9) or application of broad-spectrum biocides (see Chapter 16), facilitate the establishment of biological control agents introduced after these treatments. As the activity of the soil microbial community is reduced, the establishment and survival of biological control agents is enhanced, at least in the short term. Soil disinfection will also reduce the densities of plant-parasitic nematodes to levels that are manageable for biological control agents.

13.6.3. Soil amendments and green manures

In contrast to soil disinfection, addition of organic matter to the soil through soil amendments and green manures results in an increase in numbers and diversity of microbial populations, including beneficial ones. Application of only organic matter, without external addition of a biological control agent, often results in suppression of plant-parasitic nematode populations (see Section 13.11.1) and increase in saprophytic nematodes. The mechanism of this nematode suppression can be attributed to the release of nematicidal compounds by the organic material (e.g. glucosinolates), to the production of allelochemicals (e.g. antibiotics, chitinases) by the soil microflora that was able to increase in numbers because of the amendments, and to improved plant growth (e.g. by plant growth-promoting bacteria) (Widmer *et al.*, 2002; Thoden *et al.*, 2011). It is assumed that the incorporation of organic matter increases the abundance of microorganisms active against nematodes. However, addition of organic amendments may increase microbial competition and result in reduced nematode parasitism by poor competitors such as *H. rhossiliensis*. Addition of organic amendments may affect the switch from the saprophytic to the parasitic state and some facultative parasites are less active in soils following application of soil amendments. The presence of adequate nutrients, supplied by the organic amendments, may suppress the production of enzymes that aid in nematode infection yet provide abundant materials and energy for population increase of the biological

control agent. For example, the serine proteases from *P. chlamydosporia*, *P. lilacinus* and *P. suchlasporium* are repressed by the presence of glucose and easily metabolized nitrogen sources.

Defined amendments have also been used for nematode management. The addition of chitin causes an increase in chitinase-producing microbes in the soil and rhizosphere, which are thought to degrade the chitin-rich eggshells of nematodes. Although there may be an increase in the abundance of nematode antagonists after such applications, the major effect of chitin on nematodes, especially soon after application, is due to the release of ammonia. In general, large amounts of soil amendments are needed to reduce nematode infestations significantly and their effects on the soil microbial community are complex and difficult to interpret.

13.7. Nematode-free Planting Material

Problem avoidance should essentially form the basis of any pest management strategy. Use of nematode-free planting material provides an effective means of nematode management (or avoidance), often at a fraction of the cost fiscally and environmentally, to treating the cropping area, which may be impractical in any case. This nematode management tool requires that farmers understand the benefits of more costly, healthy planting material.

13.7.1. Production

Healthy planting material may be available through the use of certified seed and planting material, the production of seedlings/plantlets in sterile conditions and/or an effective quarantine system (see Chapter 12), which prevents the introduction of foreign species. Cropping systems using nursery-grown plants for transplantation afford an excellent opportunity to provide nematode (and other soil-borne disease) protection. Such strategies will limit nematode transmission to otherwise pest-free areas. In developing countries, subsistence farmers could prevent major losses from nematodes by using simple nurseries for nematode-free seedling production. Inert or sterilized potting media (e.g. sawdust, coconut husk, peat, vermiculite and charred rice husk) can be used to obtain nematode-free stock. It is important to treat seedling containers or boxes regularly to maintain hygiene. Where available through (international) trade at relatively low prices, healthy certified seed or nursery material can reduce the need for farmers to produce their own pest-free material. Practices that are increasingly adopted are the use of tissue-culture propagation techniques, e.g. for ornamentals and bananas, and the floatation tray method used for tobacco and flower seedlings in Africa. At the same time, such systems also provide the opportunity to deliver optimally seedling protectants including beneficial microorganisms.

Where nematode-free propagation material is not guaranteed or available, disinfection of (potentially) infected material can provide a solution. Heat treatment and mechanical methods to separate infected from healthy seeds are widely practised methods to obtain nematode-free plants. However, if planting material is not treated or infected soil is used for seedling production before distribution, the practice can result in greater distribution of nematode (and other) pests, so caution is required.

13.7.2. Heat treatment

Hot water treatment (therapy) is used to disinfest vegetatively propagated planting material such as tubers, bulbs, rhizomes, runners, woody rootstocks and also seeds. The practice relies on the application of sufficient heat over a sufficient length of time to prove lethal to nematodes without thermal injury to the planting material. The greater the difference in the thermal sensitivity between the plant host and the nematode pest, the greater is the chance of successful decontamination. In Europe, the use of hot water treatment in managing *Ditylenchus dipsaci* led to the undoubted improvement in ornamental bulb health. In combination with infected root and corm tissue removal (paring), banana and plantain nematodes (*R. similis*, *Helicotylenchus multicinctus*, *Pratylenchus* spp. and *Meloidogyne* spp.) have been successfully managed by dipping rhizomes (suckers) in water at 53–55°C for 20 min. Other tuber, corm or bulb crops have been similarly successfully treated. Where fuel is scarce or costly, or temperature and timing regulation is problematic, such as in many subsistence farming systems, this approach may not be practical. However, a simplified technique using local materials (e.g. empty oil drums) has been adapted and uses boiling water in which pared banana suckers are immersed for just 30 s (Coyne *et al.*, 2010; Fig. 13.5). Hot water treatment can also be further improved with pretreatments, such as inducing cold hardiness by storing at low temperature (e.g. roses), pre-warming (e.g. strawberry plants), pre-soaking (e.g. rice seeds) and/or immersing in cold water after heat exposure (e.g. grapevine rootings). Pre-soaking with solutions of hydrogen peroxide, sodium chloride or other salts can further improve the process. If conditions are unfavourable for efficient drying, the treatment of large volumes of seed may be inadvisable, as fungal contamination or premature germination may result. Spreading out dry seed under intense solar radiation can also help reduce nematode seed infestations, e.g. *Aphelenchoides besseyi* in rice.



Fig. 13.5. Boiling water treatment of plantain suckers in a used oil drum to disinfect them of nematodes, in Nigeria. (D. Coyne.)

When using hot water treatment for nematode decontamination, it is necessary to test and modify the technique to suit individual circumstances. Different crops, and even cultivars, differ in their sensitivity to heat, which may also differ depending on their storage or growing conditions.

13.7.3. Mechanical methods

In the case of *Anguina tritici*, which infects wheat species (*Triticum* spp.), including emmer and spelt but also rye (*Secale cereale*), the infected seeds that turned into galls (cockles) can be separated from healthy seed stocks by weight differential; as the galls are less dense, they can be winnowed or separated by floatation in salt solutions. They can also be separated using sieves, relying on size differences.

Seed-borne nematodes that may infect other crops include *A. agrostis* and *A. funesta* on grasses, e.g. on bentgrass (*Agrostis* spp.) in the USA, as well as *A. arachidis* and *D. africanus* affecting groundnut in Africa. These seeds may also be cleaned using weight or size differentials of infested seed, or simply by visual selection of the infected distorted or discoloured seed (Brown and Kerry, 1987).

13.8. Sanitation

Approaches that limit the build-up or survival of nematode populations should be practised wherever feasible. Simple sanitation measures to reduce nematode movement between sites on implements or through irrigation or waste products are probably not a primary consideration in most circumstances, but are of major importance as they contribute considerably to the spread and build-up of nematode populations. However, such practices can be time-consuming and farmers may be reluctant to implement them if the objective is not clearly understood.

Post-harvest sanitation and physical destruction of plant debris, such as straw and stubble burning, has traditionally played an important role in the management of some seed and stem nematodes such as *A. agrostis*, *A. besseyi*, *A. tritici* and *D. angustus*. Burning reduces the return of nematode inoculum to the soil after a susceptible crop. This is also the case for the traditional post-harvest burning of uprooted tobacco plants in Malawi as a means of managing *Meloidogyne* spp. However, environmental protection acts now restrict this practice in some parts of the world, limiting the use of this method for sanitation purposes.

In intensive potato production areas in Europe, outgraded potato tubers left in the field after harvest can sprout and grow among plants of the next crop in the rotation, thus enabling potato cyst nematodes (*Globodera* spp.) to reproduce despite the strict rotation scheme. Removal, most often by destruction using selective herbicides, prevents build-up of cyst populations. In the intensive potato industry, tare soil and soil adhering to tubers are transported by trucks over long distances, thereby distributing potato cyst nematodes. Washing potato tubers free of soil has proven to be effective in the USA for many years. As this practice is not always possible, disinfection of soil is receiving more attention, especially in view of international trade.

For two nematode species, *Bursaphelenchus xylophilus* and *B. cocophilus*, which affect pine and palm trees, respectively, the treatment of wood and wood products is a key measure towards reducing their spread. *Bursaphelenchus xylophilus* can be controlled in timber and wood chips by proper heating in kilns before removal from infested sites, a practice already required for international trade of selected wood products as a preventive measure. Pesticides can also be used to control *Bursaphelenchus* spp., provided the use of these chemicals is not prohibited. A principal method of containment and management is the removal and destruction by burning of dead and dying trees.

13.9. Physical Soil Treatments

Managing nematode population densities in the soil to levels below the damage thresholds can be achieved by preventing nematode multiplication or survival, or by killing them directly. Over previous decades soil disinfestation has relied heavily on broad-spectrum biocides, at least in developed countries (Chapter 16). Alternative methods for disinfesting or sterilizing soil rely on physical methods.

13.9.1. Dry heat

The use of fire is not the most efficient way to apply heat for disinfesting soil. The fuel requirements to permit lethal heat levels to penetrate sufficiently deep are uneconomical in most circumstances. However, it may be practical and effective for small areas of land, such as in the treatment of seedling nurseries. The use of slow burning of rice husks in Asia has provided nematode-free soil for use in rice and vegetable nurseries. Pre-drenching the soil, before placing fuel and igniting, further improves the depth of penetration of lethal heat and the general efficiency of the process. Dry heat can be used to treat bulk and container soil using various heating devices, e.g. metal plates heated by electricity placed at regular intervals in the soil. Depending on the method and soil type, changes in the physical and chemical nature of treated soil can cause unsatisfactory plant growth.

The use of microwave radiation for treatment of soil against nematodes has been demonstrated for *H. schachtii* and *Rotylenchulus reniformis*, but this method is still not applied widely as it appears to be an impractical method for field soil. Research continues, however, into alternatives to synthetic pesticides that may prove suitable for small volumes of soil or for nursery situations.

13.9.2. Steam

Steam heat has long been applied in heated glasshouses as an effective, if costly, means of soil sterilization. Soil type and prior soil tillage affect heat penetration and efficacy, as does water absorption capability. Dry soil has a greater capacity to absorb condensed water and to a greater depth than wet soil. Steam can be applied by pumping through a network of underground, perforated pipes (about 60 cm depth). Even distribution of heat through the soil, especially the surface layers, is improved by

covering the soil with plastic sheeting. Blowing steam under plastic sheeting (sheet steaming), anchored at the edges, provides surface soil sterilization. Sheet steaming combined with buried pipes can create ‘negative pressure steaming’, a more energy efficient and effective method that draws the steam through the soil using an extractor fan. The main drawback to the use of steam is cost. Treated soil should be set aside for some time to permit the soil to stabilize as the release of phytotoxic chemicals (nitrite, ammonia) and change in pH may affect plant growth. For less commercial systems, steam can still be effectively used for small volumes of soil, such as in nurseries. Containers, such as used oil drums, can be semi-filled with water, placed over a heat source and adapted to deliver steam beneath the soil via a pipe attached to the steam outlet on the drum. Plastic sheeting can be used to contain the steam in the soil being treated.

13.9.3. Solar heat

Solar radiation has been employed effectively to disinfest soil of nematodes, primarily in hot climates and for relatively shallow depths of soil (Gaur and Perry, 1991). Moistened soil covered with polyethylene sheeting, with the edges anchored, will significantly reduce nematode populations (Fig. 13.6). Moisture is necessary as this promotes biological activity, improving efficacy. This system has been variously termed plastic, polythene, polyethylene mulching or tarping, solar heating, solar pasteurization or soil solarization, the latter term being the more generally accepted. The effect reduces with depth, but solarization for at least 4–6 weeks will increase soil temperatures to 35–50°C to depths of up to about 30 cm, depending on soil type and prior tillage. Results can be optimized by using double layers of polyethylene, thin (25–30 µm), transparent as opposed to black sheeting, and using solarization during periods of highest solar intensity. Thinner sheeting tends to be more effective but less



Fig. 13.6. Use of plastic sheeting for solarization of small area of land in Malawi. (D. Coyne.)

durable and more easily damaged. Larger areas are also more practical to treat, as soil heating is less effective near the edges of the covered area. The length of time required for effective solarization is a great limitation and the method is most suited to nursery beds and in glasshouses rather than large field areas. Also, in areas of intensive use, the disposal of the large quantities of plastic sheeting can be a problem. Excellent nematode control in soil for use in potting composts, to raise seedlings or rooting cuttings, can be achieved for small volumes of soil if the soil is moistened, contained in sealed plastic bags and placed on a suitable surface in direct sunshine for 2 weeks. An additional benefit of solarization is the control of soil-borne pathogens and weeds.

13.9.4. Flooding

Following natural inundation areas of land are mostly free of plant-parasitic nematodes. Areas such as river flood plains, riverbanks and seasonally flooded lakes have long been exploited for crop production, primarily to take advantage of fresh sediment deposits. Extended periods of flooding provide almost nematode-free conditions. Notable exceptions are the rice root nematodes, *Hirschmanniella* spp., and the foliar nematodes, *A. besseyi* and *D. angustus*. Some species of *Tylenchorhynchus*, *Meloidogyne*, *Helicotylenchus*, *Heterodera*, *Rotylenchus*, *Pratylenchus*, *Paratylenchus*, *Hemicyclophora*, *Xiphinema* and *Criconematidae* are also known to survive periods of flooding. If small in area, flooded sites may be used for nursery purposes or, alternatively, the soil may be removed for use in a nursery sited elsewhere. Artificially flooded areas, such as rice paddies, can also provide nematode-free conditions for post-rice crops that are non-hosts for the rice nematodes. Rice-vegetable and rice-wheat production systems tend to be quite sustainable, despite the presence of nematode pests, including species of *Meloidogyne*. This is partly a consequence of the nematode suppressiveness of soils flooded for the rice crop. A disadvantage of flooding is that the field cannot be used to grow crops, and so provide income, for an extended period of time. Fields also need to be non-sloping. As the use of nematicides becomes more restricted, the practice of flooding has gained more attention in high-value crops where quarantine regulations require zero levels of certain nematode species, e.g. flower bulb fields in The Netherlands are kept inundated for 17 weeks to reduce *D. dipsaci* to undetectable levels.

13.9.5. Anaerobic soil disinfection

A technique called anaerobic soil disinfection (ASD), also called biological soil disinfection (BST), has been developed as an alternative to the use of methyl bromide. ASD consists of incorporating copious quantities of organic matter into the soil, followed by irrigation to obtain a moisture level above field capacity and carefully sealing the amended soil with impermeable polyethylene sheeting for several weeks. The decomposing organic matter (e.g. rice husks, grass or crop by-products) and the wet conditions result in anaerobic conditions while several toxic gaseous compounds are produced. The method requires sufficiently high temperatures and careful sealing to trap the gases and establish the anaerobic conditions necessary for

killing soil pathogens, including nematodes. ASD is applied on sandy soils for strawberry and asparagus production in The Netherlands and California. The method still needs optimization, but is promising (Thoden *et al.*, 2011).

13.10. Biologically based Practices

13.10.1. Crop rotation

Seasonal rotation on the same area of land with different crops remains one of the most important methods of nematode management. Rotation with crops that have different nutritional demands on the soil and have different pest problems has obvious benefits to the maintenance of the system. Supposedly, the Incas practised crop rotation in response to damage caused by the host-specific potato cyst nematodes. However, intensification of cropping systems and the success of pesticides has seen reduced reliance on crop rotation in modern agriculture. The basic premise of crop rotation is to distance the time between susceptible hosts to the same nematode species using resistant, poor or inhibitory hosts, in order that population densities do not increase to damaging levels, or decline below damage thresholds before the next fully susceptible crop is grown. The number of cropping sequences or the period between susceptible hosts can depend on, in particular, the host status of the rotation crops (and cultivars), the nematode species and the nematode population density at harvest of the last susceptible crop. A rotation of at least 7 years between potato crops was traditionally necessary to prevent losses due to *G. rostochiensis* and *G. pallida* in Europe but, with the integrated use of nematicides and cultivars resistant to *G. rostochiensis*, this has since been shortened (Phillips and Trudgill, 1998). While crop rotation is widely practised and is environmentally appealing, it has important limitations. The occurrence of nematode communities containing multiple pests or polyphagous species with wide host ranges, such as some species of *Meloidogyne* or *Pratylenchus*, limits the potential of using acceptable non-host crops for the rotation. Rotation crops may also facilitate the increase of alternative nematode pest species. The degree of control is dependent on the level of resistance of the rotation crops and the length of the rotation cycle, which may be too long to be acceptable, especially for specialist producers in intensive production systems and for subsistence farmers with limited land. Further difficulties arise where the non-host crops have no local market or are of limited value. Correct nematode identification and knowledge of the host range and cultivar susceptibility, therefore, determine the introduction of successful rotations. *Meloidogyne* spp. as a group presents the most formidable challenge to the implementation of successful rotations because of their broad host range. Accurate identification of species present is essential but the development of ‘resistance-breaking’ populations, the emergence of virulent strains/races or pathotypes and communities of multiple species of *Meloidogyne* all pose a challenge. Poor control of weeds, which host polyphagous nematode pests, will also reduce the effectiveness of crop rotations. Nevertheless, crop rotation as a nematode management strategy can be employed efficiently and can provide an essential measure in integrated pest management programmes.

Heterodera glycines, *G. rostochiensis*, *D. dipsaci* and even some species of *Meloidogyne* have limited host ranges amongst crop species or, alternatively, occur in situations where they tend to be the dominant nematode species. They provide

examples where crop rotation has provided effective nematode management. *Heterodera glycines* has few alternative host crops and cultivation of non-hosts such as maize and groundnut for 2 years can be sufficient to provide a full yield of soybean. This can be further reduced, or the number of consecutive soybean crops increased, through the integration of resistant cultivars (see Chapter 14). In California, successful management of *M. naasi* on barley has been accomplished through barley-oat rotations. The development of cultivars with resistance to key nematode pests continues to improve the flexibility of nematode management programmes based on crop rotation.

13.10.2. Trap cropping

Certain situations may accommodate the implementation of trap cropping, where a highly susceptible host is planted and grown for sufficient time to permit nematode invasion and development, but not to complete its life cycle. This strategy is primarily employed for sedentary endoparasitic nematode management. The trap crop must be physically removed or destroyed (with herbicide or ploughed in) before the nematodes reproduce.

An alternative approach involves the use of resistant crops or cultivars, which stimulate nematode activity and/or support nematode invasion but do not permit completion of the life cycle. Such plants are also termed trap crops. Examples of resistant crops that do attract nematodes are sunn hemp (*Crotalaria spectabilis*) for *Meloidogyne* spp., *Sesbania rostrata* for *Hirschmanniella oryzae* in rice and *Solanum sisymbriifolium* for the control of *Globodera* spp. on potato in Europe (see Chapter 8).

The trap crop is preferably planted quite densely so that roots and root diffusates reach as many nematodes as possible. Success depends upon proper planting techniques, precise timing and total crop destruction in the case of susceptible crops. Trap cropping is therefore rather costly and inconvenient but can be a very effective tool for nematode management.

13.10.3. Antagonistic plants

Plants with nematode antagonistic properties due to root diffusates can be used in rotation or as intercrops with susceptible crops against certain nematode species. Species of marigold (*Tagetes erectus*, *T. patula* and *T. minuta*) are good examples, which successfully reduce populations of *Pratylenchus* spp. and *Meloidogyne* spp. as well as other species. The mode of action of *Tagetes* spp. is attributed to alpha-terthienyl, a very toxic compound contained in the root cells, but also simply to its non-host status for several nematode species, although some Trichodoridae are reported to increase on marigold.

The value of the crop and of the antagonistic plants determine the suitability of this approach, although antagonistic crops with a potential market, such as asparagus (*Asparagus officinalis*), will undoubtedly improve the acceptability. For example, use of *Tagetes* spp. for nematode management has increased with its increased use as a source of xanthophylls for food colourants.

13.10.4. Cover crops

Numerous examples of plants that are antagonistic, resistant, suppressive or detrimental to the population development of plant-parasitic nematodes are available (see Whitehead, 1998; Luc *et al.*, 2005). However, many such plants often have no direct commercial value, limiting their appeal, compared with those that have an alternative use. Additional functions may include use as cover crops for soil conservation during the off season (winter or dry period), or as forage for livestock (e.g. joint vetch (*Aeschynomene* spp.), hairy indigo (*Indigofera hirsuta*), stylo (*Stylosanthes* spp.)). Leguminous crops contribute to soil nitrogen availability, whilst crops that produce extensive foliage may be ploughed in as a green manure. Cover crops with nematode-suppressive qualities vary with geographical location and target nematode species. Noteworthy examples include *Aeschynomene* spp., horse bean (*Canavalia ensiformis*), butterfly pea (*Centrosema pubescens*), *Crotalaria* spp., kudzu (*Pueraria phaseoloides*), castor (*Ricinus communis*) and particularly velvet bean (*Mucuna pruriens* and *M. deeringiana*). In tropical regions, grasses and cereals are generally poor hosts of *Meloidogyne* spp. and are often successful in reducing *M. javanica* and *M. incognita*. Additionally, when ploughed in, some crops produce or release nematotoxic compounds upon decomposition. Brassica crops such as rapeseed (*Brassica napus*) and mustard (*B. campestris*) contain glucosinolates, which become hydrolysed to the volatile isothiocyanate and other products with broad biocidal activity. These act as biofumigants following incorporation of brassicaceous residues into the soil, reducing soil-borne pathogens including plant-parasitic nematodes. Brassica crops and cultivars vary in their glucosinolate content and their effects on nematodes vary according to the physiological stage of the nematode and the environmental conditions. Their role in reducing pathogen densities is also attributed to merely adding organic matter to the soil, thereby stimulating diverse soil microbial activity, which in turn can have a positive effect on plant growth while reducing the share of plant pathogens.

Taking advantage of the off-season to grow a poor host as a cover crop has numerous merits for the farmer. However, if this period coincides with a period of natural nematode dormancy (see Chapter 8) or low activity, only limited nematode management may be achieved.

13.10.5. Fallow

The term black fallow is used when no vegetation is allowed to grow on land, whereas fallow refers more to a period without agricultural cropping, but where weeds can still maintain or even increase nematode populations. Keeping land free of vegetation through frequent tillage or herbicide application can reduce nematode populations primarily through starvation, although desiccation and exposure to solar heat may contribute. In general, the practice is not particularly attractive due to the increased risk of soil erosion and loss of production during the period of black fallow.

In traditional subsistence cropping systems in developing countries, smallholders rely on natural fallow periods for several seasons following crop production, for restoration of soil fertility, maintenance of soil structure and suppression of pests. However, this practice can be maintained only where sufficient land is available. Intensification of cropping practices and rising human populations increasingly limit the extent of this practice.

13.11. Amendments

Application to soil of fertilizers or organic matter is a readily accepted practice for improving crop production, primarily with regard to improving soil fertility and structure but which can also lead to reduced plant-parasitic nematode population densities.

13.11.1. Organic matter

Amending soil with various sources of organic matter (Table 13.1) has led to reduced nematode problems, either by reducing population levels or by increasing yields without affecting populations (Widmer *et al.*, 2002; Thoden *et al.*, 2011). The specific mechanisms of control are not clearly understood. Nematode suppression is certainly attributable to increased saprophytic and antagonistic soil biota activity following application of organic matter. It is also recognized that crops are less stressed and more tolerant of nematode parasitism when grown with mulches than in less favourable conditions. The release and consequent build-up of organic acids, phenolic compounds, ammonia or other compounds to concentrations toxic to nematodes are also prime factors in the nematicidal activity of different soil amendments. Numerous nematode genera are reported affected, although most studies deal with species of *Meloidogyne*. Application of oilseed waste (cakes), such as castor, neem (also known as margosa) (*Azadirachta indica*), cotton (*Gossypium* spp.), groundnut and mustard, amongst others, appear particularly effective at reducing nematode population levels. Waste crop by-products, such as sawdust, fruit pulp, coffee husk, oil palm debris and molasses, are also attractive amendments. Although such waste products tend to be inexpensive, they are often only locally available and require transportation to the field. Consequently, their practical use is of limited value for widespread field implementation. However, locally they can offer an effective means of nematode management and soil fertility improvement. Amendment with animal waste products such as manures, bonemeal and chitin can also lead to reduced nematode populations. The addition of crustacean chitin has proved highly effective, leading to the registration of commercial products. Chicken manure also appears particularly effective and, as with chitin, its activity probably depends on the release and build-up of nematotoxic levels of ammonia. In some banana production systems mulching with a range of organic material has led to reduced damage by *R. similis*, caused by several factors, including suppressing temperature increases, which would otherwise be more optimal for nematode multiplication. Applying animal manure or cultivating a green manure crop is a regular agricultural practice. The additional benefit of nematode management may thus be an important and useful supplementary gain. However, although the mechanisms surrounding the reduction of nematode populations or damage appear complex, in general, large quantities of material are necessary to be effective. Therefore, the material must be locally available and inexpensive.

In addition to amending soil with plant material, aqueous extracts or leachates from plants with nematicidal properties has received considerable attention. Commonly termed a botanical pesticide, the principle exploits nematicidal compounds from certain

Table 13.1. Plants most readily noted for field nematode management.

Plant	Method	Plant part used
American jointvetch (<i>Aeschynomene americana</i>)	Rotation	—
<i>Brassica</i> spp.	Incorporated	Whole plant
Bahiagrass (<i>Paspalum notatum</i>)	Rotation	—
Butterfly pea (<i>Centrosema pubescens</i>)	Rotation	—
Castor (<i>Ricinus communis</i>)	Incorporated/rotation	Oil cake
Cotton (<i>Gossypium</i> spp.)	Incorporated/rotation	Oil cake
Sunn hemp (<i>Crotalaria</i> spp.)	Incorporated/rotation	Whole plant
Giant star grass (<i>Cynodon nlemfuensis</i>)	Rotation	—
Groundnut (<i>Arachis hypogaea</i>)	Incorporated/rotation	Oil cake
Hairy indigo (<i>Indigofera hirsuta</i>)	Rotation	—
Horse bean (<i>Canavalia ensiformis</i>)	Rotation	—
Lemon grass (<i>Cymbopogon flexuosus</i>)	Incorporated	Leaves
Marigold (<i>Tagetes</i> spp.)	Incorporated/rotation	Whole plant
Mexican sunflower (<i>Tithonia diversifolia</i>)	Incorporated/rotation	Leaves
Mustard (<i>Brassica campestris</i>)	Incorporated	Leaves
Neem [margosa] (<i>Azadirachta indica</i>)	Incorporated	Leaves, oil cake, seeds
Pangola grass (<i>Digitaria decumbens</i>)	Rotation	—
Kudzu (<i>Pueraria phaseoloides</i>)	Rotation	—
Rye (<i>Secale cereale</i>)	Incorporated	Leaves
Sesame (<i>Sesamum indicum</i>)	Incorporated/rotation	Oil cake
Sorghum (<i>Sorghum bicolor</i>)	Incorporated/rotation	Leaves
Sunflower (<i>Helianthus annus</i>)	Rotation	—
Velvet bean (<i>Mucuna</i> spp.)	Rotation	—

Note: Plants grown in rotation are often incorporated in due course, but are included here as their cultivation in rotation (in some cases intercropped) or as a cover crop provides nematode pest reduction. Incorporation refers to the fact that plant parts are transported to the site for the purpose of mulching and incorporated into the soil. Organic mulches/amendments in general, irrespective of plant origin, tend to result in nematode suppression.

plants, which are applied as a pesticide following extraction. Many plants with nematicidal properties are also applied as organic amendments, in the form of leaves, bark or pounded seeds, for example to the base of the planting hole or as a mulch. Neem (*A. indica*) is perhaps the most studied, with some convincing results against various nematode species when applied as a mulch, dip or drench. Other notable examples include *Tagetes* spp., *Crotalaria* spp. and lemon grass (*Cymbopogon flexuosus*), whilst some seaweeds are less well known but produce a range of bioactive compounds.

13.11.2. Non-organic matter

Various levels of nematode control following application of mineral fertilizers have been observed. Applications of certain fertilizers may be toxic to nematodes or suppress their multiplication and damage through changes in host nutrition. The interactions

between N, P and K availability and nematode populations and/or damage has probably received most attention, especially K, which is also understood to have a general balancing effect on N and P. However, results are often contradictory or highly variable in the levels of control obtained (Coyne *et al.*, 2004). Indeed, this may depend on several factors such as the fertilizer type and rate of application, the minerals applied and their chemical formulation. However, differences in biotic and abiotic factors between sites, in climate and environmental factors, which affect nematode population dynamics, undoubtedly compound the influence of mineral availability on nematodes. Moreover, the magnitude and complexity of the probable interactions make it difficult to provide general recommendations on the use of mineral fertilizers for nematode management.

13.12. Time of Planting

Planting to avoid periods of peak nematode activity can be exploited in certain circumstances to reduce nematode damage. Crops planted during periods when temperatures are sub-optimal for nematode development can enable seedlings to be sufficiently advanced to withstand nematode attack. Crops may still be affected but, due to delayed nematode maturation, peak activity may occur too late in the crop cycle to result in heavy yield losses. Autumn sown, as opposed to spring sown, cereals in Europe suffer lower yield losses to *H. avenae*; carrots are sown late in California to avoid *M. incognita* damage. Care should be taken, however, not to cause a shift in nematode species that are better adapted to the time of planting. Early planting of potatoes in north-west Europe to avoid damage by *G. rostochiensis* contributed to the shift towards *G. pallida*, the potato cyst species that hatches at colder temperatures.

13.13. Other Control Practices

Various other practices can also incidentally affect nematode multiplication. For example, weeding may affect nematode densities either positively or negatively, depending on whether the dominant weeds are nematode hosts, antagonists or trap crops. If dominant weeds are a preferred host of key nematodes, their removal may result in increased nematode damage to the crop due to removal of the main host, or less damage to the crop if the weeds can be exploited as trap crops. The level of nematode attack on fruit trees can also be related to the timing of pruning. Knowledge of such interactions may help growers improve their cropping practices simply by adjusting the timing of their regular activities. In other cases, nematode damage can influence breeding criteria for particular traits, such as shallow rooting systems in citrus trees, which may be more conveniently treated with nematicides than deep-rooted trees.

14 Nematode Resistance in Crops*

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14.1. Introduction

For many important crops that are damaged by nematodes, there are constraints on the management options available to reduce potential crop losses. Such constraints may be the potential or actual harmful environmental side effects of pesticides, as well as practical difficulties in their application to soil for nematode control. Other limitations are imposed by the cropping system, including economic and social factors affecting costs of treatment or avoidance of damage through crop rotations. Crop damage can be economic or have a direct impact on household food security, depending on the nature of the crop and the production system employed. Growers and agricultural scientists increasingly look to the use of resistant cultivars as an environmentally benign, durable and cost-efficient way of avoiding nematode-induced

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crop loss. Pressure to provide alternative nematode management technology has increased exponentially over the past decade or so as a result of the withdrawal of highly effective synthetic nematicides, a massive movement to ‘greener’ cultivation technologies such as conservation, precision and/or organic production approaches, as well as greater awareness among particularly first-world growers of soil health restoration and preservation. Even so, the development and use of resistance requires time and planning if these potential benefits are to be attained. In this case technology could be packaged in seed or other plant-propagation material to the advantage of anyone who could access the particular variety or cultivar that contains a desired trait.

Host ranges differ among nematode species, with some showing a high degree of specialization. Cyst nematode species, in particular, tend to have host ranges restricted to one or a few related plant families. Major pest root-knot nematode species, notably those reproducing asexually, have wide host ranges that contribute to their economic importance (see Chapter 3). Many other non-sedentary nematode parasites also have wide host ranges with some showing preferences for particular plant taxa on which they reproduce most and may become significant pests of crops (Chapters 5 and 6). These differences in host ranges can be exploited to manage nematode populations and reduce yield losses through rotation of crop species (Chapter 12). It is within plant taxa that are generally hosts of a particular nematode species that resistant plants may be found and exploited as sources of resistance.

In this chapter, we describe the impacts of resistant genotypes on host plant growth and nematode populations and provide specific examples to indicate some successes, opportunities and challenges.

14.2. Concepts of Resistance

Resistance is the term used to describe one aspect of the outcome of the interaction between a plant and a parasitic nematode. In the context of resistant cultivars, it refers to plants that have a phenotype different from at least some other plants of the species, and usually different from the commonly grown crop cultivars. In many cases, resistance is towards one extreme of a host efficiency range shown by an array of plant genotypes. Figure 14.1 illustrates not only resistance but also other theoretical potential outcomes of interactions between plants and nematodes.

The phenotypic continuum **susceptible–resistant** is a measure of **host efficiency**. This is measured by comparing nematode reproduction on a number of host plant genotypes. Those that support no or little nematode reproduction are **resistant**; those that allow substantial reproduction are **susceptible**. Note that this phenotypic classification is used as an adjective to describe the plant, but is in fact derived from the genetic interaction between plant and nematode. The important practical relevance of this range of phenotypes is that resistance can be used in crops to manage nematode populations.

The other aspect of the interaction is how a nematode affects its host, the **tolerant–intolerant** (or **sensitive**) continuum in the upper part of Fig. 14.1. This is a measure of **host sensitivity** and may be determined by assessing plant growth in the presence of the nematode, or in crop terms, measuring yield loss caused by nematodes. In practice, the sensitivity of a single plant usually not only depends upon its genotype, but also is related to how many nematodes attack it. At very great nematode population

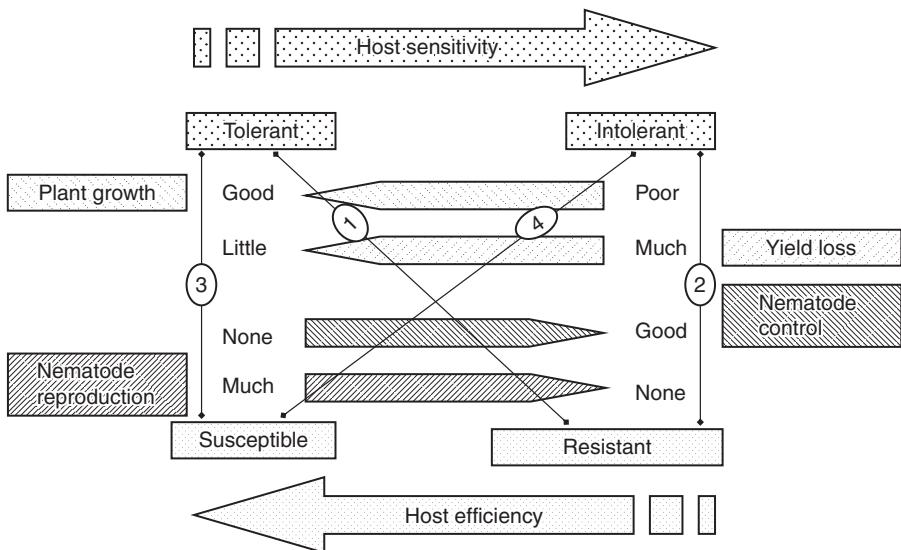


Fig. 14.1. The interrelationships between plants and nematodes defining resistance and susceptibility as extremes of host efficiency (measured by amount of nematode reproduction) and tolerance and intolerance as extremes of host sensitivity measured by plant growth (or by yield). The numbered lines represent potential extreme outcomes. (1) A resistant and tolerant cultivar – the ideal for nematode population management and crop production. (2) Resistant but intolerant, controlling nematode populations but liable to damage when sown in heavily infested fields. (3) Susceptible but tolerant, of limited value as nematode populations can increase to damaging densities. (4) Susceptible and intolerant, of no value where nematodes occur.

densities, plants suffer measurable growth reduction, whereas when only a few nematodes attack the roots most plants grow well. This is of course why we write of nematode management, or more strictly of nematode population management, rather than population control. The objective is to reduce nematode populations and then maintain them at densities less than that at which economic crop loss occurs.

Similarly, host efficiency is affected by nematode population density. Nematode population densities are usually expressed in relation to the volume or mass of soil but a better expression in relation to host efficiency would be nematode density per unit of available host tissue. As nematode density per unit of plant increases, a threshold is reached (governed in part by environment, host crop and nematode species) at which there is too little host resource to support maximum nematode reproduction. At populations greatly exceeding this threshold value or carrying capacity of the host, the final population density of nematodes per unit of soil is less than the initial density. This multiplication threshold is invariably greater than the damage threshold so the observation is of limited practical value but is important in quantifying plant-nematode relationships. Chapter 10 includes a full treatment of population dynamics, different types of threshold values and yield responses.

The outcome of the host sensitivity continuum is also subject to environmental influence through the often interacting effects of conditions on nematodes and plants. Thus, factors that favour nematode activity tend to increase plant sensitivity, whereas

those that favour plant growth tend to increase tolerance. Often such influences may be favourable for both plant and nematode; soil moisture parameters, for example, may promote root growth and nematode movement and hence invasion. At other times, when nematodes have invaded and established feeding sites within roots, dry soils may adversely affect plant growth whilst not affecting the nematodes, thereby reducing the tolerance of the plants relative to growth in moister soils. Host efficiency is also subject to environmental influences although usually to a lesser degree, except that high temperatures may erode the effectiveness of some resistance.

Finally, in this section defining the concepts that are important to developing and using resistant cultivars, we draw attention to the variety of combinations of host efficiency and host sensitivity that exists in plants. These are indicated by the numbered connecting lines in Fig. 14.1 as the following four extreme combinations. (i) A **tolerant and resistant plant** – the ideal for management of plant-parasitic nematode populations. (ii) An **intolerant and resistant plant**, controlling nematode populations effectively but liable to damage when nematodes are numerous. (iii) A **tolerant but susceptible plant**, of limited value where nematode reproduction may increase the population density above the damage threshold. (iv) An **intolerant and susceptible plant**, of no value where nematodes occur. Few cases of high levels of resistance coupled with high levels of intolerance have been documented. Tobacco carrying the *Rk* gene for resistance to *Meloidogyne incognita* exhibits stunting when young plants are transplanted into soils infested with a large population of the nematode. Similarly, upland cotton genotypes (*Gossypium hirsutum*) with resistance to the reniform nematode, *Rotylenchulus reniformis*, introgressed from *G. longicalyx* are intolerant of the nematode. In practice, particularly in subtropical and tropical conditions where a nematode species can complete multiple cycles of reproduction on a single annual crop, effective resistance typically reduces nematode population densities per unit of plant such that, compared to the susceptible plant, resistant cultivars have significantly greater yield. In such situations, and when initial nematode densities are below the damage threshold, intolerant but resistant cultivars will yield more than susceptible ones.

14.3. Inheritance and Mechanisms of Resistance

Resistance to nematodes is expressed in many forms and is conditioned by a wide variety of genetic systems (Williamson and Kumar, 2006). In some cases resistance is conditioned by a single dominant gene with a major effect, typically a classic hypersensitive necrotic reaction by affected plant cells. The *Mi* gene for resistance to *Meloidogyne* spp. in tomato is a well-characterized example of this type of resistance. Other forms of resistance may be conditioned by multiple genes and may also involve either a hypersensitive plant response or less dramatic responses. In some cases the resistant plant fails to support well-developed giant cells (for root-knot nematodes) or syncytium complex (for cyst and reniform nematodes) and rates of nematode development and fecundity are reduced. In some soybean and groundnut accessions, resistance to root-knot nematodes is expressed as a reduction in the number of invading juveniles that establish feeding sites. Initial penetration of the roots is nearly equal between the susceptible and resistant plants but then there is a high rate of emigration from the roots of a resistant plant. Juveniles that do establish a feeding site in such

resistant plants typically have a slower rate of development than do juveniles on susceptible plants. Roots of immune or resistant cassava cultivars form a callous tissue complex in response to nematode infection, thus making giant cell formation difficult.

In some plants resistance is conditioned by recessive genes. This has been observed in several cotton accessions that express moderate levels of resistance. Further, resistance in cotton is often transgressive, where progeny of crosses between two moderately resistant parents express a level of resistance that has greater than expected additive effects. In both groundnut and cotton, there are some resistant accessions where the best evidence suggests that resistance is conditioned by two genes. Further, it appears that one gene in each system is primarily responsible for suppression of nematode reproduction, whereas the other gene is primarily responsible for suppression of root galling.

The observed phenotypic resistance to *Ditylenchus africanus*, the groundnut pod nematode, in groundnut breeding lines point in a different direction (Steenkamp, 2008). AFLP analyses of reciprocal two-way crosses between a susceptible (cultivar) and resistant (line) parent suggested that the resistance is quantitatively inherited (thus, is likely to be polygenic), although the phenotypic expression of resistance is relatively very strong. The linkage analysis indicates that three quantitative trait loci (QTLs) on two separate linkage groups are associated with the observed groundnut resistance to this nematode. Heritability of the trait and maintaining it in progeny is a challenge that would benefit from more in-depth knowledge of the genes or loci that are associated with the trait. Another nematode associated with groundnut, the testa nematode, *Aphelenchoides arachidis*, was originally discovered in Nigeria in 1970 (Dickson and De Waele, 2005) and was regarded as of only local importance until it was found more recently in Egypt and South Africa. Unfortunately, there is no record of resistance to this nematode in groundnut.

Plant resistance is conditioned by a variety of genetic mechanisms, which may be mono-, oligo- or polygenic. The genes involved may be further classified by their effect on phenotypic expression, ranging from major genes (large effects) to minor genes (small effects). The apparent inheritance of resistance genes can be influenced by the genetic background in which they exist. Resistance to root-knot nematodes in the two accessions of upland cotton, Clevewilt-6 (PI65358) and Wild Mexico Jack Jones (PI593649), is conditioned by a single recessive gene. Mapping studies have confirmed that they are two distinct genes and, further, progeny from a cross of these parents exhibit transgressive segregation. There is evidence that this transgressive segregation is due to recessive genes present in some susceptible genotypes (Wang *et al.*, 2008). A further issue with these sources of resistance is that after multiple backcrosses of progeny from this cross to susceptible parents the resistance appears to be inherited as a dominant trait governed by two genes. Thus, that which initially appeared to be a recessive trait is expressed as a dominant trait after introgression into different genetic backgrounds.

The physiological and molecular basis of resistance to most nematodes is still poorly understood. Phytoalexins and other similar plant-produced compounds with antimicrobial activity have been implicated in some plant resistance responses. In lima bean (*Phaseolus lunatus*) and soybean (*Glycine max*) the accumulation of such compounds in necrotic lesions in response to *Pratylenchus scribneri* or *M. incognita* has been observed. These compounds appear to act as repellents to the nematodes.

The *Mi-1.2* gene from tomato is one of several genes for resistance to root-knot nematodes in the *Solanum peruvianum* germplasm and has been studied extensively (see Chapter 15). This gene was introgressed into cultivated tomato, *S. lycopersicum*, in the 1940s and is now widely used in commercial tomato production. The *Mi-1.2* resistance gene has several unique characteristics. It conditions resistance to three nematode species (*M. arenaria*, *M. incognita* and *M. javanica*, which may be due more to how closely related these species are to each other than to any unique attribute of the resistance gene), it is temperature sensitive and non-functional at temperatures exceeding 28°C. This gene also conditions resistance to potato aphids and whiteflies. *Mi-1.2* belongs to the NB-LRR class of plant resistance genes (contain a nucleotide binding site (NB) and a leucine-rich repeat (LRR)). The resistance is similar to resistance to other pathogens that is conditioned by this class of resistance genes. The interaction of the resistance gene with an effector molecule (avirulence gene product), either directly or indirectly, initiates a signal transduction pathway in the host cell. The end result of this pathway is cell death due to a complex of biochemical reactions. An important component of this signal transduction pathway is the protein Rme 1. This protein may act at the same step as the *Mi-1.2* protein or upstream (Fig. 14.2). A key characteristic of such resistance responses is an oxidative burst with production of oxygen-free radicals, which then react with a host of other compounds. Affected host cells also respond with the production of various pathogenesis-related (PR) proteins and phytoalexins. Membrane integrity is compromised. The resulting host cell death inhibits further giant cell development and results in death of the invading nematode. In the case of the *Mi-1.2* gene this host response is rapid, occurring within the first day or two after root penetration. In other resistant plants a similar necrotic response may not be initiated for several

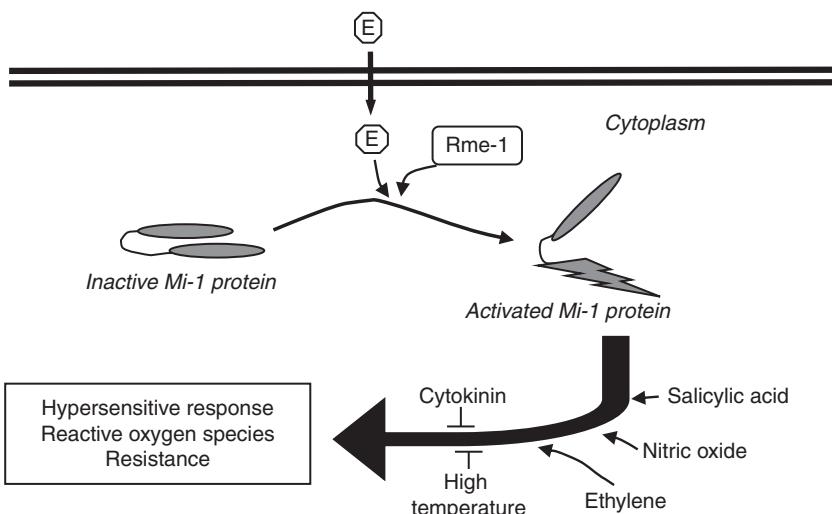


Fig. 14.2. Schematic representation of the interaction of the *Mi-1.2* resistance gene protein in tomato with effector molecules from the nematode and other host components to initiate the resistant host response. The Rme-1 protein from the host may be involved in the activation of the resistance response. (From Williamson and Roberts, 2009.)

days after invasion by the nematode. In such cases the initial development of the syncytia or giant cells appears normal before initiation of the necrotic response. The genes *Mi*-2 through to *Mi*-9, which are also from accessions of *S. peruvianum*, have additional attributes (Williamson, 1998). Some of these genes are not temperature sensitive and others are active against nematode populations that are virulent on *Mi*-1.2. Unfortunately, none of these genes has yet been introgressed into cultivated tomato. The effector molecule from the nematode that initiates the signal transduction pathway has not been definitively identified but one candidate has been reported: the silencing of the *Cg*-1 gene in a nematode renders them virulent on *Mi*-1.2-mediated resistance.

Not all major effect dominant resistance genes condition a necrotic hypersensitive host response. The resistance in groundnut is conditioned by a single dominant gene that confers near immunity but no hypersensitive necrotic response is observed.

The identification of nematode resistance genes has been made difficult by the fact that they often reside in regions of the plant genome that have limited recombination. Further, these regions of the plant genome are often rich in resistance genes and resistance gene analogues. In several cases, the location of the resistance gene has been mapped to a particular region of a chromosome but it has been difficult to identify the precise gene sequence responsible for nematode resistance. In the case of *Mi*, there are actually three closely linked genes with a high degree of homology designated 1.1, 1.2 and 1.3, with only *Mi*-1.2 being the active resistance gene. The genes *Gpa* 2 and *Gro* 1 for resistance to the potato cyst nematodes *Globodera pallida* and *G. rostochiensis*, respectively, have been sequenced and are also NB-LLR type resistance genes. However, the *H1* gene for resistance to *G. rostochiensis* remains elusive. *H1* resides in a large cluster of genes of the CC-NB-LRR type and precise identification has not yet been achieved.

Similarly, resistance to the soybean cyst nematode, *Heterodera glycines*, is complex with several QTLs contributing to the resistant phenotype, some of which are dominantly inherited (*Rhg*4) and some as recessive genes (*rhg*1). Although there are many markers linked to these loci the identification of actual genes has been difficult (Concibido *et al.*, 2004). Initial reports that they were receptor kinases have not been confirmed. One report (Cook *et al.*, 2012) provides evidence of two genes, an α-SNAP protein and a WI12 wound-inducible protein being involved in the resistance response (see Chapter 15). Interestingly, susceptible soybean has a single copy of these two linked genes, whereas resistant genotypes have ten tandem repeats of these genes. Another recent report provides strong evidence for *Rhg*4 being a serine hydroxymethyltransferase involved in serine ↔ glycine interconversions and essential for one-carbon metabolism in the plant (Lui *et al.*, 2012). Although the mechanism by which this protein confers resistance is yet to be characterized, the authors offered three alternative hypotheses for the resistance mechanism. Alterations in folate homeostasis may trigger a necrotic hypersensitive host response. The alteration of one-carbon metabolism may lead to production by the plant of a nematicidal compound. Lastly, alteration in the plant's folate metabolism may result in a severe nutrient deficiency to the nematode parasite. Thus current evidence for resistance to cyst nematodes in soybean points to very novel mechanisms based on genes not previously associated with resistance.

14.4. Virulence in Nematodes

The development of resistant cultivars has largely been based upon single, major genes (barley, groundnut, potato and tomato) or on a few genes (cotton, potato and soybean). During the course of breeding, it has become apparent that many plant–nematode interactions conform to predictions of the gene-for-gene hypothesis (Box 14.1). This postulates that for each plant resistance gene (*R*-gene) a pathogen has a corresponding avirulence gene (*Avr*-gene). The interactions between the products of these genes condition whether the plant is resistant and the nematode is avirulent or conversely the plant is susceptible and the nematode is virulent. When resistance and avirulence are conditioned by the dominant allele, the four possible genotypic combinations give rise to the two phenotypes of each organism (Fig. 14.3). Such genetic interactions have been formally demonstrated for potato and soybean cyst nematodes. It is also likely that they apply to interactions with root-knot nematodes, many species of which reproduce asexually so that formal genetic proof is more difficult, requiring cloning of *Avr*-genes.

Box 14.1. Small grain cereals and cyst nematodes.

The use of resistance in cereals to *Heterodera* spp. is complicated by the diversity of the cyst nematodes. Resistant cultivars have been developed in Europe and India but have been most successful in Australia. It is likely that cereal cyst nematodes in Australia are introduced and are of limited genetic diversity. Australian populations appear to have a single virulence phenotype that is distinct from virulence phenotypes prevalent in Europe, although there are similarities with those in Spain and Germany.

The resistances used in wheat and barley in Australia are also effective against some, but not all, European nematodes. Oat that is fully resistant in Australia is not highly resistant in Europe. The use of resistant cereals allows the intensive culture of wheat in much of Australia, and after 20 years there has been no emergence of virulence. However, in fields in which cyst nematode populations have been controlled, species of migratory root-lesion nematodes (*Pratylenchus* spp.) increase and cause damage.

In Europe, notably in the UK and in Scandinavian countries, resistant cultivars of oat and barley have been bred and grown successfully for short periods. Virulent pathotypes, and also new species, have emerged on cultivars with major gene resistance. These virulent nematodes have not become a general problem because other factors control cereal cyst nematodes. These factors include soil suppressiveness by fungal parasites of nematode females and eggs, coupled with the greater tolerance of winter-sown crops and good growing conditions.

Rice has a notorious history of susceptibility to aggressive species of cyst, rice-root and root-knot nematodes. Little resistance has been found in Asian rice (*Oryza sativa*) but most African rice genotypes (*O. glaberrima*) are regarded as resistant to some of these nematode species. Resistance to *Heterodera sacchari* appears to be a qualitative trait in progeny of crosses between the rice species, whereas resistance to *Meloidogyne graminicola* appears to be quantitative.

Although there are numerous reports of resistance to various nematodes in maize, sorghum and pearl millets, little effort has been expended in the development of that resistance. In many instances the nematodes are not viewed as pests of sufficient importance on these crops for resistance to nematodes to warrant attention from plant breeders.

	Avr/Avr	Avr/avr	avr/Avr	avr/avr
R/R	Ra	Ra	Ra	rV
R/r	Ra	Ra	Ra	rV
r/R	Ra	Ra	Ra	rV
r/r	rV	rV	rV	rV

→

	Avr	avr
R	Ra	rV
r	rV	rV

Genotype symbols

R, r = dominant, recessive alleles of plant *R*-gene

Avr, avr = dominant, recessive alleles of nematode *Avr*-gene

Phenotype symbols

R, r = resistant, susceptible plant

a, V = avirulent, virulent nematode

Fig. 14.3. How plant–nematode interactions conform to predictions of the gene-for-gene hypothesis: for each plant resistance gene (*R*-gene) a nematode has a corresponding avirulence gene (*Avr*-gene). The interactions between the products of these genes condition whether the plant is resistant and the nematode is avirulent or conversely the plant is susceptible and the nematode is virulent. When resistance and avirulence are conditioned by the dominant allele, the four possible genotypic combinations give rise to the two phenotypic combinations (resistant/avirulent and susceptible/virulent).

These genetic interactions and the phenotypes they control are the basis of the specificity that is found in some resistant cultivars. A key feature is that populations of both plants and nematodes differ in the numbers and frequencies of their *R* and *Avr*-genes. Resistant cultivars of self-pollinated crops developed by modern plant breeding are pure lines and homozygous at their *R*-gene loci. Hybrid and out-crossing crops may be homozygous or heterozygous at the *R*-gene locus. Nematode populations may similarly be homozygous or heterozygous for their *Avr*-gene(s) or may have different alleles of more than one gene. A single *R*-gene will confer resistance only to avirulent individual nematodes, those with the matching *Avr*-gene. Where the crop and the pest have only a single gene pair interaction, the crop is resistant to the whole nematode population (perhaps in a field, or in all the fields of a region). Where the nematode population is more variable, there may be virulent individuals present that have the selective advantage of reproducing fully on that cultivar. If this occurs then the cultivar will appear to be less resistant. The degree and rate of erosion of the effectiveness of resistance depends upon the frequency of virulent nematodes in the population and rate of growth of the nematode population. A shift in the nematode population structure (frequency of virulence) will occur more rapidly in a species completing multiple generations per season than in species completing one generation per season.

Different terms have been used to describe this variation in virulence within nematode species. For cyst nematodes, populations distinguished by their virulence have been called pathotypes or races (see Chapter 4). In some cases, a field population may correspond precisely with a single pathotype (race). Other fields may have nematode

populations with several virulence genes that have the potential to be selected by *R*-genes. The frequencies of these genes will determine the virulence phenotype of the population, exposure to different resistance genes will then cause changes in *Avr*-gene frequency and thus the phenotypic behaviour of the nematode population. Root-knot nematodes differing in responses to *R*-genes from more than one host plant species have been categorized using a concept of the biotype, which groups genetically similar individuals with a common phenotype (Table 14.1). Nematode pathotypes and races based on specific virulence traits should not be confused with 'host races' identified based on variation in host range. Variation in host range (plant species on which the nematode population will reproduce) is typically unrelated to the ability to reproduce on hosts carrying a specific *R*-gene.

This summary of the genetic basis of variation in resistance and virulence has been developed from the practical experiences outlined in the examples in this chapter and in the light of theory and experiments with plant interactions with other pathogens. This underpins the development of schemes to categorize plant host status and nematode virulence spectra. Pathotype or race identification schemes use selected plants with known resistance and at least one known susceptible as a control to test nematode populations of interest. The plants used are known as differentials. In practice, specific attributes of the individual crop and nematode have been major influences in shaping the schemes used to describe particular nematode population virulence phenotypes. Thus, schemes to categorize potato cyst nematode pathotypes include as differentials potato clones that are heterogeneous and nematode populations that were subsequently proven to be two species (Box 14.2). Schemes for cereal cyst nematode were developed using plants of several small grain cereal genera (barley, oat, rye and wheat) and applied to nematodes that subsequently have been allocated to several quite distinct species. *Heterodera glycines* (HG) types are now the preferred designation for variation in virulence in phenotype in *H. glycines* to different sources of resistance in soybean (Box 14.3).

The function of pathotype classifications is to provide evidence as to the potential effectiveness of resistance against a range of populations of particular crop nematode pests. The information from these schemes has to be useful in plant breeding in directing the choice of resistance sources. Such a practical function means that the classifications do not always provide precise evidence on gene-for-gene interactions. Thus, many of the differential potatoes and cereals have 'cryptic' resistance genes whose existence was only revealed as the differentials were tested against more populations

Table 14.1. Hypothetical example of biotype designations for four isolates of a *Meloidogyne* spp. on tomato (*Solanum lycopersicum*; S.lyc) based on virulence on different *Mi* resistance genes (from Roberts, 1993).

Isolate	<i>mi</i>	<i>Mi1</i>	<i>Mi2</i>	<i>Mi3</i>	<i>Mi4</i>	<i>Mi5</i>	<i>Mi?</i>	Biotype
ABC	+	–	–	–	–	–	?	S.lyc 0/1.2.3.4.5
DEF	+	+	+	–	–	–	?	S.lyc 1.2/3.4.5
GHI	+	+	–	+	–	+	?	S.lyc 1.3.5/2.4
JKL	+	+	+	+	+	+	?	S.lyc 1.2.3.4.5/

mi, susceptible genotype; *Mi1* to *Mi5* are genes for resistance to *M. arenaria*, *M. incognita* and *M. javanica*; +, a compatible interaction, –, an incompatible interaction.

Box 14.2. Potato and cyst nematodes.

Potato cyst nematodes (PCNs) are distributed worldwide but originate from South America, the centre of origin of the potato. *Globodera rostochiensis* has a more southerly native distribution than *G. pallida*. Both species were distributed around the world on tubers either directly from South America or as secondary spread from Europe.

Resistant cultivars with gene *H1* from *S. tuberosum* ssp. *andigena* CPC1673 have been widely grown in north-west Europe from about 1970. In The Netherlands 55% of ware and 99% of the starch potatoes are resistant to one or more PCN pathotypes. In the UK about 45% are resistant to pathotype Ro1. These cultivars still control *G. rostochiensis* Ro1 but growing them has led to the emergence, recognition and current dominance of *G. pallida*. Cultivars with partial resistance derived from *S. vernei* are grown with nematicides to control *G. pallida*. Pathotypes of *G. pallida* have not been clearly distinguished because of incomplete dominance of resistance genes and because nematode virulence is not fixed in field populations (Nijboer and Parlevliet, 1990). This is consistent with the observed selection for virulence in European populations of *G. pallida* on partially resistant potato cultivars (Turner *et al.*, 1983) and which threatens durable use of such cultivars. Nine genes for resistance to PCNs have been mapped and are located in regions of the genome harbouring genes for resistance to other pathogens. Remarkably, both qualitative and quantitative genes map to the same *R*-gene clusters, suggesting control by similar molecular mechanisms. Like the empirical evidence, this indicates that characterization of resistance as 'quantitative' may be the result of genetic variation in the nematode populations (Bakker *et al.*, 2004).

Resistance is clearly widespread in wild and cultivated *Solanum* species, with 52 of 63 species showing PCN resistance, and with 53 and 57% of 213 accessions resistant to *G. rostochiensis* and *G. pallida*, respectively, and 37% to both. Resistance sources to *G. rostochiensis* appeared to be centred in accessions from Bolivia and Argentina, whilst those resistant to *G. pallida* came from the whole geographic range, indicating selection not only by *G. pallida* but possibly also by the related cyst nematode species from the northern range, *G. mexicana* (Castelli *et al.*, 2003).

In Europe, potato and cyst nematode interactions have been simplified by the genetic bottlenecks resulting from the introduction of limited genotypes of both organisms, followed by strong selection pressure in agriculture. Where there has been secondary spread from Europe to North America genetic diversity amongst the nematodes is further reduced. In South America, genetic interactions are more complex so that it is more difficult to distinguish pathotypes and to exploit simple resistance. The evidence of co-evolution, represented by the widespread incidence of resistance in *Solanum* spp. matched by diversity in virulence in the nematode species confirms that there is a multiplicity of resistance genes. Single resistance genes can be used effectively only where there is little variability in the nematode populations.

from wider areas. In the cereal cyst nematode scheme, it was even found that the susceptible control for the barley differentials was resistant to certain cereal cyst nematode pathotypes. Differential series for pathotype/race testing must be amenable to continuous revision in order to accommodate new sources of resistant plants and nematodes and new genetic information about them.

Box 14.3. Soybean and cyst nematode: classification of virulence phenotypes.

Populations of *Heterodera glycines*, the soybean cyst nematode, exhibit considerable variation in virulence phenotype. The history of classification of virulence phenotypes illustrates the importance of the genetic interactions between plants and nematodes. In the USA, initially four and subsequently 16 'races' were recognized, based on differential development of females on four host genotypes. Unfortunately, the system was flawed from the beginning because: (i) the genes conditioning resistance in the differential hosts were not characterized, with two hosts probably carrying the same resistance genes; and (ii) because the system actually measures frequency of virulence phenotypes (the genetics of which are still inadequately known) within the group of individuals being tested. The race concept in most of plant pathology is based on genotype of an individual, whereas the *H. glycines* race system measured the average virulence 'behaviour' of a population. Because there could be substantial variation in frequency of specific virulences within any of the races, there was little similarity in behaviour of populations with the same race designation from different fields or when tested at different times. Researchers and others tried to infer more genetic information from the original system than was warranted.

A new classification scheme (Niblack *et al.*, 2002) defines 'HG Type' based on the development of females on a set of differential soybean accessions actually used as sources of resistance in formally released cultivars. The differentials are numbered sequentially based on the date of their release as a source of resistance; currently, there are seven differentials, with numbers 1, 2 and 3 being the same host genotypes as three differentials used in the original race classification scheme. This system was developed with the clear understanding that it is not a genetic system but rather based on phenotypic behaviour of a specific population at a given point in time. It is intended to guide the selection of resistant cultivars carrying different combinations of resistance genes at the time at which the nematode population was characterized for HG Type.

Both the original race and the HG Type classification system use a Female Index (FI = number of females developing on the differential/number of females developing on the susceptible standard, expressed as a percentage) to measure virulence on a given differential host (see also Chapter 4). An arbitrary separation of virulence and avirulence was set at FI = 10%. A population with FI >10% on differentials 1, 4 and 7 (and therefore virulent on these) would be designated 'HG Type 1.4.7'. Any cultivar with resistance derived only from differentials 1, 4 and 7 would thus not be highly effective. Conversely, cultivars with resistance derived from differentials 2, 3, 5 or 6 will be effective because measured virulence within the population on each of these sources of resistance was less than 10%.

The HG Type, by definition, is not a genetic tool. It can be expanded as new sources of resistance are introgressed into soybean cultivars. The system is cumbersome, costly and lacks the efficiency needed routinely to type large numbers of populations. However, used correctly, the HG Type system will reduce errors that commonly arose when the old race scheme was used to infer more from the race designation than was possible or appropriate. It has to be recognized that HG Type indicates population behaviour at one point in time. An avirulence rating does not mean an absence of the virulence phenotype in that population, rather it is present at a low frequency (<10%) at that point in time. The HG Type may change as the population evolves in response to environmental influences, in particular to the frequency at which different sources of resistance are deployed in a field.

14.5. Origins and Functions

As agriculture has developed to present-day highly technological forms, there is increasing uniformity of cropping practices over quite large scales. At the same time the degree of genetic diversity in crops has become increasingly restricted. Nematode pathotype and race schemes indicate that there is a diversity of resistance in crop plants and avirulence genes in their nematode pathogens. More *R*-genes are found in ancestral farmer-selected ‘land races’ of crops and in related wild species than in modern cultivars (see Section 14.6.1).

It seems certain that the resistance exploited in crop cultivars is derived from plant–nematode interactions, such as occur in natural ecosystems, but is utilized in crops in agricultural ecosystems that are very different in a number of significant ways. First, the uniformity of crop species in fields provides increased host density that leads to high population densities of fewer nematode species than are encountered in natural ecosystems. Second, the narrow genetic base of the crop tends to select for genotypes of nematodes that are well adapted to the crop and, therefore, reproduce freely on it. Where the crop has no *R*-genes, perhaps because cultivars have been bred by being grown in nematode-free soils where *R*-genes contribute no advantage and were lost during selection for yield, there may be no effect of the uniform crop on the variation in the nematode population. When single *R*-genes are introduced into such a system, it is therefore likely that it will select for increased frequencies of virulent nematode genotypes if virulence exists within the nematode population. When this happens, the resistance appears to have been eroded or ‘broken down’. This is a practical shorthand term because the gene is still effective in controlling nematodes lacking the matching virulence traits. The resistance gene is not broken; rather, the nematode no longer produces a functional avirulence gene product that can interact with the resistance gene product to initiate the resistance response.

In nature, it is likely that resistance developed between co-evolving plants and nematodes. The incidence of extensive resistance sources among plant species that are ancestors of or related to crops suggests that the co-evolution is ancient. Comparisons of gene sequences thought to be involved in the parasitism of two cyst nematodes (*H. glycines* and *G. rostochiensis*) and a root-knot nematode (*M. incognita*) indicates that nematodes have diverged greatly from other organisms, also suggesting an ancient lineage (Baum *et al.*, 2004; Smart *et al.*, 2004). Molecular evidence suggests that two morphologically similar cyst nematodes (*H. glycines* and *H. schachtii*) diverged from each other between 7 and 4 million years ago (Radice *et al.*, 1988). This is additional evidence that indicates the very long timescale of plant-nematode co-evolution, compared to the domestication of most crops during the development of agriculture in the past 10,000 years.

Nematode crop pests have also been affected by the development of agriculture and some may be as different from nematodes in natural vegetation as are their crop hosts from wild plants. This may well be the case with the parthenogenetic root-knot nematodes (see Chapter 3; Trudgill and Blok, 2001). These have apparently undergone hybridization and polyploidization that has striking parallels with the evolution of several crop plant species. In some cases, the nematode pests have been introduced with their crop hosts into areas well beyond the apparent region or common centre of diversity where co-evolution occurred. This has certainly happened with potato cyst nematodes introduced into Europe and North America with potatoes from South

American agriculture that had domesticated the potato close to the centres of diversity of both plant and nematode. Also, the cereal cyst nematode seems to have been introduced into Australia along with cereal cropping from Europe. Similarly, clover cyst nematode appears to have been introduced from Europe into New Zealand, where it thrives on introduced white clover. Soybean and its cyst nematode have also been introduced from Asia into North and South America. These cyst nematodes have spread with their crop hosts in three continents, becoming significant pests within periods of less than 100 years. It is possible that the parthenogenetic root-knot nematodes have also spread in cropping systems throughout the world in relatively recent years.

14.6. Exploitation

14.6.1. Sources of resistance

There is a hierarchy of plant resources in which resistance to particular nematodes may be found. The development of resistant cultivars has been quicker and easier when sources of resistance are found in plants closely related to the susceptible cultivars. This is because there are no cytogenetic barriers to hybridization and fewer undesirable characteristics genetically linked to the resistance genes. When resistance is not available in closely related sources, more distant relatives need to be examined, including older varieties of the crop and also related, perhaps ancestral, species.

There are extensive bibliographies of nematode resistance and some germplasm collections have been thoroughly screened for resistance. Much of this information is widely available and provides a first screen to increase the likelihood of identifying sources effective against local populations. The existence of variation in *R*-genes and *Avr*-genes means that local testing is essential to validate reported resistance from other regions or countries. Various national and international germplasm collections also provide a wide range of material; some indication of what is freely available may be seen at the Germplasm Resources Information Network (GRIN) via <http://www.ars-grin.gov/npgs/>, which contains links to many germplasm repositories.

In wheat, a crop created during domestication of naturally occurring hybrids between wild species, resistance to cyst nematodes has been introduced by breeding from the ancestral wild species. Similarly, resistance to potato cyst nematode and to root-knot nematodes of tomato has been introduced to the crops from related but wild species. Such resistance is more difficult to use in breeding programmes and there may be many problems to be overcome before successful resistant cultivars can be produced.

There are usually cytogenetic barriers to hybridization between related species and, if these can be overcome, generally a number of undesirable features of the wild source are also transferred. The resulting increased breeding and selection efforts increases the costs and timescale of the breeding programme. Thus, Castelli *et al.* (2003) found cyst nematode resistance in 52 out of 63 wild and cultivated species of *Solanum*, the potato genus. Although not all wild species can be easily crossed with cultivated crops, some barriers can be overcome by a variety of procedures that in combination with resistance screening can transfer the gene(s) for resistance into plant breeding programmes (Box 14.4).

Box 14.4. Groundnut and *Meloidogyne arenaria*.

Resistance to *Meloidogyne arenaria* and *M. javanica* has been introgressed into groundnut (*Arachis hypogaea*) from a wild species. The cultivated groundnut is an allotetraploid ($2n = 40$) believed to contain an A and B genome, each with 20 chromosomes. Most wild *Arachis* species are diploids ($2n = 20$) with either the A or B genome and, therefore, are not directly compatible with the cultivated type. Two approaches have been used to introgress resistance to *M. arenaria* into cultivated groundnut from the diploid A-genome species *A. cardenasi*.

In a hexaploid pathway, the diploid species was crossed with the tetraploid, resulting in a sterile triploid. Treatment of embryos with colchicine resulted in the formation of a hexaploid, which had low to moderate fertility. Typically, several generations of back-crossing to a tetraploid recurrent parent would be necessary to achieve introgression of the resistance gene from the hexaploid into a tetraploid. In this case, however, a genetic 'error' resulted in the reversion of the hexaploid to a resistant tetraploid after one generation. This individual was fully compatible with cultivated groundnut and used as a parent in a traditional breeding scheme.

Another successful approach, the tetraploid pathway, was to cross a diploid B-genome individual (*A. batizocoi*) with an interspecific diploid A-genome hybrid individual (*A. diogoi* × *A. cardenasi*) to generate a sterile AB genome diploid. Colchicine treatment of the sterile AB diploid resulted in a fertile AB-tetraploid, which was readily cross-compatible with cultivated groundnut and also carried the gene(s) for nematode resistance from the original A-genome parent *A. cardenasi*. The first groundnut cultivars with effective resistance to *M. arenaria* were subsequently developed from this tetraploid pathway by completing several backcross generations with a high-yielding cultivar as the recurrent parent.

It is interesting that in wild species resistance often appears to be less specific than that of modern cultivars. Some of these sources are resistant to several species and some to more than one genus of nematodes. There is little evidence to indicate whether such sources have a single resistance mechanism effective against more than one nematode species. Nonetheless, in some cases it seems possible that the same mechanism is involved; for example, sweet potato selected for resistance to *Pratylenchus coffeae* also proved to be resistant to *M. incognita*. It may be that in wild species such less specific resistance may be due to 'clusters' of resistance genes, which are disrupted by breeding and selection for a more specific resistance.

Resistance may lack durability because repeated use of single resistance genes often leads to a shift in the virulence characteristics of the nematode population, such that with time a specific resistance gene is no longer effective. This has been demonstrated with *Globodera* and *Heterodera* species on potato and soybean, respectively (Turner, 1990; Young and Hartwig, 1992), and for *M. incognita* and *M. javanica* with virulence to the *Mi* gene in tomato (Kaloshian *et al.*, 1996; Ornati *et al.*, 2001). However, if the nematode population in a given field or region lacks the appropriate diversity with respect to virulence, then there may be no selection for virulence with repeated use of a given resistance gene. This appears to be the case for the *H1* gene for resistance to *G. rostochiensis* in some regions. Similarly, repeated use of resistance may cause a shift in the species present in a field, with species against which the resistance is not effective becoming dominant. This has been documented for tobacco,

Table 14.2. Some food crops for which high-yielding cultivars with resistance to one or more nematode species are available (from Roberts, 1982; Sikora *et al.*, 2005).

Crop	Nematode	Crop	Nematode
Apricot	<i>Meloidogyne</i> spp.	Maize	<i>P. hexincisus</i>
Barley	<i>Heterodera avenae</i>	Peach	<i>M. incognita</i>
Bean, common	<i>M. incognita</i> , <i>M. javanica</i> , <i>Pratylenchus scribneri</i>	Potato	<i>Globodera pallida</i> , <i>G. rostochiensis</i>
Citrus	<i>Tylenchulus semipenetrans</i>	Oat	<i>D. dipsaci</i> , <i>H. avenae</i>
Clover	<i>Ditylenchus dipsaci</i>	Rice	<i>Aphelenchoides besseyi</i> , <i>D. angustus</i>
Cotton	<i>M. incognita</i>	Tobacco	<i>G. tabacum</i> , <i>M. arenaria</i> , <i>M. incognita</i>
Cowpea	<i>M. incognita</i>	Sweet potato	<i>M. arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i> , <i>R. reniformis</i>
Grape	<i>Meloidogyne</i> spp., <i>Xiphinema index</i>	Tomato	<i>M. arenaria</i> , <i>M. incognita</i> , <i>M. javanica</i>
Groundnut	<i>M. arenaria</i> , <i>M. javanica</i>	Walnut	<i>Meloidogyne</i> spp.
Lucerne	<i>D. dipsaci</i> , <i>M. hapla</i>	Wheat	<i>H. avenae</i> , <i>P. neglectus</i> , <i>P. thornei</i>

where increased use of resistance to *M. incognita* led to an increase in the frequency of *M. javanica* against which the resistance was not effective, and in potato where use of resistance to *G. rostochiensis* led to an increased incidence of *G. pallida*. The point needs to be emphasized that variability with respect to virulence must exist within a population (either variation within a species or among species) for the use of resistance to select for a greater frequency of the virulence phenotype. Where the nematode is an introduced pest, the introduction process often represents a genetic bottleneck that reduces diversity relative to that which exists at the centre of origin. Thus the number of potential virulence races in an introduced nematode population is typically fewer than the number that exists at the centre of origin for the nematode where it co-evolved with the host.

For most nematode pests there is no shortage of reports of resistance and many untapped sources are available. De Waele and Elsen (2002) and Peng and Moens (2003) list many sources of resistance to migratory parasitic nematodes. Peng and Moens reviewed 81 crop–nematode specific combinations and reported that there was resistance in 46 crops to 30 species of 12 nematode genera. Bred cultivars were said to be available for 25 of the 81 combinations, although not all are well adapted for production. Roberts (1982) and Sikora *et al.* (2005) have listed 22 crops, including annuals, trees and other perennials, for which there are high-yielding cultivars available with resistance to 19 species in nine genera of nematodes (Table 14.2).

14.6.2. Selection criteria

The criteria for selecting plant germplasm as sources of resistance include the genetic relatedness of the source to the crop of interest. This is important as it affects the ways and ease with which resistance may be transferred, as indicated in the preceding

and following sections. The aspects of selection criteria that are critical to the development of resistant cultivars derive from the definitions of resistance and tolerance and the factors that influence their expression (see Section 14.3). The application of selection criteria in breeding programmes (screening) has two main applications: the first is identification of sources, the second is selection of resistant individuals from segregating populations during breeding. The principles that are relevant to the choice of method and of assessment criteria are broadly the same for both purposes. Done well, screening not only is accurate and ensures that resistance is effective against the appropriate range of nematode pathotypes or species, but also breaks genetic linkages with undesirable attributes. This provides germplasm suitable not only for cultivar development but also for the associated and relevant evaluations of nematode population control and crop loss reduction.

Quality control is important: screening procedures should have a known degree of accuracy and must be reliably repeatable. The use of 'control' plants is essential: these are genotypes of known response, ideally including both a susceptible and a resistant control. The susceptible control assesses that the test has been successful by indicating the extent to which nematodes have invaded, developed and reproduced in the conditions of the test. This allows comparison among tests over time to ensure that nematode population densities and environmental factors are optimized to detect susceptibility. The resistant control, which obviously can only be introduced once a resistance source is identified, is the target by which other sources or breeding lines are identified. These two types of controls may include more than one genotype of each, and are also replicated throughout batches of 'unknown' plants to be screened. Replication is increased to improve accuracy or reduced to increase the amount of material that can be screened and is determined by the costs (and time) of growing and assessing each screening unit.

The nematodes used in screening may be introduced in a number of ways, as active invasive vermiciform stages, eggs, egg masses or cysts, or by use of naturally infested soil. Generally the order of this list corresponds to decreasing accuracy, but increasing robustness to environmental disturbance. The balance and choice of method depend upon technical resources available and the desired outcomes of the tests. There is also a parallel accuracy/robustness gradient related to the size and 'naturalness' of screening – a gradient with extremes represented by *in vitro* dixenic (only two organisms present) tissue culture methods to tests in fields or field plots. Eventually, and particularly for assessment of host sensitivity, relatively large scale plot experiments are needed to confirm the value of the more refined tests.

It is also important to decide what diversity of nematodes should be included in tests. The alternatives are either to screen with a mixed inoculum or to use nematodes of a known but more narrowly defined genetic base: the mixtures may be of populations, for example with the major root-knot nematode asexual species, or of pathotypes of some cyst nematodes. In using mixtures, the expectation is of identifying resistance that is more broadly based. Inoculum with a narrow genetic base has the danger of identifying sources of limited effectiveness because it may be active only against a small portion of the larger nematode population. Theory strongly supports the use of narrowly based inoculum so as to maximize the numbers of resistance genes selected. In some cases, where broad-based resistance is available, it is sensible to maintain that by screening with a more broad-based inoculum. Other considerations affecting this decision include existing knowledge of variation in virulence or the

likelihood of it occurring in the particular species. The choice is also affected by the goal of the breeding programme, for example, either to introgress a specific resistance gene into a crop or in initial screening of germplasm for new sources of resistance.

Assessments must take account of plant size to minimize false negatives by misclassifying as resistant such poorly growing plants that they are incapable of expressing their genetic susceptibility. This may occur when comparing nematode reproduction on wild species that lack the vigorous growth habit of the cultivated crop. A useful concept is one of effective nematode inoculum density to ensure sufficient root space for nematode invasion during the early stages of a screen. It is also important to consider the numbers of generations for which nematodes are allowed to reproduce as differences between tests (and even plants) may result from counting nematodes at one or more completed reproduction cycles. Generally, assessments of host efficiency are based upon a measure of reproduction by counting nematodes in the new generation. The nematodes counted may be the reproducing females or their progeny (in cysts, egg masses or even as next-generation invasive stages).

Sometimes a closely linked surrogate (marker) may be assessed, such as symptom expressions. These may include root symptoms such as galls, where these clearly indicate the establishment of reproducing root-knot nematodes, or knots on wheat roots characteristic of female cyst nematode feeding sites, or swollen buds typical of reproducing stem nematodes in lucerne. When such surrogates are assessed their relationship to nematode reproduction need to be well described if misclassifications are to be minimized. Although root-galling and reproduction of root-knot nematodes are typically positively correlated, cases of poor correlation where root-galling is not a good measure of resistance have been reported.

Identification of resistant individuals from segregating populations is increasingly reliant on marker-assisted selection (MAS) (Xu and Crouch, 2008). Markers based on identified DNA sequences derived either directly from the resistance gene or sequences closely linked to the resistance locus are used to develop specific polymerase chain reaction (PCR) primers. Although the initial costs of development of such systems can be quite high, the potential for a highly specific, high throughput system makes this technology very attractive. The rapidly increasing amount of genomic sequence data available for most crops is making the development of MAS more efficient. Successful breeding programmes screen thousands of individuals to identify those few that have the desired combination of yield potential, other desired agronomic or horticultural traits and resistance. Evaluation of such numbers of plants using phenotypic assays is much more costly and time-consuming than the use of high-throughput MAS systems. The favoured markers are those that are co-dominant with the resistance allele and thus allow one to select individuals that are homozygous for resistance, which is a highly desirable feature in a breeding programme. At present most MAS systems are for resistance conditioned by single, major effect genes. Polygenic resistance requires the use of QTL markers. Unfortunately, a single QTL recovers only a portion of the available resistance, and when the original resistance is moderate, possibly only a 60–75% suppression of nematode reproduction, then a single QTL that recovers only 50% of the resistance phenotype results in a very low level of resistance in the selected progeny. The use of multiple QTLs is thus required to recover a substantial portion of the total available resistance. An important caveat to the use of MAS is that it does not eliminate the need for verification of the resistant phenotype in the final selections from a breeding programme by direct evaluation of the nematode–host interaction.

14.6.3. Breeding processes

The traditional plant breeding approaches to cultivar development are applicable to nematode resistance. In general terms, this involves four stages: (i) crossing to introduce resistance from the source into the acceptable crop background; (ii) screening the progeny to select those that are resistant; (iii) further crossing (either backcrossing to the recurrent susceptible parent or to other desirable yet susceptible parents); and (iv) repeated screening of each generation of hybrids or progeny to retain the resistance. Eventually, a range of resistant germplasm will be available for further selection accompanied by screening to produce useful resistant plants for multiplication and registration as a new cultivar or perhaps for further hybridization within the breeding programme.

The genetic structure of the crop plant and inheritance of resistance determine the appropriate breeding strategy and its integration with resistance screening. Boxes 14.1, 14.2, 14.4 and 14.5 detail some instances where resistant cultivars have involved transferring resistance from wild species, selection from among the heterogeneous plants of an existing outbreeding cultivar (lucerne) and by backcrossing from an unadapted genotype in pure line inbred crops (barley, soybean).

Transferring resistance from wild plants can take many generations to break linkages of the *R*-gene with genes for characters that are undesirable in crop plants. In tomato, the *Mi* gene from wild *S. peruvianum* confers resistance to root-knot nematodes. After crossing the wild and cultivated tomatoes, the developing embryo had to be excised and grown *in vitro* to obtain a viable hybrid (embryo rescue). Many generations of backcrossing were necessary before the resistance was available in genetic backgrounds suitable for use in breeding programmes to produce resistant cultivars.

Outcrossing crops are selected and released as cultivars that have a more or less uniform, recognizable collection of phenotypes. Even so, these may include a range of nematode resistance genotypes. In this case, it may be possible to select enough individual resistant plants for use as the parent plants to intercross and increase the proportion of resistant plants in a new variety that retains the desirable agronomic features of the original but more susceptible cultivar. A key feature of using outbreeding cultivars with less than 100% resistant plants is that it is essential that selection for resistance is made during early seed multiplication stages if the high levels of resistance are to be maintained (Box 14.5; Fig. 14.4).

In inbred crops, modern cultivars are homozygous and homogeneous; that is, they breed true for all characteristics. Nonetheless, most inbred crops allow a degree of successful outcrossing. As a result, some of the older cultivars retain a small degree of heterozygosity in their resistance genotypes. These may be useful sources of resistance where the cultivar has been maintained by saving seed from enough plants (as saving seed from single plants leads rapidly to homozygosity in inbreds). Such mixtures may also contribute to observations that some cultivars have partial resistance when tested with heterogeneous nematode populations. In modern cultivars, the *R*-gene must be homozygous for the crop to breed true for resistance. Some crop cultivars, such as those of potato, are maintained as clones by vegetative propagation. In these, dominant resistance genes may be used in the heterozygous condition as there is no opportunity for segregation during propagation of seed tubers.

Box 14.5. Stem nematode resistant cultivars.

Resistance to the stem nematode *Ditylenchus dipsaci* has been exploited in a number of crops including inbred cereals (oat) and outbreeding herbage legumes (lucerne, clover). Stem nematode populations are characterized as host races, each associated with a few very susceptible host species or genera but usually able to reproduce to a lesser extent on a wider range of plants. Nonetheless, major gene resistance has been very effective worldwide in lucerne and in the UK and Australia in oat.

In oat, resistance sources were identified among traditionally grown cultivars and transferred by backcrossing into adapted backgrounds. In the UK, the source was Old Grey Winter and a single dominant gene is present in most successful winter oat cultivars grown there. Partly as a consequence of this resistance, but also because oat is grown less frequently than it once was, serious damage is rarely seen. The symptoms of resistance and susceptibility are so reliable that screening techniques are based upon the degree of swelling and necrosis. A similar approach is used with legumes to distinguish resistant and susceptible seedlings (Fig. 14.4). In the field, there are big differences in growth and survival of resistant and susceptible plants on heavily infested sites (Fig. 14.4).

In the USA, stem nematode resistance was identified in lucerne accessions from Turkestan, developed by mass selection as cv. Lahontan and subsequently introduced by crossing and selection. This resistance has been widely used and appears to be effective worldwide in modern cultivars. In Europe, resistant plants were identified in an older cultivar and mass selection produced parent plants for the first fully resistant cultivars. Mass selection has also been applied to red and white clover to provide cultivars with a higher proportion of resistant phenotypes. In all these outbreeding heterogeneous and polyploid crops, individual resistant plants are not heterozygous for the resistance gene. After outbreeding, progenies include some susceptible plants and it is important that selection for resistance is maintained during early generation seed multiplication.

In the field it may be difficult to recognize virulent strains, particularly on outbreeding crops, because after several generations of multiplication within a single plant genotype, nematode progeny disperse and themselves outbreed with nematodes selected on other genotypes. Reports of virulence in nematode populations in lucerne in the UK appear to be artefacts of inadequate control of the screening procedures. However, field populations virulent on resistant cultivars of field bean (*Vicia faba*) and white clover indicate that variation in virulence exists and is likely to be selected in the long term. There is also clear evidence for nematode isolate × plant genotype interactions in pea (*Pisum sativum*) (Plowright *et al.*, 2002).

14.6.4. Use of resistant cultivars

The ideal resistant cultivar has all the advantages of susceptible cultivars, especially yield potential, together with the additional benefit of controlling nematode population increase. In practice, such resistant cultivars may be difficult to produce, particularly in crops where intensive breeding is leading to a rapidly changing portfolio of cultivars with many other improved attributes. In these cases, it is essential to use resistant cultivars in situations where nematodes are present and when control is advantageous to yields (Box 14.6).



Fig. 14.4. The effects of stem nematode, *Ditylenchus dipsaci*, on growth of susceptible and resistant cultivars; (upper) lucerne seedlings after laboratory inoculation with nematodes (+) and non-inoculated controls (−) of resistant cv. Vertus and susceptible cv. Sabilt: there is necrosis and stunting in the resistant response, but great hypertrophy of infected organs in the susceptible; (lower) differences in growth and survival of winter oat susceptible (left foreground) and resistant (right) to stem nematode.



To the grower, the most important benefit of a resistant cultivar is the improved yield. In soil infested with nematode populations that exceed the damage threshold, yields of resistant cultivars are expected to be better than those of comparable susceptible cultivars. Suppression of nematode multiplication usually ensures that the crop grows better with fewer nematodes. Occasionally, some intolerant resistant cultivars may be damaged by invasion even though they prevent nematode reproduction. In cases with annual crops and nematodes that have only a single generation per season, the yield benefits may not be great. However, in warm temperate to tropical crops, where nematodes complete multiple generations on annual crops, a yield increase through nematode population control will be achieved even in intolerant crops. Succeeding crops, whether resistant or susceptible, often benefit from nematode control provided by the resistant crop. Ogallo *et al.* (1997) showed that root-knot susceptible lima beans yielded well after two successive crops of root-knot resistant cotton but very poorly after susceptible cotton.

The benefits in yield are relative to those of cultivars with similar genetic yield potential. Where breeding has made rapid progress there may be susceptible cultivars with such potential that they yield more than a resistant cultivar in non-infested fields. The apparent negative effects of resistance on yield potential are probably mostly due to linkage drag, whereby genes with negative effects on yield potential are linked to resistance loci. No data are available that show a direct effect of resistance genes on reduced yield potentials. Indeed, as breeding programmes continue to work

Box 14.6. Resistance in West Africa.

The root and tuber crops yam and cassava serve as major staples in West Africa. The major nematode pests associated with them are several root-knot nematodes and of special importance on yam is *Scutellonema bradys*. Resistance to both *Meloidogyne incognita* and *S. bradys* has been found in the yam species, *Dioscorea dumetorum*, but not in the more than 300 cultivars of the most popularly consumed species of *D. alata* and *D. rotundata*. Breeding programmes are ongoing to develop nematode-resistant cultivars, with new accessions being developed and screened for nematode resistance. More success has been achieved in development of resistance in cassava for nematode management, with some cultivars rated as immune. These were improved cultivars that were developed for resistance to diseases.

The root-knot nematodes are the major pest of vegetables in West Africa. The warm humid climate allows for the development of several nematode generations within a season, resulting in an almost continuous disease cycle. Most of the tomatoes and peppers grown in the region are susceptible. Although the resistance in tomato is effective against three species (*M. arenaria*, *M. incognita* and *M. javanica*), the resistance of pepper is limited to *M. incognita*. Unfortunately, field populations often exist as mixed species, which limits the effectiveness of the available resistance in pepper. Resistant tomato cultivars were effective in suppressing nematode populations in Ghana where there was a mix of *M. javanica* and *M. incognita*. However, in Nigeria, where *M. enterobolii* was also present, the same resistant cultivars were not as effective. The molecular techniques used for identification of these *Meloidogyne* species also revealed the presence of populations that could not be identified and it is not known whether the currently available resistance will be effective against those populations. There has often been concern that the resistance in tomato is temperature sensitive and is not expressed at temperatures $>28^{\circ}\text{C}$. This limitation can be overcome in the region by planting in the cooler months, when soil temperatures are below the critical threshold for at least the first several weeks of crop development and thus providing an opportunity for the crop to become well established before the onset of nematode parasitism.

Farmers in West Africa grow a variety of crops on small lots. Almost all the crops they grow are susceptible to one or more nematode species and the management options open to the farmers are limited. They have limited access to nematicides due to availability and/or cost. The effect of crop rotation is often not noticeable within the period they can afford to have a secondary crop growing. Further, biocontrol has so far not been successfully implemented in on-farm studies. Having resistance in some of the crops they grow has the potential to substantially increase farmers' productivity and income. Even the presence of partial resistance would increase their yields relative to susceptible cultivars.

with resistance, the yield potential of the resistant genotypes usually increases. For example, the first groundnut cultivar with resistance to *M. arenaria* was selected from the fifth backcross generation in a breeding programme in which resistance derived from a wild species was introgressed into cultivated groundnut (Simpson and Starr, 2001). Yield of that first release was superior to the best susceptible cultivars in nematode-infested fields (Fig. 14.5) but yields of the resistant cultivar were not competitive in the absence of nematode parasitism (Church *et al.*, 2000). The second resistant cultivar, released after two additional backcross generations, had yield

potentials nearly equal to that of the best susceptible cultivar and yields much better over a range of nematode population densities (Fig. 14.6). The development of soybean cultivars with resistance to *H. glycines* has been in progress for more than 40 years. Yield drag associated with nematode resistance has been reduced but not yet completely eliminated (Donald *et al.*, 2006).

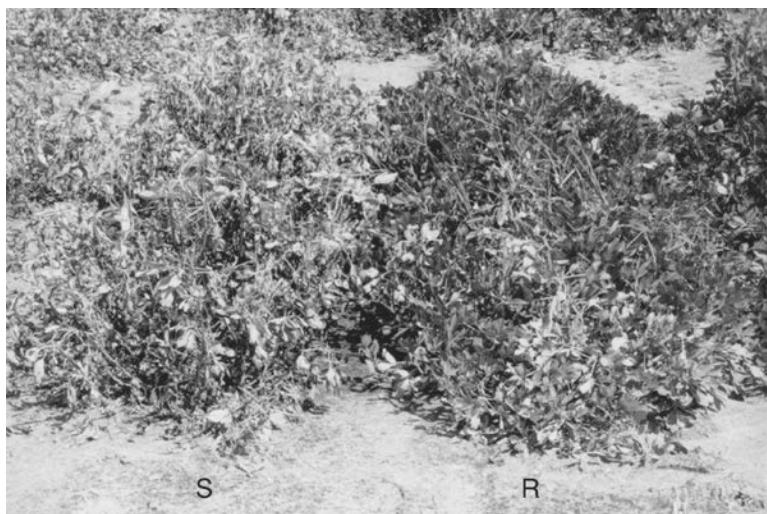


Fig. 14.5. The effects of *Meloidogyne arenaria* on the growth of susceptible (S) and resistant (R) cultivars of groundnut (*Arachis hypogaea*) in a field heavily infested with the nematode.

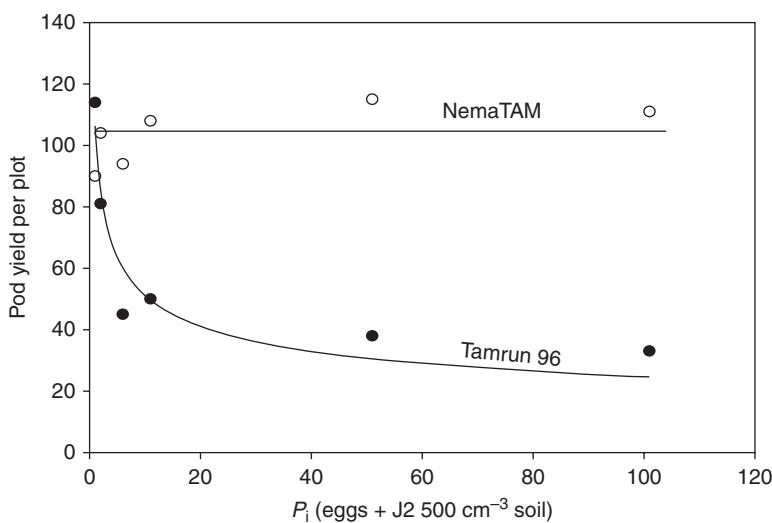


Fig. 14.6. Comparisons of the effects of *Meloidogyne arenaria* on the yields of susceptible (Tamrun 96) and resistant (NemaTAM) cultivars of groundnut (*Arachis hypogaea*) sown at a range of initial nematode population densities. (From Starr *et al.*, 2002.)

14.6.5. Resistant cultivars and integrated nematode management

Resistant cultivars are rarely the single effective solution to management of nematode populations to avoid crop losses. One limitation can be the specificity of resistance, except where the crop only has one major nematode pest species or where a field is infested with only one major pest species. Otherwise, highly specific resistance effective against only one pathotype of a species or even a single species will not control other pathotypes or species in a polyspecific community. Some of the breeding approaches to enhancing durability are discussed in other sections within this chapter. The durability of resistant cultivars may be enhanced by integration with other control measures. A current example of this is the combined use of soil treatments with nematicides and partially resistant potato cultivars to control the potato cyst nematode, *G. pallida*. In this case, the damage threshold approaches the detection limit and growers on infested soils choose to treat soils at planting with granular nematicides (see Chapter 16) to avoid invasion damage, and use partially resistant cultivars to reduce the rate of population increase. In this way, growers can prevent yield losses and also minimize intervals between potato crops to increase their overall profitability. Durability of resistance to the soybean cyst nematode is enhanced by using crop rotation schemes to reduce the frequency with which it is necessary to grow a resistant cultivar.

Biological control may provide an environmentally benign approach that would benefit from being combined with resistant cultivars. In Western Europe, monocultures of cereal crops are now grown without damage from cereal cyst nematode. In recent history, an epidemic of these nematodes seriously threatened intensive cereal production through greatly reduced yields of third and fourth successive crops. However, under continuous cropping it was found that nematode numbers declined to less than the damage threshold and susceptible cultivars could be grown (see Chapter 13). The cereal cultivars grown in much of this area have been developed from the different land races that had been grown over several hundreds of years in different agro-ecological zones of Europe. Many of these land races, particularly of barley, oat and rye, had more than one gene for resistance to cereal cyst nematode. The effects of this resistance, often not fully effective today, in modern cultivars ensures that these have a degree of resistance in comparison to fully susceptible cultivars. So in effect the natural decline of cereal cyst nematode associated with fungal parasites may be assisted by the unwitting use of partial resistance. Similar biological phenomena may be useful to control potato cyst nematodes (see Chapter 13).

In nature, root nematode populations are affected by a number of other soil organisms, including plant symbionts (mycorrhizal fungi). It is likely that the activity of these organisms, when coupled with moderate resistance, could be quite effective in suppression of nematode population densities.

14.7. Successes and Opportunities

Resistance is currently available to several nematodes in a limited number of crops (Table 14.2), and there is a great need for development of resistance to additional nematodes in numerous crops. It appears that available sources of resistance in crops are vastly under-utilized, both in highly developed and in developing countries.

Resistance to cyst nematodes is widely used in potato in Europe (particularly in The Netherlands and the UK) and in soybean in the USA, Brazil and Argentina. In both crops, diversity in virulence demands continued research and breeding to provide genes with sufficiently widespread effectiveness.

Resistance to *Meloidogyne* species in tomato is widely used commercially in California, but not in many other regions, especially in the tropics. Even though the *Mi1.2* element of the *Mi* gene is not effective at temperatures above 28°C, it is effective during cooler months in many subtropical and tropical regions. Further, even if the resistance is effective only during the first few weeks of a growing season, before higher temperatures inactivate the system, this early season inhibition of nematode activity would probably be beneficial, especially when combined with other management tactics. There is increasing use of this gene in crops under protected cultivation in the Mediterranean regions of Europe but the emergence of virulence in Europe is a threat to the continued adoption of these cultivars. Recent screening of additional accessions of *S. peruvianum*, the source of the *Mi1.2*, has revealed the presence of other nematode-resistance genes (designated *Mi-2* to *Mi-8*). These genes will be important to the more widespread use of resistance in tomato, and possibly other solanaceous crops, because some of these resistance genes are not heat sensitive and some are effective against *Meloidogyne* populations that are virulent on *Mi-1.2*.

Despite the limitations of *Mi-1.2*, Sorribas *et al.* (2005) documented its value in the production of tomato in glasshouses where the soil beds were infested with *M. javanica*. Growing three successive crops of a cultivar with *Mi-1.2* increased gross returns by €30,000 ha⁻¹ compared with three crops of a susceptible cultivar. The resistant cultivar also increased returns by €10,000 ha⁻¹ relative to susceptible cultivars when the soil was treated with methyl bromide before planting the first crop.

Cereal cultivars (mainly wheat and barley) are used to control widespread cereal cyst nematode in Australia where there appears to be a single pathotype and emergence of virulence has not been a problem. In Europe, particularly in Scandinavian countries, major gene resistance to cereal cyst nematode is used in barley and oat cultivars and resistant wheat and barley have been introduced into northern India (Nicol *et al.*, 2004). In Europe, diversity in virulence within and among species of the cereal cyst nematode complex reduces the effectiveness of these cultivars.

Recently developed resistance in groundnut to *M. arenaria* and *M. javanica* should be useful in Africa, India and South-east Asia. Resistant rootstocks in perennial crops, such as peach and citrus, have been used successfully for several decades. More recently, the grafting of susceptible scions to resistant rootstocks has been used to manage root-knot nematodes on annual crops. This system allows one to overcome cytogenetic barriers to creation of productive hybrids between more distantly related plants. This practice is being widely used on cucumber, melon, pepper and eggplant in South-east Asia, Taiwan, Morocco and Mediterranean regions of Europe.

Unfortunately, in many other cases the potential benefit of available resistance has not been realized. Cotton and common bean are examples where resistance to *M. incognita* has been identified and introgressed into modern crop genotypes but is still not widely available to growers. Considering the importance of cowpea and common bean as sources of dietary protein and their susceptibility and intolerance to *M. incognita*, one wonders what are the impediments to greater utilization of resistance.

There are at least four possible explanations of why resistance is not more widely used. First, some reports of resistance may not have been accurate. Second, the currently

available resistance is often linked to undesirable characteristics that made the growing of such cultivars unacceptable, thus requiring a substantial breeding effort to achieve a high-yielding cultivar. Third, the costs of developing resistant cultivars are not justified by the (perceived) importance of the nematode problem. The fourth, which in combination with the third is probably the chief explanation, is that naturally occurring genetic resistance tends to be too specific for use in intensive agriculture. Some of the examples (Boxes 14.1 to 14.5) illustrate the extent to which resistance has been compromised in nematode management programmes by erosion of its effectiveness by the emergence of new virulent forms of the target or other species.

One prospect is that the transfer of genes or gene combinations that provide widely effective resistance will be made easier and more predictable due primarily to MAS. This would also allow rotation of specific resistance genes to prolong durability. It is also essential to recognize that although resistant cultivars are described as cost free at the point of use, they do incur substantial costs in their development. Thus, accurate quantification of losses and benefits is essential if plant breeders are to develop and growers to exploit resistant cultivars. Their apparent environmental neutrality should be included in the balance sheet compared with chemical control approaches. We should also recognize that effective use of resistant cultivars requires substantial technological and educational inputs at the grower level.

15 Genetic Engineering for Resistance*

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15.1. Genetic Engineering for Resistance: General Introduction

The principal current methods to control plant-parasitic nematodes are discussed in Part III of this book and include the use of natural resistances, crop rotation, nematicides, biocontrol, soil solarization, flooding and trap cropping. Natural resistance and crop rotation have predominated as methods of control in recent years, certainly in developed countries, as nematicides have become less available as a consequence of their adverse environmental impact; for example, one of the most commonly used nematicides, methyl bromide, not only affected a wide range of non-target organisms but also contributed to the depletion of the ozone layer (United Nations Environment Program [UNEP], 2000). It is now no longer generally available and its use will be phased out entirely by 2015.

The use of natural nematode resistance for control can initially be highly effective. There were early successes in crops as diverse as soybean, cotton and potato (see Chapter 14). With recent developments in genomic and transcriptome sequencing coupled with cheap, high-throughput marker analyses, identification of qualitative trait loci (QTL) conferring nematode resistance is no longer the laborious undertaking it used to be. Next-generation sequencing platforms such as 454TM pyrosequencing and Illumina's sequencing by synthesis (SBS) technology allow rapid single nucleotide polymorphism (SNP) discovery, which can be used to identify QTL. This technology has yet to be exploited fully in nematode resistance (R) gene discovery but upon identification resistance genes can then be introgressed into crop plants from wild or economically less desirable relatives using traditional breeding methods or by genetic manipulation. SNP markers are also the markers of choice for marker assisted selection (MAS) in breeding programmes due to their ease of use; SNP marker technologies such as KASPar (a homogeneous fluorescent resonance energy transfer [FRET] based system) and various SNP chip formats enable inexpensive, rapid and robust results.

Whilst advances in technologies now facilitate rapid resistance gene identification and the means to select for these in breeding programmes, they may have limited use. Resistances are often highly specific; for example, the tomato *Mi* gene confers resistance to *Meloidogyne incognita* but not to virulent populations of *M. javanica* (Tzortzakakis and Gowen, 1996) and current agricultural practice, such as the use of short rotations, can act as strong selectors for resistance breaking races or species. These can take the form of related nematode species, for example selecting *M. arenaria* instead of *M. incognita*, or virulent mutants of the same species; virulent *M. incognita* can develop within five generations on tomatoes with *Mi* conferred resistance (Noling, 2000) and one of the earliest uses of nematode resistance in soybean, derived from PI 88788, a germplasm accession from China, is now ineffective against most populations of *Heterodera glycines* (Mitchum *et al.*, 2007; Hershman *et al.*, 2008).

Despite technological advances, resistance QTL discovery and the resultant breeding programme require considerable time and financial commitments. Thus, the limited scope and durability of this solution to nematode parasitism may negate the investment for such a programme. An alternative solution is an engineered resistance, but what does this provide over the currently available methods? The immediate benefits of an engineered resistance are summarized in Box 15.1.

Box 15.1. Benefits of an engineered resistance.

- A knowledge-based, specific targeting of plant-parasitic nematodes.
- Low risk to humans and animals, and the environment.
- Ease and speed with which a specific desired trait can be introduced into a preferred plant variety.
- Resistance to a broader spectrum of plant-parasitic nematodes.
- Durability of resistance by targeting key steps in the infection cycle or by stacking resistance.

15.2. Genetic Engineering for Nematode Resistance: Use of Natural Resistances

Chapter 14 examines resistance and resistant cultivars in detail, so the transfer of cloned resistance genes is summarized only briefly here. Disease resistance in plants was thought to be mediated by two complementary genes; a pathogen avirulence gene (*Avr*) and a host resistance gene (*R*). This **gene for gene** hypothesis was first proposed over 50 years ago by Harold Flor, from studying host responses to flax rust fungus (Flor, 1971). It was thought that the host *R*-gene product acts as a receptor to the pathogen's *Avr*-gene product, which functions as a ligand; binding of *Avr* to *R* activates plant defence mechanisms. This elicitor–receptor model may function in some resistance mechanisms but in recent years research has shown a decided lack of direct *R–Avr* interaction. The **guard hypothesis** proposes that the *R* proteins associate with the cellular targets of the *Avr* proteins (Dangl and Jones, 2001). These cellular targets could be proteins involved in plant defence, for example, or be required to provide pathogen nutrition. The guard hypothesis proposes that when a pathogen *Avr*-gene product binds to a target in a resistant plant cell, this complex is recognized by the *R* protein, which initiates the plant's defence. Therefore, in a susceptible host no *R* protein is present and the plant target is unguarded from the pathogen virulence elicitor resulting in disease. The *R* protein may be constitutively bound to the target but become unbound and active upon binding of the *Avr* product to the target. A further model (the **decoy model**) proposes that *R* proteins do not recognize *Avr* protein targets directly but proteins that resemble them; in the absence of an *R*-gene these decoys do not contribute to pathogen fitness, but in its presence elicit plant defence mechanisms (van der Hoorn and Kamoun, 2008). The decoy theory arose as it appears that a limited number of host cellular targets are targeted by several pathogen effectors. The major class of plant *R*-genes encode proteins containing a nucleotide-binding and leucine-rich repeat (NB–LRR). Nearly half the genes encoding NB–LRR proteins in *Arabidopsis thaliana* are single copy; thus, divergent evolution has not been necessary to provide effective resistance possibly due to the structural constraints of the pathogen elicitors imposed by the binding of plant targets. Transferring *R*-genes to susceptible hosts would seem to offer an effective and long-term solution to disease and as early as 1993 a patent protecting sequences conferring resistance to *Heterodera schachtii* in sugar beet was submitted (Sandal *et al.*, 1993). Numerous nematode *R*-genes have since been identified (see Chapter 14) and those that have been utilized in engineering resistance include *Hs1 pro* (Cai *et al.*, 1997), *Mi-1* (Milligan *et al.*, 1998),

Hero (Ernst *et al.*, 2002) and *Gpa2* (van der Vossen *et al.*, 2000). The gene products of *Mi-1*, *Hero* and *Gpa2* belong to the leucine-zipper, nucleotide-binding site (NBS), LRR-containing class of R proteins and are probably located in the cell cytoplasm, whilst the *Hs1 pro* gene encodes an extracellular protein. The first of these R-genes to be cloned was *Hs1 (pro-1)* and was identified as conferring resistance to *H. schachtii* in a wild species of beet, *Beta procumbens*, and conferred resistance to a susceptible beet variety in hairy root culture (Cai *et al.*, 1997). The second R-gene to be identified was the tomato *Mi* gene, which confers resistance to *M. incognita* and several other species of *Meloidogyne*; it had been cloned as part of a larger DNA fragment (Milligan *et al.*, 1998), of which the *Mi1.2* element was shown to be involved not only in resistance to nematodes but also to potato aphids. The potato *Gpa2* R-gene was initially isolated from a cluster of R-genes at the *Gpa2* locus; at least two of the genes in the cluster are active, one of which (*Rx1*) confers resistance to potato virus X and the other, *Gpa2*, confers resistance to the potato cyst nematode *Globodera pallida* (van der Vossen *et al.*, 2000).

The tomato resistance gene *Hero* was isolated from a region containing 14 homologous genes, of which eight appear to be functional genes. *Hero* encodes an NBS–LRR type R protein with a leucine zipper motif within the N-terminal domain (Ernst *et al.*, 2002). *Hero* is unusual amongst NBS–LRR type R proteins as it contains a negatively-charged region between LRRs 4 and 5; the resulting peptide sequence is predicted to form a coil, the likely function of which is pathogen recognition (Ernst *et al.*, 2002). *Hero* confers resistance to a number of cyst nematodes but predominantly *Globodera* spp. and resistance levels of 95% have been observed for *G. rostochiensis*. Cyst nematodes fail to progress to maturity in resistant plants as, although a syncytium is initiated by juvenile nematodes, it quickly becomes encapsulated by necrotic cells leading to its degradation (Sobczak *et al.*, 2005). This resistance mechanism is not associated with the rapid hypersensitive response (HR) initiated by many R-gene products, such as that initiated by the *Mi-1* gene where necrosis of cells adjacent to the migratory tract and feeding cell occurs within 12 h following root invasion.

Despite the identification of a number of R-genes conferring nematode resistance there have been relatively few successes using these genes as part of an engineered resistance. In particular, many confer resistance to one species only and even to a specific pathotype. Transfer of R-genes to a different host species has resulted in loss of resistance. For example, transfer of *Hero A* (from tomato) into potato resulted in no significant resistance to *Globodera* spp. (Sobczak *et al.*, 2005); similarly, transfer of *Mi-1.2* (from tomato) to tobacco resulted in no significant resistance to *Meloidogyne* species (reviewed in Williamson, 1998). A further limiting factor of *Mi* resistance is that it is temperature-sensitive and breaks down over 28°C, limiting its use to temperate climates.

Two studies have identified entirely novel plant-nematode resistance mechanisms; one comprises altered host gene expression, the other altered protein regulation. The first of these studies identified resistance conferred by gene copy number variation, resulting in overexpression of three disparate genes encoding an amino acid transporter, an α-SNAP protein and a WI12 (wound-inducible domain) protein (Cook *et al.*, 2012). These genes are located at the *rhg1-b* locus, which confers resistance to the soybean cyst nematode *H. glycines*. In susceptible soybean varieties one copy of the genes is found per haploid genome but ten tandem copies are found in the *rhg1-b* haplotype. Overexpression of all three genes was shown to be required for

Rhg1-mediated *H. glycines* resistance. The mechanism by which overexpression confers nematode resistance is unknown, but sequence comparisons and structure predictions of the encoded proteins suggests that they may combine to produce an unfavourable environment for nematodes; overexpression of the amino acid transporter may result in altered auxin biosynthesis or distribution, possibly impacting on feeding cell initiation and maintenance, the α -SNAP protein may have a role in exocytosis, thus increased abundance may result in removal of products required by the nematode for development or for feeding cell maintenance, while the WI12 protein may be involved in the production of phenazine-like compounds, which are toxic to nematodes (Cook *et al.*, 2012).

The second study identified resistance conferred by altered regulation of a serine hydroxymethyltransferase (Shmt) (Liu *et al.*, 2012). The Shmt encoding gene was identified at the *Rhg4* (resistance to *H. glycines* 4) locus and was shown to confer nematode resistance. Shmt has an essential role in one carbon folate metabolism and while alleles of *Rhg4* encode functional enzymes, polymorphisms are predicted to disrupt Shmt regulation. Folate one-carbon metabolism is essential for nematode feeding cell development and maintenance and loss of Shmt regulation leading to nematode resistance may occur via a number of mechanisms as a consequence of folate deficiency in syncytia; nematode starvation may result with subsequent degeneration of the syncytia, or a hypersensitive response may be generated to the folate-deficient syncytia leading to nematode death (folate deficiency has previously been shown to induce apoptosis of mammalian cells). Further explanations of the resistance mechanism are that the compound produced by the altered Shmt acts either as a nematicide or effector protein, triggering resistance signalling pathways (Liu *et al.*, 2012).

Both studies offer insights into further engineered solutions for nematode resistance; overexpression of a combination of genes by either increasing copy number or altering transcript levels using promoters to produce elevated levels of transcripts and, hence, protein products can create an environment noxious to nematodes. The same can be achieved by removing regulatory elements from proteins, which would result in the production of compounds necessary for nematode parasitism being less abundant or functionally altered.

15.2.1. Broadening *R*-gene resistance

There is evidence to suggest that the products of different *R*-genes share common downstream signalling cascades, the intermediates of which could be engineered to respond to a wider range of nematode species. Research has identified some of these convergent signalling molecules; for example, RAR1 was identified as a common convergence point in signalling pathways initiated by fungi and viruses (Liu *et al.*, 2002), SGT1 was shown to interact with Rar1 (Azevedo *et al.*, 2002) and the heat shock protein HSP90 was identified as their molecular chaperone (Takahashi *et al.*, 2003). However, only HSP90 was shown to be essential for *Mi-1* mediated resistance to aphids and nematodes (Bhattarai *et al.*, 2007). With an increase in the understanding of how resistance genes exert their function, it may be possible to broaden the specificity of a resistance or increase its durability by directed modification.

15.2.2. An alternative strategy of using natural resistances

In theory, determining *Avr* structure and utilizing ligand–receptor binding modelling may suggest engineering solutions that confer less receptor specificity, thus enabling R-gene mediated response to a wider range of nematode species. However, this approach may not be possible as a putative *Avr*-gene product (MAP-1) thought to interact with *Mi* was found exclusively in avirulent populations of *M. incognita* and other *Meloidogyne* species (Semblat *et al.*, 2001). The presence of this protein only in avirulent populations discounts R protein modification as no ligand would appear to exist in virulent populations. However, this may not be the situation for other *Avr*-gene products, which may differ between virulent and avirulent species by amino acid sequence, thus offering a means of receptor modification to achieve control. RBP-1 is a SPRY domain containing protein secreted by *G. pallida*, which elicits an HR response in potato plants expressing the *R*-gene *Gpa2*. RBP-1 is highly polymorphic and initiates a resistance response only if a proline residue is present at position 187 in the SPRY domain. However, whilst the LRR domain of *Gpa2* recognizes RBP-1 variants and initiates activation of the HR response, the interaction of *Gpa2* with RBP-1 is entirely dependent on a third protein RanGap2 (Sacco *et al.*, 2009).

The tacit assumption is that recognition of nematode *Avr* effector proteins triggers plant NB–LRR protein mediated cell death. However, in one case the opposite is true; the *G. rostochiensis* effector protein SPRYSEC-19 was shown to associate with the LRR domain of a SW5 resistance gene in tomato; this association did not result in programmed cell death and thus resistance, but conversely was shown to suppress it (Postma *et al.*, 2012). This was true for other (SW5B, Rx1, *Gpa2* and RGH10) but not all NB–LRR resistance proteins (where presence of SPRYSEC-19 did result in cell death). This new insight into nematode effector protein function offers a further means of control as targeting nematode effector proteins that suppress cell death would enhance resistance.

Currently very little is definitively established as regards *Avr*–*R*-gene product interactions; however, genomic sequences are now published for *M. incognita* and *M. hapla*, providing new opportunities to identify *Avr* and other genes involved in parasitism.

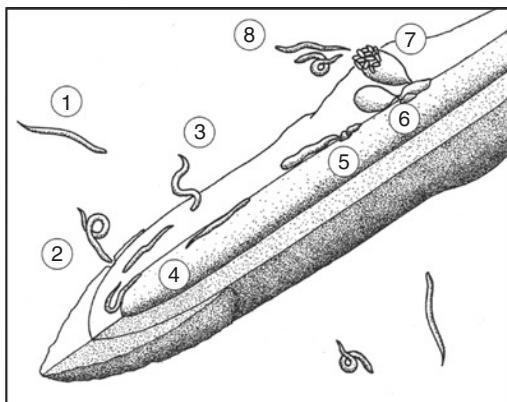
15.3. Targets in the Early Nematode–Plant Interaction for Engineered Resistance

The first step in a knowledge-based approach to engineered resistance is to identify which steps in the interaction of nematodes with plants can be targeted to achieve an effective control. Figure 15.1 illustrates different stages of nematode plant parasitism that offer potential opportunities for control.

15.3.1. Host-induced nematode hatching and attraction

Previous chapters have described how various plant-parasitic nematodes locate host plants, penetrate and migrate through host tissues, feed and progress through their life cycle. There are several mechanisms whereby nematodes are attracted to plants (see Chapter 8); these range from detection of CO₂ gradients from plant respiration to recognition of plant root diffusates, which in some species of nematodes can also

Fig. 15.1. Plant-parasitic nematode life cycle stages that are targets for genetically engineered resistance strategies. 1: Attraction of the nematode to the plant. 2: Grazing on the root (which is relevant for many ectoparasitic nematodes). 3: Penetration of the nematode into the plant. 4: Migration of the nematode through plant tissues. 5: Establishment of a specialized feeding site (as with endoparasitic nematodes). 6: Maturation and moulting with continued feeding from the plant. 7: Mating and egg production. 8: Maturation of eggs and hatching of juveniles (which begin the cycle of re-infection).



stimulate hatching. In cereals, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) is a naturally occurring hydroxamic acid which serves as a defence against a wide range of pests including insects, pathogenic fungi and bacteria, but acts as an attractant to the plant-parasitic nematode *Pratylenchus zeae* (Friebe *et al.*, 1998). *Pratylenchus scribneri* is attracted to the roots of tall fescue (*Festuca arundinacea*) by N-formylloline but only at concentrations of below 20 µg ml⁻¹ of root extract as higher concentrations act as a repellent (Bacetti *et al.*, 2009). A potent attractant for the pinewood nematode, *Bursaphelenchus xylophilus*, is β-myrcene, an olefinic compound that is also an attractant for pinewood beetles of the genus *Monochamus* that vector *B. xylophilus*. Relatively few attractants have definitively been identified, but resistance could be achieved in plants that have been engineered to mask their attraction to nematodes or actively deter nematodes.

A novel defence that disrupts location and invasion of host roots by plant-parasitic nematodes has been developed to field efficacy. Nematodes must sense and respond appropriately to a range of chemical signals in order to achieve a successful parasitic interaction. Two distinct, synthetic peptides interfere with cyst nematode chemoreception by binding to either acetylcholinesterase or nicotinic acetylcholine receptors (nAChRs), both targets in the nematode cholinergic nervous system (Winter *et al.*, 2002). Transgenic plants were subsequently developed that secrete the peptides from their roots (Liu *et al.*, 2005; Green *et al.*, 2012). The acetylcholinesterase-inhibiting peptide suppressed the number of female *H. schachtii* that developed on *A. thaliana* by more than 80%, whilst expression in the root tips of potato plants resulted in almost 95% resistance to *G. pallida* (Lilley *et al.*, 2011). The nAChR-binding peptide is taken up from the environment by certain chemosensory sensilla within the anterior amphidial pouches and undergoes retrograde transport along some chemoreceptive neurons to their cell bodies and a limited number of interneurons. Chemoreception was only impaired when that transport had been completed (Wang *et al.*, 2011). Potato plants secreting this peptide from their root tips provided effective resistance (up to 77%) against potato cyst nematode in both containment glasshouse and field trials (Green *et al.*, 2012). This resistance strategy may effectively target other nematode species in addition to cyst nematodes as nAChRs are common to all nematodes studied (Sattelle, 2009). The infective stages of sedentary

endoparasitic nematodes are vulnerable to sensory intervention prior to feeding cell initiation, whilst migratory endoparasites and ectoparasites remain motile and may be affected throughout their life cycle.

15.3.2. Penetration and migration

Plant-parasitic nematodes are either endo- or ectoparasites; those species (such as species of trichodorids) that fall into the latter category graze opportunistically on plant roots and present very difficult targets for any resistance mechanism as individuals can move onto fresh root material relatively quickly and before a response specific to the feeding site can take effect. However, possible defence mechanisms could include the induction of a more systemic resistance throughout the whole plant, or more preferentially the root system, which would act against subsequent grazing nematodes.

Penetration into the root and migration inside the root would appear to be prime targets for an engineered resistance, especially where damage to root tissue is generated by feeding, such as with the stem nematode *Ditylenchus dipsaci* where no complete natural resistance has been found. The second-stage juveniles (J2) of *Meloidogyne* do not feed during migration and minimize the damage they cause within the root by migrating between cells. Thus, they evade inducible resistance mechanisms during their migration. Disrupting migration would impede feeding cell initiation and result in fewer established females; additionally the site of feeding cell initiation may be suboptimal, resulting in smaller females and reduced egg production. Migrating nematodes, in particular *Meloidogyne* spp., may follow auxin (indole-3-acetic acid [IAA]) gradients; penetration is usually behind the root cap, nematodes then migrate to a point in the root tip that appears to correspond to the quiescent centre (an area of maximum auxin concentration within the root; Petersson *et al.*, 2009) before turning and migrating back up the root and selecting a feeding site (see Chapter 3). The nematode migratory period is between 8 and 12 h as the natural resistance conferred by the *Mi* gene in tomato appears to begin within 8–12 h of initial nematode penetration, which coincides with the timing of feeding site initiation post-migration.

15.4. Genetic Engineering to Target the Nematode Directly

Direct targeting of a pathogen in order to kill it or impair its function sufficiently to prevent reproduction is a strategy commonly used to engineer resistance to fungi, bacteria and insect pests. Plant-parasitic nematodes present a variety of targets for such an approach, including the exoskeleton, the feeding tube, digestion, essential metabolic processes and reproduction; some of the methods used to attack these targets are discussed in the following sections.

15.4.1. *Bacillus thuringiensis* (*Bt*) crystal (Cry) proteins

Bacillus thuringiensis (*Bt*) crystal (Cry) proteins are best known for their insecticidal properties. The *Bt* toxin causes the death of insect larvae by binding to receptors in

the epithelial cells of the larval gastrointestinal tract, which leads to pore formation and cell lysis (reviewed in Koziel *et al.*, 1993). Insect resistance conferred by the *Bt* gene has been used to target cotton bollworm, maize borers and potato beetles. Crops, predominantly maize and cotton, genetically engineered to express the *Bt* toxin now cover over around 66 million ha worldwide (James, 2011). The nematicidal potential of the *Bt* toxin was first demonstrated in *Caenorhabditis elegans*, where exposure resulted in reduced fecundity and viability (Marroquin *et al.*, 2000). However, uptake of the *Bt* Cry protein as a possible control mechanism for plant-parasitic nematodes was not initially investigated as *H. schachtii* was shown to be unable to ingest proteins larger than 23 kDa (Böckenhoff and Grundler, 1994; Urwin *et al.*, 1997a) and *Bt* Cry (Cry6A) is a 54 kDa protein. However, this ingestion limit does not apply to all other plant-parasitic nematodes and expression of *Bt* Cry (Cry6A) in tomato hairy root culture was shown to intoxicate *M. incognita* as demonstrated by a fourfold reduction in progeny (Li *et al.*, 2007). By contrast, Cry5B expressing roots supported significantly reduced numbers of galls. This was reflected in a reduced total egg production but there were no significant differences in the number of eggs per egg mass between transgenic and control lines (Li *et al.*, 2008). Therefore, Cry5B appears to exert its strongest effect on juvenile stages, whilst reproduction is most sensitive to Cry6A. It is likely that root-knot nematodes are able to ingest larger molecules than cyst nematodes as their feeding tubes differ significantly (Sobczak *et al.*, 1999). Thus, the use of *Bt* as a nematicide has limitations due to the range of plant-parasitic nematodes affected but additionally as the resistance may have limited durability; the widespread and intensive use of *Bt* has resulted in the emergence of resistant pests in some crops in Australia, India and China and resistance occurs frequently under artificial selection pressures in several species, including *C. elegans* (Barrows *et al.*, 2007).

15.4.2. Plantibodies

Plants can be engineered to produce functional antibodies, or antibody fragments known as plantibodies. The potential use of plantibodies in inactivating pathogen-derived biologically active molecules is self-evident and they have been used to control both viral and bacterial pathogens (reviewed in Schillberg *et al.*, 2001). They have also been investigated as a means of controlling plant-parasitic nematodes. Some species of nematodes alter plant cell cycle and cell differentiation to form specialized feeding cells, and the factors that redirect the fate of the plant cells originate from three nematode pharyngeal glands (the sub-ventral glands and the dorsal gland; see Chapter 9). Therefore, inactivation of the active components in these secretions would result in the nematode failing to initiate a feeding cell. This was first assessed in tobacco with the expression of the heavy and light chains of a murine monoclonal antibody specific to stylet secretions of *M. incognita* (Baum *et al.*, 1996). Unfortunately, despite the antibodies binding to the nematode pharyngeal glands and stylet secretions, no reduction in the ability of the nematode to parasitize tobacco roots was seen. Although it is not entirely clear why the plantibodies failed to disrupt giant cell formation by inhibiting stylet secretion activity, one feasible explanation is that the antibody and antigen may not have coincided spatially, as the plantibodies accumulated in the endoplasmic reticulum (ER) and apoplastically, while nematode stylet secretions were delivered to the

cytoplasm. It is also possible that the antigen targeted by the antibody was not essential to feeding cell initiation or that the antibody failed to inactivate its function.

Plantibodies have also been used in an attempt to inhibit early nematode colonization by targeting penetration and migration. Plantibodies were generated that recognize secretory-excretory proteins on the cuticle surface and amphids of *G. pallida* and *G. rostochiensis*. The plantibodies were shown to affect nematode movement and resulted in delayed root penetration. However, these effects were temporary as turnover of the secreted proteins resulted in loss of the bound antibody (Sharon *et al.*, 2002). To date, plantibodies are yet to be a proven nematode control strategy. Additionally they are likely to be of limited use as antibodies raised against one species are unlikely to cross-react with another.

15.4.3. Lectins

Lectins are sugar binding proteins that bind specific monosaccharides or oligosaccharides; they are found naturally in plants, animals and fungi (reviewed in Lam and Ng, 2011). Lectins can be categorized according to their structure (merolectins, hololectins, chimerolectins and superlectins), their carbohydrate specificity or by family (legume lectins, type II ribosome-inactivating proteins, monocot mannose-binding lectins, and other lectins). Lectins have been ascribed various functions, including symbiotic recognition, seed storage, growth regulation, plant development and defence against pathogens. Lectins are thought to play a major role in plant defence as they are produced in response to various pathogens, including nematodes (Jammes *et al.*, 2005; Fuller *et al.*, 2007). Lectins are of particular use against insect pests as many plant lectins bind glycans, such as chitin, which are rarely found in plants, and lectins have been used to engineer control of insect pests in wheat, rice, tobacco and potatoes (Powell *et al.*, 1995). The potential of lectins as nematode control agents was first demonstrated by a reduction in galling of 75% in *M. incognita* infected tomato roots treated with concanavalin A lectin (Con A) (Marban-Mendoza *et al.*, 1987). Using an engineered approach, constitutive expression of snowdrop (*Galanthus nivalis*) lectin (GNA) in potato and oilseed rape achieved partial resistance to *G. pallida* and to the migratory nematode *Pratylenchus boliviensis*; although results were varied, some lines showed 80 and 100% resistance, respectively (Burrows *et al.*, 1997, 1998). Partial resistance to *M. incognita* was also achieved in *A. thaliana* expressing GNA with 50% less galling in comparison to controls (Ripoll *et al.*, 2003). The mechanism of pathogen resistance is not known but it is thought that lectin may bind glycoproteins localized on the surface of the nematodes, on the chemoreceptors in the amphid sensory organs themselves, or in the amphidial secretions, thereby interfering with nematode sensory perception and its ability to establish feeding cells. Although this has yet to be proven in plant-parasitic nematodes, ConA has been found to be associated with the anterior amphids of the nematode *Strongyloides ratti* (a mammalian parasite) resulting in disruption of its chemokinetic response (Tobata-Kudo *et al.*, 2005). Lectins are considered a leading substance in engineering phytopathogen resistance due to their broad-spectrum activity, but unfortunately unfavourable publicity about the use of lectins was generated when adverse effects were reported in rats fed on raw potato containing snowdrop lectin (Ewen and Pusztai, 1999), and even though the report was later discredited by The Royal Society

of London (http://royalsociety.org/uploadedFiles/Royal_Society_Content/policy/publications/1999/10092.pdf), the use of lectins still generates some concern. Additionally, lectin expression levels and degree of pathogen resistance can be highly variable and hyper-susceptibility to nematode infection in some lines (Ripoll *et al.*, 2003) and with some species (Kaplan and Davis, 1991) of nematode has been observed.

15.4.4. Protease inhibitors

Transgenic expression of proteinase inhibitors (PIs) in plant roots is the most widely explored approach to engineered resistance to plant-parasitic nematodes. A range of different inhibitors, most of them naturally-occurring plant proteins, have been shown to be detrimental to feeding nematodes, reducing their growth and fecundity. Inhibitors of all four main classes of proteinase (serine, cysteine, aspartic and metallo-proteinase) occur in plants and are often induced in response to wounding or herbivory. Correspondingly, proteinase genes and activity have been identified in plant-parasitic nematodes (Lilley *et al.*, 1996, 1997; Urwin *et al.*, 1997b; Neveu *et al.*, 2003; Fragoso *et al.*, 2009). A digestive role has been proposed for these enzymes, corroborated for some by expression in the intestine. With digestion of protein being a common requirement of nematodes, PI-based control could have efficacy against a wide range of species, irrespective of their parasitic strategy. This would have particular utility in those field situations where a number of different nematode pests occur concurrently.

Cysteine proteinase inhibitors, termed cystatins, have received the most attention. Initial experiments utilized the rice cystatin Oc-I, modifying its coding region to remove an amino acid and improve its inhibitory activity 13-fold over the native protein. Expression of this engineered variant (Oc-IΔD86) in tomato hairy roots using the cauliflower mosaic virus (CaMV35S) promoter resulted in significantly smaller female *G. pallida* after 6 weeks when compared to control roots (Urwin *et al.*, 1995). Expression of Oc-IΔD86 in a second model system *A. thaliana*, using the same promoter, allowed the cystatin to be tested against additional nematode species. The size of female *H. schachtii* and *M. incognita* was considerably reduced relative to controls with growth arrested before egg laying. This effect was correlated with detection of the cystatin in the feeding nematodes and reduced cysteine proteinase activity in the intestine of female *H. schachtii* recovered from plants (Urwin *et al.*, 1997b). The same *A. thaliana* plants also suppressed growth and egg production of the reniform nematode, *Rotylenchulus reniformis*, with cystatin expression level influencing reproductive success (Urwin *et al.*, 2000). Although *A. thaliana* is not a favoured host for this nematode, the study is an example of a model system providing preliminary data to support later cystatin expression in crops of interest such as pineapple (Wang *et al.*, 2009b), where transformation is limited by a slow rate of regeneration. An alternative model host plant, lucerne (alfalfa), was used to demonstrate that the rice cystatins Oc-I and Oc-II expressed at a low level in lucerne under the control of a wound-inducible promoter conferred some resistance to the root-lesion nematode *Pratylenchus penetrans* (Samac and Smigocki, 2003).

A rather different approach was taken to inhibit cysteine proteinases of *H. glycines* parasitizing transgenic soybean hairy roots (Marra *et al.*, 2009). The propeptides that are cleaved from cysteine proteinase precursors can often act as inhibitors of their

cognate enzymes (e.g. Silva *et al.*, 2004). The propeptide region of the *H. glycines* HGCP-I cathepsin L enzyme was expressed in roots and caused a reduction in the number and fecundity of female nematodes (Marra *et al.*, 2009). The prodomain inhibitor displays greater specificity for target enzymes than do typical plant PIs (Silva *et al.*, 2004) and whilst this may limit the utility of the approach to control a wide range of nematode species, it could have biosafety advantages for non-target organisms.

Although less widely studied, serine proteinase inhibitors have also demonstrated potential for nematode control. In another model system study, transgenic expression of the sweet potato serine PI, sporamin, inhibited growth and development of female *H. schachtii* parasitizing sugar beet hairy roots (Cai *et al.*, 2003). In this case, the severity of the effect was clearly correlated with the level of trypsin-inhibitory activity detected in the transformed root lines.

Engineered resistance based on PIs has been extensively tested in potato, primarily against *G. pallida*. The potential of plant PIs as anti-nematode effectors was first explored using the serine PI cowpea trypsin inhibitor (CpTI). CpTI expressed in transgenic potato influenced the sexual fate of newly established *G. pallida* (Hepher and Atkinson, 1992) and as a result the population was biased toward a predominance of the much smaller and less damaging males. Subsequent work focused on cystatins and culminated in successful field trials of transgenic potatoes. The best transgenic line of the fully susceptible potato cv Désirée, expressing chicken egg white cystatin from the constitutive CaMV35S promoter, displayed 70% resistance to potato cyst nematodes in the field (Urwin *et al.*, 2001). When the same construct was used to transform two potato cultivars, Sante and Maria Huanca, that each display natural partial resistance to *G. pallida*, the best transgenic lines of each were enhanced to full resistance (Urwin *et al.*, 2003). Subsequent field trials demonstrated that both the modified rice cystatin (OcIΔD86) and a sunflower cystatin expressed in cv Désirée afforded similar levels of protection to chicken egg white cystatin (Urwin *et al.*, 2003). Potato plants in which expression of the OcIΔD86 cystatin was limited mainly to the roots and, in particular, to the syncytia and giant cells induced by *G. pallida* and *M. incognita*, respectively, were shown to have similar resistance levels to those achieved with constitutive expression for both nematodes (Lilley *et al.*, 2004).

Proteinase inhibitor strategies are being tested in banana and plantain. Cavendish dessert bananas that express the OcIΔD86 engineered variant of rice cystatin under the control of the maize ubiquitin promoter displayed $70 \pm 10\%$ resistance to *Radopholus similis* in a glasshouse trial (Atkinson *et al.*, 2004). Plants expressing the same cystatin under the control of a root-specific promoter that is upregulated in giant cells (Green *et al.*, 2002; Lilley *et al.*, 2004) were resistant ($83 \pm 4\%$) to *M. incognita* (H.J. Atkinson, personal communication). The approach is now progressing to cooking varieties of *Musa*. East African Highland banana plants constitutively expressing a maize cystatin support reduced multiplication of *R. similis* and the plantain cv Gonja has been transformed to express both a cystatin and a repellent peptide (Roderick *et al.*, 2012). Similar additive cystatin plus repellent constructs have been introduced into East African Highland banana varieties (NARO, Uganda). There could be an additional advantage to cystatin-mediated nematode resistance in banana as cystatin impairs feeding and development of banana weevils (Kiggundu *et al.*, 2010).

To date, the only nematode resistance technology introduced into rice is the cystatin-based defence. Transgenic plants of four elite African rice varieties constitutively

expressing the modified rice cystatin *OcIΔD86* displayed 55% resistance to *M. incognita* (Vain *et al.*, 1998). Only a low level of cystatin expression was observed, possibly due to a suboptimal CaMV35S promoter or homology-dependent silencing of the transgene in combination with the endogenous *OcI* gene. In subsequent work, a maize cystatin has been expressed in the rice variety Nipponbare under the control of a root promoter from *A. thaliana* (TUB-1) that is known to be upregulated in the feeding cells of *M. incognita* parasitizing rice (Green *et al.*, 2002).

Inhibitory activity of a potato serine proteinase inhibitor (PIN2) expressed in transgenic wheat showed a positive correlation with plant growth and yield following infestation with the cereal cyst nematode *H. avenae* (Vishnudasan *et al.*, 2005). A protective effect on the plant against nematode infection was inferred; however, the effect of the PI on nematode development was not investigated. In a further demonstration of the potential of PIs, a cystatin from the tropical root crop taro (*Colocasia esculenta*) was expressed constitutively in a root-knot nematode-susceptible tomato cultivar. There was a 50% reduction in the number of galls formed by *M. incognita* on the transgenic plants compared with wild-type plants and a larger reduction in the number of egg masses produced per plant (Chan *et al.*, 2010).

15.4.4.1. Biosafety of protease inhibitors

Plant protease inhibitors are attractive as effectors against nematodes because, while they have a negative effect on the parasite, they present no harm to humans and livestock and are present in a normal diet. Considerable effort has been put into determining the effect on the environment of their use in agriculture and studies have determined no effect on other insect feeders, their predators or soil microorganisms (Cowgill *et al.*, 2002a,b, 2004; Cowgill and Atkinson, 2003).

15.4.5. RNA interference (RNAi)

RNA interference (RNAi) is the process in which double-stranded RNA (dsRNA) triggers the silencing of specific target genes through mRNA degradation. It has been adopted as a tool for functional analysis of plant-parasitic nematode genes (Rosso *et al.*, 2009), and delivery of dsRNA from a host plant to bring about RNAi silencing of genes in the feeding nematode is being explored as a nematode resistance strategy (Lilley *et al.*, 2011). To date, the approach has shown potential against both cyst and root-knot nematodes and screening methods have been developed to allow the evaluation of many gene targets. *Arabidopsis thaliana* plants expressing dsRNA from hairpin inverted repeat constructs at least partially reduced transcript abundance of targeted parasitism genes in the pharyngeal gland cells of feeding *H. schachtii* (Patel *et al.*, 2008, 2010; Sindhu *et al.*, 2009). For six of eight genes tested this led to a significant reduction in female numbers of between 23% and 64%, with considerable variation between lines for some constructs (Sindhu *et al.*, 2009). Variable, non-significant effects were also observed when a fibrillin gene of *H. glycines* was targeted from chimeric soybean plants (Li *et al.*, 2010a). In the same study, RNAi of a coatomer subunit of this nematode resulted in a significant reduction in egg production. Soybean composite plants derived from hairy root cultures engineered to silence either of two

ribosomal proteins, a spliceosomal protein or synaptobrevin, of *H. glycines* by RNAi resulted in 81–93% fewer females developing on the transgenic roots (Klink *et al.*, 2009), whilst a similarly high reduction in egg production was achieved by targeting mRNA splicing factor *prp-17* or an uncharacterized gene *cpn-1* (Li *et al.*, 2010b).

A high level of resistance to root-knot nematode was achieved by targeting a parasitism gene expressed in the sub-ventral gland cells of *M. incognita* (Huang *et al.*, 2006). dsRNA complementary to the *16D10* gene was expressed in transgenic *A. thaliana* and the resulting lines displayed a significant reduction (63–90%) in the number of galls and their size, with a corresponding reduction in total egg production. The high level of homology between the *16D10* sequences of different *Meloidogyne* species led to broad-range resistance against *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. Almost complete resistance to *Meloidogyne* infection was reported in tobacco plants expressing dsRNA corresponding to splicing factor or integrase (Yadav *et al.*, 2006) and of four genes targeted from transgenic soybean roots, two (encoding a tyrosine phosphatase and mitochondrial stress-70 protein precursor) reduced gall number by >90% (Ibrahim *et al.*, 2011). However, not all host-delivered RNAi targeting of *Meloidogyne* genes results in a resistance phenotype. Partial silencing of a putative transcription factor of *M. javanica* (*MjTis11*) did not significantly affect either nematode development or fecundity (Fairbairn *et al.*, 2007). Partial resistance resulted from targeting either a dual oxidase gene with a probable role in cuticle formation or a subunit of signal peptidase, a protein complex required for the processing of secreted proteins. Crossing transgenic lines expressing these two defences provided higher levels of resistance to *M. incognita* than either parent plant (Charlton *et al.*, 2010). Such additive resistance may raise the efficacy and durability of RNAi-based defences but a combinatorial RNAi targeted at *H. glycines* did not deliver that benefit (Bakhetia *et al.*, 2008). Possibly short interfering RNAs may sometimes compete for the RNA-induced silencing complex as occurs in mammals. One strength of the RNAi approach is that ongoing (Lilley *et al.*, 2010) and completed genome sequencing for plant-parasitic nematodes (Abad *et al.*, 2008; Opperman *et al.*, 2008) provide a large range of potential targets that can be screened *in vitro* to select those for plant transformation constructs.

Cyst and root-knot nematodes are the two most important economic nematode groups but some crops such as banana are also severely damaged by migratory endoparasites including *R. similis*, which causes rotting of banana roots. It is susceptible to RNAi, although the extent of silencing can vary according to the region of the nematode gene targeted and the experimental occasion. Its infection of *Medicago truncatula* was reduced by 60% after soaking it in dsRNA homologous to a nematode gland cell xylanase gene (Haegeman *et al.*, 2009). It remains to be seen whether or not the efficacy of host-generated RNAi will work efficiently against all species of nematodes in the field.

15.5. Targeting the Nematode Feeding Site

In the case of endoparasitic nematodes, such as species of root-knot and cyst nematodes, an alternative strategy to targeting the nematode directly is to disrupt the feeding sites that these nematodes initiate within the plant. Once the J2 has established a feeding site it then becomes sessile and its continued existence and development is entirely dependent on the feeding cells. Therefore, killing the feeding cell would result in the death of the nematode and thus confer resistance to the host plant. In theory,

this could be achieved by expressing a cell death gene specifically within the feeding site upon its induction. Primarily, this approach would require a promoter that would drive the cell death system specifically in the nematode feeding cell; the value of such a promoter is self-evident.

The concept of using promoters of genes upregulated in nematode feeding sites and linking them to effectors that could either target the nematode or the feeding site was stated by Gurr *et al.* (1991), who identified transcripts upregulated in nematode feeding sites from differential cDNA screening. However, as the nematode feeding cells are derived from normal plant cell(s), it is unlikely that any gene, and by association its promoter, would have an expression profile specific to the feeding cell. It is more likely that the gene/promoter will be expressed in other tissues. This is likely to cause unacceptable damage to the plant but may have advantages; for example, if the expression elsewhere were only in pollen, this would confer male sterility, which could actually be a bonus in a GM crop. An alternative strategy is to use combinations of promoters whose only expression overlap is within the feeding cell. This strategy would require a binary cell death system.

15.5.1. Multiple component strategies and cell death systems

Multiple component strategies can be achieved in a variety of ways, a few of which are outlined in Table 15.1. Figure 15.2 illustrates the general principles that underlie three of the mechanisms.

15.5.1.1. Barnase and barstar (enzyme and inhibitor)

Barnase is an extracellular ribonuclease produced by *Bacillus amyloliquefaciens* and barstar is its intracellular inhibitor. The tacit assumption has been that barnase serves the soil bacterium by degrading RNA for nutritional use. However, it could act as a toxin for predators or competitors within the soil environment. The bacterium protects itself from the highly effective barnase before export by producing the inhibitor barstar. The barnase–barstar combination was utilized in the early 1990s to achieve plant sterility (Mariani *et al.*, 1992). A male sterile line is maintained in a hemizygous state, which is with only one copy of the barnase gene under the control of an anther-specific promoter. Barnase expression in the anther results in cell death and no pollen is produced. To restore male fertility, the hemizygous line is crossed with a line homozygous for the gene encoding barstar, which is also under the control of an anther-specific promoter. Within the F1 population, plants hemizygous for both barnase and barstar will be able to produce pollen due to the neutralizing effect of barstar. A similar system was utilized in *A. thaliana* to engineer resistance to *H. schachtii*; by using a promoter with an expression profile mostly limited to the syncytia to express barnase and constitutive expression of barstar a 70% reduction in susceptibility was achieved (Ohl *et al.*, 1997).

Clearly expression of barnase and not barstar within the nematode feeding cell and nowhere else in the plant is necessary. However, expression of barstar alone may be elsewhere in the plant. A promoter that is turned off when a nematode feeding cell differentiates would be useful for driving expression of barstar.

Table 15.1. Summary of targets of engineered nematode resistance, possible strategies and expected outcomes.

Resistance mechanism	Engineered resistance	Example	Outcome
<i>Natural resistance</i>			
Resistance (<i>R</i>) gene	Transfer of <i>R</i> -gene	<i>Mi</i> from resistant to susceptible cultivar	HR response
<i>R</i> -gene mediated signalling	Modification of signalling activator	<i>Hsp90</i> activation in response to nematode species	HR response
Avirulence factor (<i>Avr</i>) recognition	Modification of R protein or <i>Avr</i> interacting protein	<i>Gpa2</i> responds to all RBP-1 variants	HR response
<i>Targeting early nematode parasitism</i>			
Hatching and attraction	Plants produce chemical deterrents	Over-production of N-formyloline in roots	Nematodes avoid plants
Penetration and migration	Disruption of chemical cues utilised by nematodes during migration	Modification of auxin influx/ efflux carrier expression in response to nematode invasion	Migration disrupted, poor establishment
<i>Targeting the nematode</i>			
Intoxication	Expression of <i>Bt</i> crystal (Cry) protein	Constitutive or local expression of <i>Bt</i> gene	Nematode death
Immobilization	Production of antibodies against nematode proteins	Plantibodies that bind to nematode cuticle proteins	Migration disrupted, poor establishment
Sensory disruption	Expression of a lectin	Nematode sensory disruption due to binding of ConA to amphids	Failure to establish
Disruption of digestion	Expression of a protease inhibitor	CpTI inhibits nematode serine proteases	Poor growth/reduced fecundity
Inhibition of protein production (RNAi)	Expression of dsRNA homologous to target transcripts	Targeted destruction of nematode major sperm protein (MSP) transcripts	Impaired viable egg production
<i>Targeting the nematode feeding site</i>			
Cell death agent and inhibitor	Expression of barnase within feeding cell	Expression of barnase at feeding sites and barstar elsewhere	Feeding cell death/death of nematode
Inactive cell death agent and activator	Expression of active RIP within feeding cell	Expression of inactive PAP-S and activator (TEV 1a protease) within the feeding cell	Feeding cell death/death of nematode

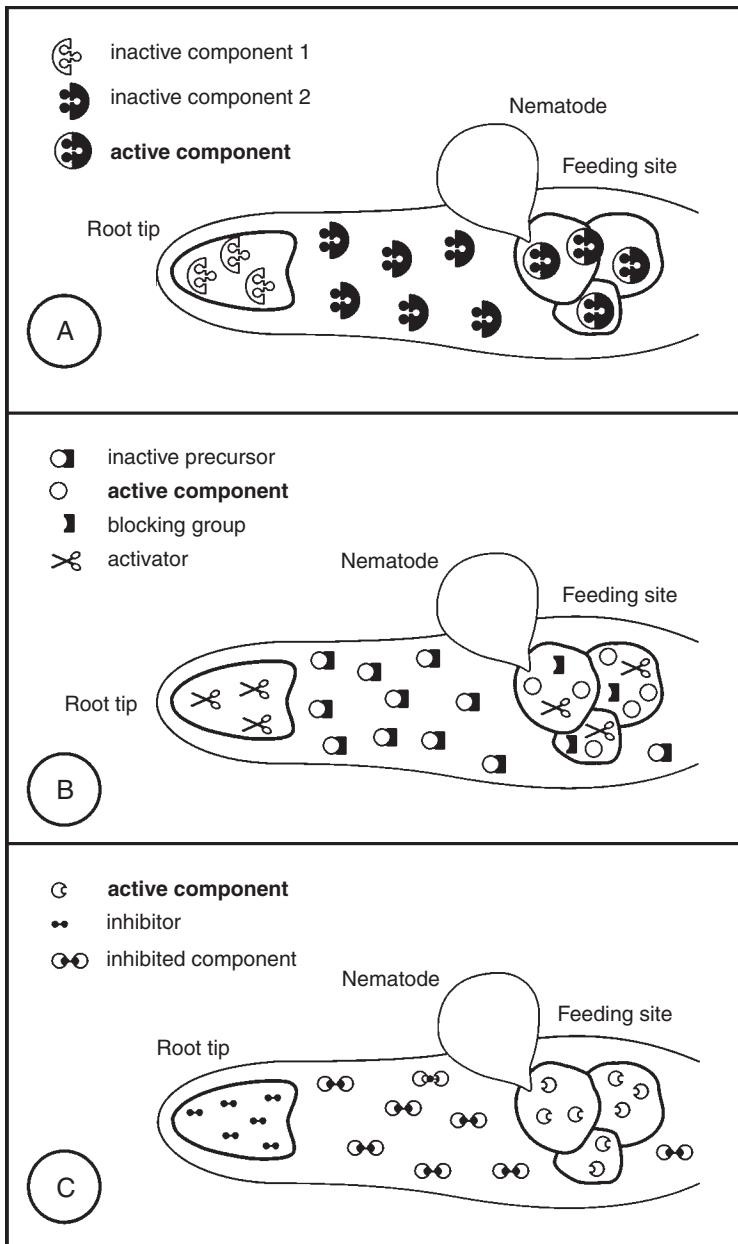


Fig. 15.2. Three examples of binary systems to induce nematode feeding cell death.
A: An active component is produced from two inactive components when both are co-localized within the feeding cell (e.g. maize RIP in Section 15.5.1.2). **B:** An inactive precursor is activated by removal of a blocking group by an activator when both are co-localized within the feeding cell (e.g. PAP-S with blocking group in Section 15.5.1.2). **C:** An active component, within the feeding cell and elsewhere, is inactivated by an inhibitor when both are co-localized outside of the feeding cell (e.g. barnase–barstar in Section 15.5.1.1).

15.5.1.2. Ribosome-inactivating proteins (binary action and activation systems)

Ribosome-inactivating proteins (RIPs) have been linked to plant defence as long ago as 1925 when inhibition of viral infection by an extract of pokeweed, *Phytolacca americana*, was demonstrated. RIPs are N-glycosidases that depurinate the conserved α -sarcin loop of large rRNAs, which leads to the inactivation of ribosomes resulting in blocked protein synthesis. The RIPs have great potential as cell killing agents as their ability to inhibit translation is extremely effective. RIPs are classified into three types (Box 15.2).

Type 3 maize RIP is synthesized as an inactive precursor (RIP α –[25 AAs]–RIP α), which is cleaved to its active form by the removal of 25 central amino acids (Walsh *et al.*, 1991). The feasibility of using the two RIP polypeptide chains as a ‘binary’ cell death system was investigated by quantifying reporter gene (*uidA*) activity in tobacco protoplasts (patent number: WO 02/33106). The uncleaved protein (RIP α –[25 AAs]–RIP α) did not affect reporter gene product activity, while a recombinant RIP (RIP α –RIP α) (with the central, cleavable residues removed) resulted in loss of activity indicating loss of tobacco ribosomal function. Both polypeptide chains were evaluated separately; RIP α showed no effect whilst RIP α resulted in some loss of reporter gene activity. Loss of activity was also observed if both chains were synthesized separately and then combined. In transgenic tobacco plants both the recombinant RIP and the two component RIP systems (RIP α and RIP α), under the control of promoters predominantly active in the root and/or the nematode feeding site, appeared to have some inhibitory effect on the development of female *M. javanica* as measured by nematode size. RIP PAP-S, the seed form of the pokeweed antiviral protein of *P. americana*, was also shown to be capable of inhibiting reporter gene product activity, attaching a blocking group to the N-terminus of PAP-S inactivated RIP activity which could be partially restored by cleaving off the blocking group with a tobacco etch virus (TEV) NIa protease, thus offering potential as a binary cell death system. The maize RIP and the PAP-S two-component systems appear feasible as binary cell death mechanisms but require further development and are likely to be dependent on the identification of promoters to enable sufficient and more specific expression (A. Neelam, C. Thomas and M.J. McPherson, unpublished data).

Box 15.2. Types of ribosome-inactivating proteins (RIPs).

Type 1 RIPs, such as the pokeweed antiviral protein (PAP), are monomeric enzymes and most plant RIPs fall into this category. They are highly effective against ribosomes *in vitro* but are poorly transported in biological systems.

Type 2 RIPs, like ricin and abrin, are highly toxic heterodimeric proteins as their enzymatic chain is linked to a lectin, facilitating uptake into the cell.

Type 3 RIPs are synthesized as inactive precursors (proRIPs) and require proteolytic processing for activation.

15.5.2. The need for promoters

Many genetically engineered solutions, for example the use of plantibodies, require expression early on in the nematode parasitism process at either invasion or migration. To achieve the best results, expression of the transgene should be activated by these nematode activities and expression should be as limited as possible to the nematode location. A number of promoters have been reported that fit these criteria; both the *wun-1* promoter from potato (Logemann and Schell, 1989) and a tobacco extensin gene promoter (Niebel *et al.*, 1993) drive gene expression during *G. pallida* invasion in response to wounding caused by the nematode; however, expression is lost upon syncytia induction. The *wun-1* promoter was not activated during *M. incognita* invasion and migration, presumably because the nematode penetrates root tissues between cells (Hansen *et al.*, 1996). The rice cystatins Oc-I and Oc-II expressed at a low level in alfalfa under the control of a wound-inducible promoter conferred some resistance to *P. penetrans* (Samac and Smigocki, 2003). Invasion by cyst and root-knot nematodes occurs behind the root cap in the zone of elongation and disruption of chemoattraction and invasion requires promoter expression in outer root cells, so that peptides can be secreted into the rhizosphere. A promoter region (*AtMDK4-20*) of an *Arabidopsis* gene with homology to a maize gene known to express in the root cap was used to deliver a chemodisruptive peptide in both *Arabidopsis* and potato; in the case of the latter significantly improved resistance (approximately 60% greater) to *G. pallida* was achieved in comparison to expressing the peptide from the CaMV35S promoter (Lilley *et al.*, 2011).

Biotechnological control strategies of sedentary endoparasitic nematodes have used the CaMV35S promoter, but more refined technology has utilized promoters with a more restricted pattern of expression. Promoters of TUB-1, a β -tubulin gene of *A. thaliana*, RPL16 that encodes an *Arabidopsis* ribosomal protein L16 and ARSK-1, a likely serine/threonine kinase, all direct expression of sufficient cystatin to provide partial resistance to *G. pallida* in the field and to *M. incognita* in containment. The ARSK promoter lines provide more resistance to *G. pallida* than *M. incognita* whereas the other promoters were associated with less resistance against the cyst nematodes. All three promoters were active in the giant cells induced by *M. incognita* but only ARSK1 was also active in the syncytium of the cyst nematode (Lilley *et al.*, 2004). Molecular engineering can lead to promoters that are extremely specific. Deletion of the 5'-flanking region of a root-preferential promoter *TobRB7* resulted in a 300 bp promoter fragment just upstream of the coding region that remained active within the giant cells induced by *M. incognita* and silenced in root meristems (Opperman *et al.*, 1994). While such promoters may have specificity, the strength of expression must also be considered. When Fairbairn *et al.* (2007) targeted a transcription factor of *M. javanica* in an *in planta* RNAi biotechnological strategy (see Section 15.4.5) they tested the CaMV35S promoter and the $\Delta 0.3TobRB7$ promoter. None of the lines containing the $\Delta 0.3TobRB7$ promoter, in sharp contrast to those harbouring CaMV35S, showed any signs of silencing the targeted gene. When reporter plants made with GUS plants under the control of $\Delta 0.3TobRB7$ were analysed only a small percentage of galls showed GUS activity and those that did revealed only weak activity (Fairbairn *et al.*, 2007).

By contrast, if the nematode feeding cell is to be targeted then the expression of the effector, or overlap of the binary cell death system, must only occur within the

feeding cell. Expression elsewhere would result in cell death in other plant tissues and may lead to a loss of viability of the host. As nematode feeding cells are derived from normal root cells it is unlikely that a feeding cell-specific promoter exists and, as yet, there has been no demonstration of such a promoter. However, a combination of promoters whose overlap is specific to the feeding cell is a distinct possibility. In the past, the search for nematode-responsive promoters assumed that they might be active in both the feeding cells of root-knot and cyst nematodes; although there are some similarities in the feeding sites, with the increase in number of nematode-responsive genes isolated it is now apparent that the different feeding structures also have their own unique gene expression profiles (see Chapter 9).

The wealth of *A. thaliana* resources makes this plant an attractive tool for identifying promoters that express in nematode feeding cells. These include primarily a fully sequenced genome, micro-arrays, which have been used to identify syncytium and giant cell expressing transcripts (e.g. Jammes *et al.*, 2005; Fuller *et al.*, 2007; Szakasits *et al.*, 2009), and enhancer trap lines, which have been used to identify feeding cell expressing promoter elements. Reporter tagged lines were instrumental in the identification of the *pyk20* gene, which encodes a transcription factor specific to Cruciferae (Puzio *et al.*, 1999) and is expressed in response to IAA and kinetin. The tagged line showed reporter activity in early syncytia of *H. schachtii* (Puzio *et al.*, 1998). A syncytial-specific promoter from a tagged line that may represent this promoter has been used to achieve partial resistance to the potato cyst nematode (Ohl *et al.*, 1997). As the full genomic sequence of *Arabidopsis* is known, it is a relatively simple task to isolate putative promoter sequences from nematode-responsive genes using a PCR approach. In *Arabidopsis*, 2 Kbp of nucleotides 5' to the initiation codon are generally sufficient to drive reporter gene expression. Clearly, this is not always the case as the promoter may exceed 2 Kbp or proteins bound to sequences within introns, exons or 3' untranslated sequences may interact with promoter elements and be necessary for a full expression profile. The promoters of myo-inositol oxygenase genes are predominantly expressed in syncytia induced by *H. schachtii* in *Arabidopsis* roots and may have a use in biotechnological strategies (Siddique *et al.*, 2009). A promoter of a defensin with strong expression in the syncytia of *H. schachtii* in *Arabidopsis* and limited expression elsewhere (in siliques) has been identified (Siddique *et al.*, 2011). The promoter was isolated from a defensin gene *Pdf2.1* which was previously identified as upregulated in syncytia from GeneChip array data (Szakasits *et al.*, 2009). Many *Arabidopsis* promoters, when transferred into other plant species, do exhibit an identical expression profile but this is not always the case. For example, the AtSUC2 promoter has been reported to express in the syncytia of *H. schachtii* in *Arabidopsis* (Juergensen *et al.*, 2003); in both *Arabidopsis* and potato the promoter drives reporter gene expression in the phloem and a high level of expression is observed in the syncytia of *H. schachtii* in *Arabidopsis* but in the syncytia of *G. pallida* only weak transient expression was seen (A. Cottage, unpublished data).

Many promoters are reported in the literature as expressing in the feeding cells of nematodes; however, caution should be exercised when utilizing these data in the context of engineering nematode resistance in crop plants. Frequently nematode infections are carried out in culture in media that may contain sucrose; when plants are grown in potting medium (soil, sand or compost) a different expression profile to that reported is often seen, particularly in root tissues.

15.6. Stacked Defences

Whilst there may be an immediate benefit in utilizing a successfully engineered resistance on its own, one should consider the longer-term implications and plan for a more sophisticated approach. Single resistances generate a strong selective pressure, especially in the predominant intensive farming/monoculture currently used in agriculture. For example, when resistance to *G. rostochiensis* was introduced (in the form of potato cultivars that are naturally resistant to *G. rostochiensis* as their genomes encode the *R*-gene *H1*), the initial success in the UK was soon negated by a shift in nematode populations. Currently *G. pallida* is prevalent, for which there is no commercial full resistance and 64% of the UK potato growing areas are now affected.

There are at least two approaches, which are not mutually exclusive, to try and ensure that the balance is shifted towards successful plant protection. The first approach is to stack several resistances to the pathogen in the plant, preferably targeting different aspects of the nematode's life cycle. Combining partial natural resistance with a protease inhibitor based resistance has been shown to improve the overall resistance in comparison to that of single engineered resistance (Urwin *et al.*, 2003). The second approach is to utilize the engineered resistances as part of a panel of methods that can be used in integrated pest management that may include pesticides (if available), natural resistances, crop rotation, trap cropping and biocontrol measures.

15.7. The Research Approach to Engineering Nematode Resistance

When investigating a genetically engineered resistance a number of factors need to be considered. Firstly, the effective delivery of defined and quantified nematode inocula is important. In order to achieve this, nematodes should be relatively easy to culture and propagate *in vitro*, in containment and in field conditions. Nematode species and strain must be correctly identified and defined; for example, *M. javanica* rather than *M. incognita* or *G. pallida* pathotypes 2/3 rather than pathotype 1. Quantification of a viable inoculum may take the form of number of cysts/eggs per volume of soil, or a defined number of hatched juveniles applied in suspension. Secondly, the selection of a plant species or cultivar is not trivial; considerations are the ease of transformation and regeneration and the propagation time from parent to offspring. The obvious choice for proof-of-concept type research is the use of *A. thaliana* with *H. schachtii* or *Meloidogyne* spp. *Arabidopsis* has a short generation time of approximately 6 weeks, is easily transformed (by floral dip) and numerous plants can be grown in small areas *in vitro*. Similarly, crop plants of the family *Solanaceae*, e.g. potato and tobacco, have been used extensively as experimental models over the past two decades and can be readily transformed and regenerated. Before nematode resistance can be evaluated all putative transformants should be verified as containing the transgene (by PCR) and transgene copy number determined (using real-time quantitative PCR), and expression levels of resistance products assessed (i.e. using real-time quantitative reverse transcription PCR). The transgene insertion site should also be determined to ensure that it has not interrupted a plant gene.

Symptoms of infection can vary considerably from plant to plant; additionally, the level of activity of an engineered resistance can vary considerably between plants.

Therefore, it is necessary to try to standardize infection and cultivation as much as possible. The resistance trial design should include the test plants, susceptible controls and resistant controls (if available), populations of which should be infected and non-infected. All populations must have originated and been grown in the same way, i.e. in the first instance through tissue culture. The resistance is then measured as a population effect; instead of looking for one or two apparently highly resistant lines, an overall increase in resistance in the engineered population relative to the control population is sought.

Trial design enabling meaningful statistical evaluation necessitates large numbers of plants and scoring methods must either be simple enough to assess an experiment within a day or allow immediate fixing of plants and nematodes to facilitate assessment at a later date. Nematodes infecting plants can be counted on material grown and infected *in vitro* using standard techniques. Effects on nematode development due to the resistance mechanism can be assessed using image capture and measurement techniques (Atkinson *et al.*, 1996). Once resistance has been established in contained trials the next step is field trial evaluation, which evaluates not only the resistance in an agricultural setting but also enables the interaction of the transgenic crop with the environment to be assessed.

The ultimate aim of a genetically engineered resistance is to make that resistance commercially available, and freedom to operate should be determined as in many cases methodologies and target sequences are protected by intellectual property rights (reviewed by Rommens, 2010).

15.8. The Future

With the increase in understanding of plant–nematode interactions more sophisticated resistance mechanisms will become available. The major challenges will be to gain market acceptance, especially in a sceptical European environment. However, nematode resistance is probably one of the best mechanisms for demonstrating the benefits of a GM technology if it is soundly based. In the long term, engineered resistances should just be seen for what they are – another tool in an armoury against persistent and damaging pests that are best used in an integrated pest management approach.

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16 Chemical Control of Nematodes*

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16.1. History and Development of Nematicides

The UK's Chemicals Regulation Directorate defines a pesticide as 'any substance, preparation or organism prepared or used for controlling any pest. A pesticide product consists of one or more active substance (a.s.) co-formulated with other materials' and a nematicide is classed as 'a pesticide used to control unwanted nematodes' (<http://www.pesticides.gov.uk/guidance/industries/pesticides>). However, whilst some biological control agents for nematodes are classified for registration purposes as

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nematicides within the UK and EU, they will not be discussed here as they are considered separately in Chapter 13. Nematicides are chemical compounds that are lethal to nematodes, whereas the terms ‘nematistat’ or ‘nematostatic’ (see Chapter 10) are frequently used for compounds that provide sub-lethal dosages, which disrupt nematode behaviour. These latter chemicals work by paralysing the nematodes for a variable period of time, during which they may deplete their lipid reserves to such an extent that they are unable to invade the plant. Nematode recovery is possible from the sub-lethal effects of nematostats. The term nematicide is commonly used as an umbrella term encompassing both types of activity and will be used in this sense here.

Nematicides are applied primarily to reduce root/plant damage caused by nematodes and to increase productivity (Tobin *et al.*, 2008), which is achieved by reducing the numbers of nematodes feeding on (e.g. *Trichodorus* spp.) or in (e.g. *Meloidogyne* spp., *Globodera* spp.) plant tissues. Nematicides are also applied to prevent or reduce nematode reproduction and to limit the transmission of nematode-borne viruses to the plant (Dale *et al.*, 2004). The economic benefit to the crop is seen as a reduction in yield loss (and/or an increase in quality), maintenance of future production and profitability. Nematicide application has been shown significantly to reduce a wide number of plant-feeding nematodes (Eisenhauer *et al.*, 2010); however, reductions in nematode population densities do not always occur, particularly where the nematode’s reproductive rate is high or multiple generations of the nematode occur during the growth period of the crop. For example with the potato cyst nematode, *Globodera pallida*, in the UK the damage threshold for an intolerant cultivar is approximately 1–3 eggs g⁻¹ soil. The greater the initial population density the greater the level of control required to achieve this low level. Population multiplication on a susceptible cultivar is inversely density dependent (Chapter 11), but in practice is rarely more than 50-fold, so that a reduction in initial population density of 98% should result in full control (Whitehead and Turner, 1998). This degree of control is rarely achieved and the use of nematicides will need to be combined with other measures such as crop rotation and plant resistance as part of an integrated management strategy. Modelling of *G. pallida* populations has shown that egg survival following nematicide treatment is often sufficient to allow large populations to ‘rebound’. This has been supported by field experiments showing that *G. pallida* population increase was almost as great in nematicide treated as in untreated plots (Trudgill *et al.*, 2003).

Nematicides have been in use since the late 19th century when the fumigant carbon disulphide was introduced. Development of further fumigants took place in the first half of the 20th century with the introduction of chloropicrin, 1,3-dichloropropene (1,3-D), methyl bromide, 1,2-dibromo-3-chloropropane (1,2-DBCP), 1,3-dichloropropene and 1,2-dichloropropane mixtures (DD), formaldehyde, metam sodium and dazomet. The remaining uses of methyl bromide were revoked for developed countries in 2005 under the Montreal Protocol for the reduction of gases contributing to global warming, although critical use exemptions still apply (Section 16.4.3). Similarly, 1,3-dichloropropene (1,3-D) is now banned in the EU but continues to be used in the USA (http://iaspub.epa.gov/apex/pesticides/f?p=PPLS:102::NO::P102_REG_NUM:62719-32) and is being registered for use in China (Qiao *et al.*, 2012). The second half of the 20th century saw the development of the organophosphates such as fenamiphos, ethoprophos and fosthiazate, together with the carbamates carbofuran, aldicarb and oxamyl (Table 16.1). The most recent addition to the nematicide options is fluensulfone, a fluoroalkenyl systemic non-fumigant (Oka *et al.*, 2012). The process of nematicide discovery and testing is outlined in Box 16.1. The late 20th and early 21st century

Table 16.1. Globally important nematicides.

Active substance	Chemical group	LD ₅₀ (acute oral male rats)	Year of discovery	Example trade name	State of formulation	Manufacturer
Abamectin	Avermectins	10 mg kg ⁻¹	1979	Avicta 500FS	Liquid	Syngenta http://www.Syngenta.com
Aldicarb ¹	Oxime carbamate	0.93	1965	Temik 15G	Microgranule	Bayer CropScience http://www.bayercropscience.com
Carbofuran	Carbamate	8	1965	Furadan 15G	Microgranule	FMC Corporation http://www.fmc.com
Cadusafos ¹	Organophosphorus	37	1982	Rugby 200 CS	Liquid	FMC Corporation http://www.fmc.com
Dazomet	Methyl isothiocyanate ² liberator	77–220 ²	1897	Rugby 10G	Microgranule	BASF Corporation http://www.agriculturalproducts.basf.com
1,3-dichloropropene	Halogenated hydrocarbon	150	1956	Basamid	Microgranule	Dow AgroSciences http://www.dowagro.com
Ethoprophos	Organophosphorus	62	1966	Telone II	Liquid	Bayer CropScience http://www.bayercropscience.com
Fenamiphos	Organophosphorus	6	1967	Telone EC	Liquid	Bayer CropScience http://www.bayercropscience.com
Fosthiazate	Organophosphorus	73	1992	Mocap 10G	Microgranule	Bayer CropScience http://www.bayercropscience.com
Iprodione	Dicarboximides	>2000	1974 (a.s.)	Mocap EC	Liquid	Syngenta http://www.syngenta.com
Metam sodium (sodium N-methyldithiocarbamate)	Methyl isothiocyanate liberator	77–220 ²	1951	Nemacur 15G	Liquid	Devgen nv http://www.devgen.com
Oxamyl	Oxime carbamate	3.1	1974	Nemacur 3	Liquid	Amvac Chemical Corporation http://www.amvac-chemical.com
				Nemathorin 10G	Microgranule	Du Pont http://www.1.dupont.com
				Vapam	Liquid	
				Vapam HL	Liquid	
				Vydate 10G	Microgranule	
				Vydate L	Liquid	

¹Chemicals such as aldicarb and cadusafos have now been banned, restricted or are under licence revocation in several countries.²LD₅₀ is for methyl isothiocyanate.

Box 16.1. Nematicide discovery and evaluation.

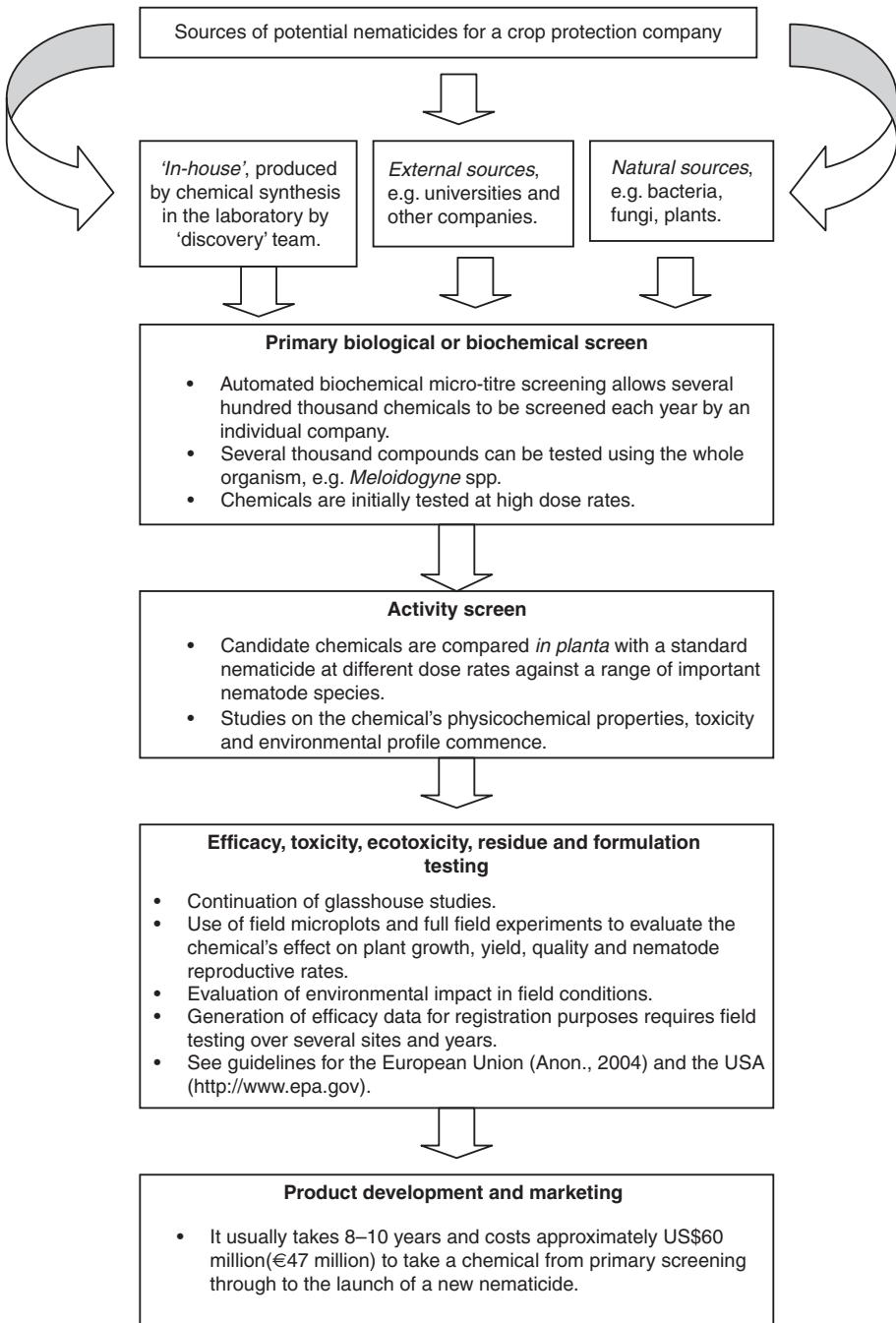


Table 16.2. Estimated market share of nematicides for key nematodes and crops.

Nematodes	Market share (% of total value)	Crop	Market share (% of total value)
Root-knot nematodes	48	Vegetables	38
Cyst nematodes	30	Potatoes	25
Others	22	Bananas	9
		Tobacco	8
		Sugar beet	6
		Others	14

has seen more research and development to improve the efficiency of use of nematicides, to minimize their environmental impact and to reduce their cost to growers. There has also been increased interest in and development of ‘natural’ nematicides, derived mainly from plant extracts and bacteria (Section 16.6). Avermectins (B1a and B1b) derived from the soil bacterium *Streptomyces avermitilis* are the components of the nematicide abamectin.

Nematicides continue to be an important part of nematode management programmes, whether used as part of an integrated management approach or as the sole control component (Hillocks, 2012; Qiao *et al.*, 2012). The global market for nematicides is suggested to be worth US\$1 billion (approximately €755 million) per annum, utilizing in the order of 350,000 t of active substance each year, with the annual cost from loss of production due to nematodes at US\$80–125 billion. Approximate usage by crop and nematode group is given in Table 16.2. Vegetable crops account for the greatest proportion of nematicide use where *Meloidogyne* spp. are the target for approximately half of the world’s usage and so are of major concern when developing new products.

16.2. Active Substances: Chemical Groups and Modes of Action

Nematicides can be classified according to their chemical group (e.g. carbamates, organophosphates), their mode of action (e.g. acetylcholinesterase inhibitor) and mode of application (e.g. fumigant, non-fumigant). In this chapter, nematicides will be grouped according to their mode of application and discussed by chemical group and mode of action. Whilst there is much data to support the efficacy of nematicides, there is a dearth of information to understand their precise activity in nematodes, most of the information being taken from their known effects in insects and mammals.

16.2.1. Fumigant nematicides

The fumigant nematicides consist of compounds based on halogenated hydrocarbons and those that release methyl isothiocyanate. The halogenated hydrocarbons include 1,3-D and methyl bromide. These fumigants are thought to directly affect

biochemical pathways in protein synthesis and respiration. In soil, sodium N-methyldithiocarbamate (metam sodium) and dazomet degrade to release methyl isothiocyanate. Once inside the nematode, cyanide prevents the utilization of oxygen, presumably carried by oxygen-transporting globins, and so prevents respiration.

16.2.2. Non-fumigant nematicides

Organophosphate (e.g. fenamiphos, ethoprophos and fosthiazate) and carbamate (e.g. oxamyl, aldicarb) nematicides are applied to the soil as granular or, for some, liquid formulations. At low concentrations they disrupt chemoreception and the ability of the nematode to locate the host plant root; at higher concentrations they disrupt nematode hatch and movement. The active substance is absorbed through the nematode's cuticle where it disrupts functioning of the nervous system by binding with the enzyme acetylcholinesterase (AChE) in the synapse, AChE being essential to hydrolyse acetylcholine at the synaptic cleft (see Chapter 7). At field rates, the biochemical effects of carbamates and organophosphates are reversible, explaining why they are often termed 'nematostats' or 'nematostatics'. However, even if recovery does follow exposure to a sub-lethal dose, nematodes may not be sufficiently vigorous to locate the host plant. In this situation it is not necessary to kill the nematode directly in order to reduce its harmful effects on plant growth. To maximize nematode control, exposure (nematicide concentration \times time) is maximized by the correct timing of application and thorough incorporation (solid formulations) or dissipation (liquid formulations) of products in the target zone of the soil. For further information on nematicide mode of action consult Wright (1981).

16.3. Formulation and Application

16.3.1. Liquid formulations

Globally there are now only a few non-fumigant nematicides available in liquid formulations, e.g. fenamiphos and oxamyl (Table 16.1). These active substances control a wide range of plant-parasitic nematodes and are marketed in various formulations dependent on target organism, delivery system and crop. The range of crops protected by these products is as diverse as potatoes, bananas, cucurbits and tobacco. Product availability and use is dependent on country and, in certain instances, can vary greatly between regions within a country. It should not, therefore, be assumed that because a nematicide is registered for use in one country/area that it would automatically be registered or available elsewhere.

Generally, liquid nematicide formulations are soil applied. Methods of application include overall spray followed by incorporation to a specified depth, sprayed over the width of the furrow or row, shank injected followed by irrigation, or added to drip, trickle or overhead irrigation in a process known as chemigation (Fig. 16.1). Foliar application is also possible with some products such as Vydate L® (a.s. oxamyl), which can be applied to tomatoes for the control of root-knot, sting, stubby-root, stunt and reniform nematodes. Vydate L® can be applied through low-pressure sprinkler-type equipment including centre pivot, lateral move, solid-set, mini sprinklers and

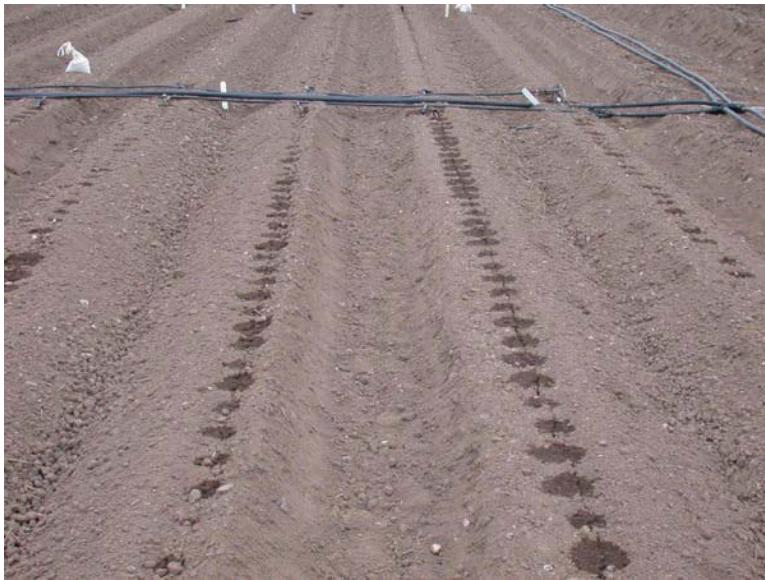


Fig. 16.1. Application of nematicide via trickle irrigation.

drip tape. Operators should always check with current recommendations from the manufacturer to ensure that each formulation and application method is legal for each nematode problem, crop and country/state of use.

Where irrigation water is used as carrier for the nematicide it is of paramount importance that sufficient safety systems are used to prevent backflow into, and contamination of, the water source. As with all irrigation, but even more so with chemigation, it is essential to ensure that run-off and leaching do not occur as a result of either poor application technique or lack of knowledge of the soil moisture status at the time of irrigation. From an environmental standpoint it can be argued that trickle or drip irrigation offer the most environmentally acceptable forms of chemigation as these methods can reduce exposure of non-soil-dwelling organisms, such as birds, to nematicides.

16.3.2. Microgranules

Microgranules are virtually dust-free, free-flowing granules of approximately 1 mm in diameter. A range of active ingredients including aldicarb, oxamyl, fosthiazate and ethoprophos are available, formulated onto microgranules formed from materials such as sepiolite, brick dust and clay. Microgranular formulations can range in active substance content from 5 to 15%. This formulation is manufactured for ease of direct application to soil, and to make products as safe as possible to handle, thereby reducing the risk of operator contamination. Engineering controls have also been introduced to reduce human exposure to the products such as the Surefill™ (www.syngenta-crop.co.uk) and Ecolite™ (www2.dupont.com) closed transfer systems, which come in sealed containers. These containers have been designed to keep the products within a sealed environment, and have been an important development in

the safe handling of microgranular nematicides and insecticides. The containers are also ‘returnable’ to the manufacturer for re-use, which satisfies the requirements of the EU Packaging and Packaging Waste Directive 94/62/EC to increase recycling and reduce packaging waste.

Microgranules must be metered onto the ground at the correct rate, and a number of methods have been developed to achieve this. The most reliable way of metering granules is by using a ground wheel driven method such as the Horstine Farmery Microband Applicator® (Fig. 16.2). This positive displacement machine eliminates the risk of over-treating when the tractor is stationary because dosing is generated by distance travelled and not the forward speed of the tractor. Cartridge inserts containing the correct metering unit for a particular product are now available (Fig. 16.3). Modern electronic and hydraulic systems are also now in use to achieve variable rate and precision application using differential global positioning system (DGPS). Whilst the economics of field sampling for nematodes often does not allow for the sampling intensity required to provide the necessary detailed field information to complement the accuracy of the application technology, electrical conductivity soil mapping can be a useful aid (Ortiz *et al.*, 2010; Hbirkou *et al.*, 2011). Therefore, issues regarding whether or not to treat low nematode populations and the potential for higher nematode population multiplication are a very real concern. Some product stewardship issues are apparent with respect to granule application and these are discussed in Section 16.5.3.

The application of granular nematicides for the control of plant-parasitic nematodes varies according to the nematode species and the crop to be protected. Factors such as root growth habit, i.e. tap rooted or fibrous rooting systems, nematode mobility and soil characteristics, will all affect the success of granular nematicide application. The high financial cost of nematicides means that they must be used efficiently and provide maximum benefit in terms of crop yield and nematode control.



Fig. 16.2. Horstine Farmery Microband Applicator showing hoppers and ‘fishtail’ distribution units.

This is obtained by optimizing the distribution of the chemical in soil in the area of early root development (Fig. 16.4).

Free-living stages of plant-parasitic nematodes generally require a localized treatment of granules combined with the seed because, to some extent, such stages can be expected



Fig. 16.3. A selection of granule metering cartridges. (Courtesy of Horstine Farmery, Lincoln, UK.)



Fig. 16.4. Incorporation of granules is best achieved by rotary cultivation. (Courtesy of Horstine Farmery, Lincoln, UK.)

to migrate towards the early root growth, following root exudate chemical gradients, and as a consequence towards the nematicide. Methods used to achieve this type of application can be considered as spot treatments or in-furrow treatments. Treatment of seed furrows with nematicides is used with crops such as onions and field beans to control the stem nematode *Ditylenchus dipsaci*. However, nematicide application to the furrow can produce large, potentially phytotoxic, concentrations of nematicide around the seed or developing plant and will only control nematodes in the immediate vicinity.

Sedentary nematode species such as the potato cyst nematode (*Globodera* spp.) and ectoparasitic nematodes parasitizing crops with fibrous root systems may not be well controlled by a localized application method. The lower mobility of the sedentary nematodes requires a root growing near to the nematode, and the diffuse nature of a fibrous root system under attack by ectoparasitic nematodes will explore a greater volume of soil than a tap-rooted plant. In both cases, the whole volume of soil explored by the root system requires treatment. This would be too expensive and would require very large volumes of soil to be treated. Therefore, a compromise between obtaining good plant establishment while achieving some nematode population control is the aim. For example, assuming a bulk density of 1 g cm^{-3} a 1 ha field has 1500 t of soil in the top 15 cm of the soil profile. To provide an effective concentration of nematicide to this quantity of soil with between 30 and 100 kg of nematicide granules is a challenge.

At present, the recommendation given by nematicide manufacturers is to incorporate nematicide granules to a depth of 5–15 cm, depending on the crop, nematode problem and the nematicide concentration required in the soil water. In the field, different machines are used to achieve this recommendation and it is likely that some methods used by growers fail to achieve this objective. It is essential to know the incorporation characteristics of tillage machinery as shallow incorporation will not treat enough soil to give satisfactory returns in terms of crop yield and nematode control. Deep incorporation is also undesirable as the a.s. of the nematicide is diluted in the greater volume of soil. Achieving the optimum depth of nematicide incorporation in relation to root growth is critical in order to maximize nematode control (Fig. 16.5). Further, if new tillage techniques are introduced into cropping systems, the introduction of any new systems should be evaluated against the recommended

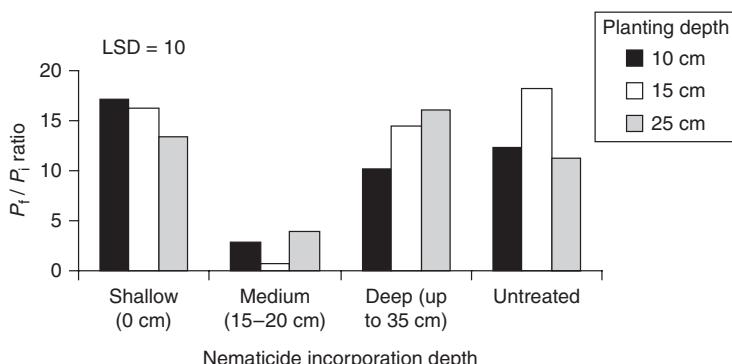


Fig. 16.5. Effect of depth of granular nematicide incorporation and depth of potato tuber planting on the multiplication of *Globodera pallida*. (Adapted from Woods and Haydock, 2000.)

granule incorporation depth. A technique based on the use of pigment-coated micro-granules that fluoresce when exposed to ultraviolet light has been developed to allow the placement of microgranules to be assessed (Woods *et al.*, 1999).

Application timing is critical for microgranule application and is normally timed for ‘as close to planting’ as possible. This is to enable the product to provide effective doses for as long as possible during early phases of plant growth when growth retardation can be severe. Additionally, the ‘harvest interval’ (HI; days between application and the harvest of the crop) may also be critical for crops such as potatoes that are destined for ‘early season’ markets. The HI for ethoprophos, oxamyl and fosthiazate when used for potatoes is 56, 84 and 119 days, respectively. With maximum residue levels of 0.01 mg kg⁻¹ in the EU for oxamyl, the HI can sometimes be problematic.

16.3.3. Fumigants

Soil fumigation is a technique whereby chemical liquids, or solids, are incorporated into the soil where they volatilize, move through air spaces in the soil as a gas and provide control of a range of pests, weeds and disease. Fumigant nematicides are usually either halogenated hydrocarbons, e.g. chloropicrin, methyl bromide, 1,3-D, or methyl isothiocyanate liberators, e.g. metam sodium and dazomet. There are often several commercial products containing these active substances individually or as a combination of two actives designed to target specific pest, disease and/or weed problems. Methyl bromide is an odourless gas above 4°C and chemicals such as chloropicrin (tear gas) may be added to a product for safety reasons. In this instance chloropicrin adds an odour to the otherwise odourless methyl bromide and thus provides an indicator to operators of the presence of the chemical.

The three fumigant nematicide formulations are as liquefied gases, volatile liquids and solids. The liquefied gases are held in liquid state under pressure but convert quickly back to gas when released into the soil or atmosphere, e.g. methyl bromide. Volatile liquids are chemicals that remain as liquids while contained at normal atmospheric pressure but readily convert to gases in the soil environment, e.g. 1,3-D. Solids are normally granular or microgranular formulations that ultimately degrade to release the active substance, e.g. methyl isothiocyanate, when placed into moist soil.

The two physical properties of a fumigant that affect its effectiveness are volatility and solubility. Volatility is affected by boiling point and vapour pressure. There is an inverse relationship between these two properties and thus a chemical with a low boiling point will have a high vapour pressure. When comparing the properties of two products it can be demonstrated how these properties affect fumigation characteristics and use. Methyl bromide has a boiling point of 3.6°C and a vapour pressure of 190 kpa (at 20°C), whereas 1,3-D has a boiling point of 108°C and a vapour pressure of 3.7 kpa (at 20°C) (Tomlin, 2009). Due to the much higher volatility of methyl bromide it has excellent diffusion characteristics and can diffuse through well-structured soils for up to 1 m. The volatility does not indicate flammability, however, as methyl bromide is classed as non-flammable. The drawback associated with this high volatility is that soil fumigation demands a good surface seal to prevent rapid loss of the chemical to the atmosphere. Consequently, methyl bromide fumigation often requires the use of impermeable sheeting to create a contained environment, a difficult task to achieve on a large field scale. By contrast, the lower volatility of 1,3-D and dazomet

can negate the need for additional sheeting, and an adequate surface seal can be achieved via the less expensive techniques of rolling or smearing the soil surface with contra-rotating drums (Fig. 16.6). However, for this type of seal to be effective, trash from the previous crop needs to be incorporated well and there should be sufficient moisture in the soil to allow a 'seal' to be achieved. The low volatility of 1,3-D does not overly hinder its movement in soil but it has a flash point of only 25°C, which makes it flammable and, therefore, care is needed when handling. In addition, the equipment and containers used for 1,3-D should not contain aluminium, zinc, cadmium or magnesium alloys as corrosion and hazardous decomposition (production of hydrochloric acid) may occur. Equipment or containers should not be washed out with water as similar decomposition to hydrochloric acid occurs.

The effectiveness of soil fumigants can be greatly influenced by several soil factors: texture, structure, organic matter, moisture content and temperature at application. Coarse-textured soils can generally be fumigated more easily than fine-textured (clay) soils, as the coarse sandy soils contain more voids per unit volume. Unfortunately this also makes them more reliant on good sealing. Clay and organic matter can adsorb large quantities of the applied chemical, either making it necessary to apply higher rates of chemical or unrealistic to use for control purposes in these soil types. Similarly, poor soil structure, e.g. compaction, can restrict gaseous movement and result in poor fumigant penetration and thus give poor nematode control. Soil moisture at the time of application can also be important for the efficacy of the fumigant. In general, it is useful to maintain soil moisture between 30 and 70% of field capacity, dependent on product and use (some need 40–60%). Consequently, there must be sufficient air spaces within the soil to allow for rapid dissemination of the gas but the soil must not be too dry as to allow rapid loss of the fumigant. There must also be sufficient moisture to interact with the fumigant



Fig. 16.6. Application of 1,3-dichloropropene using contra-rotating drums to seal the soil surface.

and then act upon the organism in question. Air vapour phase activity of 1,3-D in laboratory experiments has been shown to require a greatly reduced dosage to achieve 100% kill of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* (Grove and Haydock, 2000). It would, however, be impossible to achieve the sealed environment of these laboratory experiments in a field situation. Suitable temperature is also of great importance for effective fumigation. Most fumigants require a range of 7–27°C to work well; below 7°C the fumigant may not volatilize or diffuse adequately and above 27°C volatilization may be too rapid and degradation or loss may occur.

Application of the various fumigant formulations will vary according to the fumigant, pest and target zone, and most should be applied several weeks or months before planting to avoid crop damage due to their inherent phytotoxicity. Granular formulations can be broadcast on to the soil surface or injected, or incorporated by cultivation. There may be a requirement to then seal with sheeting, irrigation or surface consolidation. The application of the volatile liquids will greatly depend on the label recommendations and uses. They may be injected via chisel (shank) or subsoiler-type ploughs and commonly sealed with mechanical consolidation of the soil surface or suitable sheets. Liquefied gases can be injected into the soil with suitable equipment for field scale fumigation or released into evaporation pans via tubes under sealing sheets for small scale fumigation in glasshouses, etc. However, it is important to note that when the pests to be controlled exist in the upper soil horizons, up to 10 cm depth, the use of topsoil consolidation should be avoided as this consolidation effectively removes the pore spaces necessary for effective fumigant movement. In this case, the only option is to use a sealing sheet above the soil. Some dedicated product formulations also exist, such as 1,3-D as Telone EC®, which is a liquid formulation fumigant designed specifically for use in drip irrigation only.

Whether liquid or gas phase is considered, the dosage of the chemical is important. The dosage can be given as the amount of chemical required (concentration) and the time period over which it is required (time) to achieve kill of the nematode. This is given then as the concentration × time product (CT). A useful review of fumigant movement, dosage and differential effects can be found in Munnecke and Van Gundy (1979).

16.4. Nematicides in the Environment

16.4.1. Degradation

As soon as nematicides are applied to the soil (or to the plant), degradation starts to occur. It is important that nematicides degrade into harmless compounds so that they do not persist in the environment. However, it is essential that nematicides are sufficiently persistent to control the target nematode population effectively.

Once applied to the soil, losses by volatilization directly to the atmosphere may occur. This is mainly of concern with fumigant applications where losses can be great if the soil surface has not been effectively sealed (see Section 16.3.3). Certain nematicides, e.g. oxamyl, are systemic and the nematicide is taken up by the plant and hydrolysed to the corresponding oximino compound. Total degradation, leaving no detectable residues in plants, has been demonstrated.

Once applied to the soil some of the nematicide may be degraded by microbial or chemical activity or physically lost from the soil by leaching or surface run-off before nematode control has been achieved. Soil microbes play an important role in the degradation of nematicides where they are broken down to provide a source of carbon or energy. Microbial degradation is a beneficial process as it allows nematicides to be broken down into environmentally benign molecules. However, if the breakdown occurs too fast then efficacy may be reduced. Breakdown of nematicides occurs most rapidly in warm, moist, alkaline soils, conditions associated with optimal environmental conditions for microbial activity. Repeated application of nematicide to the same soil can result in reduced persistence. This has been called enhanced or accelerated microbial degradation and has been documented for several carbamates. Karpouzas and Giannakou (2002) reviewed enhanced biodegradation of nematicides. Losses of nematicides from the soil by surface run-off (relatively rare) or by leaching are highly undesirable as it may enable nematicides to enter groundwater. This has been reported for several nematicides including aldicarb and 1,3-dichloropropene. For a review of the environmental fate of fumigant nematicides see Seiber *et al.* (1996).

16.4.2. Non-target organisms and groundwater

Nematicides are usually non-selective pesticides and their use will normally affect non-target organisms. The most obvious effect is where non-target organisms receive lethal or damaging levels of the active substance by direct ingestion/contact or exposure. However, a less obvious effect is where the application of a nematicide alters the environment in which the organism lives, thereby destroying its habitat or its food source. A useful starting point to identify the potential of a nematicide to affect non-target organisms is their toxicity classifications. The Pesticide Manual (Tomlin, 2009) gives the World Health Organization (WHO), Environmental Protection Agency (EPA) and European (EC) classifications, e.g. aldicarb WHO Ia (extremely hazardous) and the EC classification of T+ (very toxic) with additional classifications including N R50 (very toxic to aquatic organisms). Further toxicology information can also be gained from the individual pesticide labels, material safety data sheets or regulatory authority websites, e.g. EPA in the USA and the Chemicals Regulation Directorate (CRD) in the UK.

The organophosphate ethoprophos, the carbamates oxamyl, aldicarb and carbofuran (or their metabolites), and the avermectin abamectin (AVM B1) are all toxic or highly toxic to birds and fish, whilst oxamyl and ethoprophos are only moderately toxic to fish. Van Straalen and van Rijn (1998) outline the work that shows carbofuran to be lethal to a wide range of soil organisms including collembola, carabid beetles and earthworms. Stenersen (1979) studied the effect of several chemicals on earthworms and reported that aldicarb was the most toxic, whereas oxamyl was not toxic to any of the species tested. This demonstrates that even though chemicals may come from the same group and have similar modes of action they do not necessarily have the same environmental impact.

Direct contact or exposure is also not necessary for a chemical to have an impact on a non-target organism. For example, birds may be directly exposed by

ingesting spilt granules but they might also eat contaminated earthworms and thus be affected by the chemical as a secondary effect. In contrast to the non-fumigants, some of the fumigant nematicides offer an additional threat to non-target organisms: exposure to the gaseous state of the chemical. In this respect the toxicity classification can be based on inhalation studies on mammals and from this the EC classification for methyl bromide and chloropicrin is ‘toxic’, 1,3-D is ‘harmful’ but metam sodium and dazomet have no inhalation classification. However, metam sodium and dazomet do pose high risk to aquatic organisms and the aquatic environment.

Contamination of water sources by nematicides can occur by two main routes. Firstly with the actual nematicide application, spills or washings from equipment, which occur into or near to water sources provide a simple route to contamination. The second, less noticeable, route is leaching from the soil into the ground-water below. In a simplistic view it can be suggested that the more soluble the chemical the more likely it is to be leached. However, the chemical interaction with soil colloids, organic matter and pH alone make this far too simple an approach and consequently several simulation models have been developed to help predict leaching potential (Herbst *et al.*, 2005). It is well documented that oxamyl, fenamiphos and aldicarb are all readily mobile in soils and, at present, the licence for aldicarb in the USA is due to be revoked by 2015. The effects of this contamination will then depend on considerations including the toxicity of the chemical and its persistence.

16.4.3. Ozone depletion and methyl bromide

Methyl bromide is a toxic substance and is not selective when used, so non-target species are also affected. This can include human operators at the site of application. It is also classified as an ozone-depleting compound and the Montréal Protocol on Substances that Deplete the Ozone Layer (1992) sets out a timetable for developed and developing nations to reduce and eventually cease the use of methyl bromide. After the phase-out date in 2005 when usage should cease in developed countries, and 2015 in developing countries, some exemptions for ‘critical’ uses will exist. Methyl bromide will still be used for quarantine and pre-shipment of goods to comply with export-import regulations and allow free trade between nations. Critical use exemptions still exist, e.g. in the USA (EPA, 2011: <http://www.epa.gov/ozone/mbr/cueinfo.html>), where no suitable alternative to methyl bromide can be proven. Emergency use exemptions will also occur.

Problems are emerging as to the suitability of replacements for methyl bromide. An expensive mixture of pesticides is often needed to obtain the same broad spectrum of activity that methyl bromide exhibits, and no single viable alternative has been developed to replace this valuable fumigant. Much research work has been done to find solutions to the withdrawal of methyl bromide and the alternatives are often more toxic than methyl bromide, but do not harm the ozone layer. Indeed, one such alternative, 1,3-D, is restricted in some areas due to concerns about it affecting air and water quality close to residential areas. The EPA website (<http://www.epa.gov/gateway/learn/pestchemtox.html>) has a wealth of useful

information on this topic. Other potential replacements being examined include 1,3-dichloropropane and iodomethane. As well as stimulating research to find alternative conventional chemicals, there has also been an increase in research looking at the effects of 'natural' nematicides, some examples of which are considered in Section 16.6.

16.4.4. Legislation

Pesticides are amongst the most heavily regulated products used by man, and approval and registration processes are similar to those required for pharmaceuticals. In addition, pesticides must be rigorously tested for their environmental impacts and those with poor environmental profiles are no longer approved for use.

The use of pesticides was an issue debated at the Earth Summit in Rio de Janeiro in 1992. The outcome was a requirement to reduce the use of pesticides and many governments subsequently adopted policies to meet this objective. One of the most radical solutions had already been taken by the Danish government, who in 1986 through their Pesticide Action Plan, had called for a 50% reduction by weight in the sales of pesticides. Other countries such as the UK encouraged a reduction in pesticide use through the implementation of integrated crop management, a system that aims to optimize all aspects of the production process resulting in an overall reduction in pesticide use. Approval processes for pesticides are complex and the data requirements for safety as well as efficacy are extensive. Before approval is likely to be given, regulators must be satisfied with the pesticide's profile in the following areas: chemistry of the a.s. and product, mammalian toxicity, non-dietary human exposure, residues, fate and behaviour in the environment, ecotoxicology and efficacy, and that any risks posed by its use are acceptable. Individual countries may have their own legislation to deal with pesticide approval. However, in Europe issues specific to the active substance approval and subsequent plant protection product authorizations are dealt with on a coordinated basis in accordance with EU Regulation (EC) No 1107/2009. Active substances are approved for use within Europe as a whole and plant protection products are authorized initially for use in EU Member States on a zonal basis and then subsequently by mutual recognition. The EU has been divided into three zones: the northern zone, the central zone and the southern zone. Initial product authorizations may be granted in multiple Member States within a zone following acceptance by a lead Member State acting on behalf of other Member States within the zone. This process reduces the bureaucratic requirements that existed under the now superseded EU Directive 91/414/EC, where the product approval process had to be performed in each Member State. Mutual recognition allows for an authorization of a product based on an existing authorization in a neighbouring Member State within a zone. The specifics of this system and its implementation in the UK in particular can be found on the CRD website (<http://www.pesticides.gov.uk>). A similar system of pesticide approval based on an evaluation of chemistry of the active substance and product, mammalian toxicity, etc. is operated in the USA and information on this procedure can be found on the EPA website (<http://www.epa.gov>).

16.5. Human Safety

16.5.1. Exposure during application

Nematicides have the potential to harm humans, and often have high mammalian toxicities. Toxicity can be determined by experimentation, and a measure of toxicity such as an LD₅₀ can be calculated and is used to derive a hazard classification for the product. An LD₅₀ is a statistical value which represents the lethal dose required to kill 50% of a test population (often rats); the lower the LD₅₀ the more toxic the nematicide (Table 16.1). There are three potential routes of contamination: inhalation, ingestion and absorption. Contamination through inhalation is uncommon under field conditions as particles small enough to be breathed into the lungs are produced in only small volumes during pesticide application.

Toxicity is not synonymous with hazard, and it is hazard that is more important when determining the risk to those using nematicides. Hazard is the potential of a substance to cause harm. A nematicide's hazard classification can be determined by its toxicity and its form, e.g. whether it is a gas, solid or a liquid, and how concentrated the formulation is. Therefore, the hazard of the nematicide-containing product must be determined. Hazard classifications are determined with reference to sets of trigger values, which result in a given warning symbol being placed on the product's label. Commonly used hazard symbols are a skull and crossbones or a black cross with associated toxic, poison or harmful risk phrases. Examples used in the USA and UK can be found on the websites of the EPA and the CRD. The WHO has produced recommendations for the classification of pesticides by hazard, which is the basis for their Globally Harmonized System of Classification and Labelling of Chemicals. Details of the WHO hazard classification and labelling systems can be found on their website (<http://www.who.int>).

The risk to the operator can be estimated from their exposure to the product. In the UK, the Control of Substances Hazardous to Health Regulations (COSHH) requires a risk assessment to be performed before using hazardous pesticides. Risk should be reduced by using the least hazardous product and then by reducing exposure. Exposure can be reduced in three principle ways: correct use of the product, engineering controls and the use of personal protective equipment (PPE). Correct use of the product can be achieved following adequate training and guidance in the use of the product. In addition, certificates can be obtained to demonstrate competence – these may be a legal requirement in some situations. Engineering controls can come in many forms but the main control measure would be an enclosed cab with an air filtration system on the sprayer. This provides a physical barrier to the spray and reduces the reliance on PPE such as gloves, coverall and face shield. Another important engineering control measure is a closed transfer system. A closed transfer system allows the pesticide to be mixed into the sprayer or delivered to the applicator while reducing the possibility of splashing or accidental spillage (see Section 16.3.2 for microgranules). There are many different types of closed transfer system that have been designed to be used with either single or multitrip containers. The benefit of a multitrip container system is that the container does not need to be disposed of as it is returned to the manufacturer for refilling. Use of such systems reduces the requirement for operators to wear PPE. However, use of the appropriate PPE for a given operation is essential to provide an effective barrier to contamination. The topic of

operator exposure, and the use of engineering controls and PPE, is covered in greater detail by Matthews (2008) and practical advice on the use of PPE is given in the Code of Practice for Using Plant Protection Products (<http://www.pesticides.gov.uk>).

Reducing exposure to nematicides is very important as contamination can lead to poisoning. Symptoms of poisoning should be known before using a specific product, and these will be given on the product's label and materials safety data sheet (MSDS) together with first aid instructions. In addition, there should be guidance on specific first aid measures an emergency action plan should be written before nematicide application takes place. A section on poisoning by pesticides and first aid measures is included in Lainsbury (2012).

16.5.2. Residues in foodstuffs

Humans can also be exposed to pesticides through the consumption of contaminated food and water. Not all pesticide applications leave residues in the harvested crop, but some do. The issue of residues in animal feeds and foodstuffs is covered within pesticide approval processes. Potential residue levels should be toxicologically acceptable and maximum residue levels (MRLs) are established to monitor the correct use of a pesticide. Individual countries can set MRLs in foods and international MRL standards are given in the Codex Alimentarius, although in the EU MRLs are set by the EU and are common to all Member States. The MRL is used as a trading standard. If the MRL is exceeded it does not necessarily mean that the residue will cause harm, but it does indicate that the pesticide has probably not been used correctly. If the pesticide has been applied to an approved crop in an approved way, i.e. correct dose rate and timing of application, the MRL should not be exceeded.

The toxicological acceptability of a residue is based on a 'no observed adverse effect level' (NOAEL), which is calculated from experimental observation of the most sensitive test species. From this value, the acceptable daily intake (ADI) (the amount of residue that could be consumed every day with the reasonable certainty that no adverse effect will occur) is calculated. The ADI is used to assess chronic exposure to a pesticide's residues. The NOAEL is divided by 10 to account for differences in sensitivity between humans and the test species, and 10 again to account for variation between individuals within the human population to give the ADI. The ADI is compared to the theoretical maximum daily intake (TMDI), which is calculated from the MRL and data on the 97.5th centile for consumption of the foodstuff. If the ADI is greater than the TMDI, i.e. the amount eaten is less than that likely to cause harm, the residue is deemed to be toxicologically acceptable. This is discussed, together with issues relating to acute exposure to residues in food, in detail by the CRD on their website.

Pesticides can end up in surface and groundwater, both of which may be used for drinking water supply. Ultimately, if not degraded, pesticides will end up in the sea. The amount of pesticide permitted in drinking water is based on a maximum admissible concentration (MAC). The MAC in the EU is set at $0.1 \mu\text{g l}^{-1}$ for an individual pesticide and $0.5 \mu\text{g l}^{-1}$ for the total pesticide content. These values were set using the 'precautionary principle', whereas the WHO has issued MACs for individual pesticides based on their toxicology. Nematicides must be kept from water by reducing contaminations from both point and diffuse sources. Following the guidance for use given on the product's label and any stewardship guidelines should minimize water contamination.

16.5.3. Product stewardship

There is greater need to justify the use of a nematicide and to improve the accuracy of nematicide application, which has resulted in an increase in grower administration and the complexity of crop protocols required by the largest retailers. Growers and manufacturers of nematicides are working together to satisfy these new challenges set by the retailers. This partnership takes the form of product stewardship programmes. Such programmes are concerned with operator competence, correct identification of the nematode problem and the accurate and safe use of nematicides. Providing advice on operator training, certification in the use and application of pesticides and refresher training for latest developments are important, as educating the user is often the most effective way to improve the efficiency and safety of product use. Sampling soil to establish nematode population densities (see Chapter 11) is important before a decision on the appropriate treatment can be made. Decisions on nematicide usage should be made within the context of an integrated nematode management approach. Product manufacturers can provide calibration of nematicide applicators as a service to nematicide users. Detailed guidance on the correct application of nematicides will be found on the product label. The analysis of treated produce for residues, ensuring that MRLs are not exceeded, is a useful end check on the quality of the nematicide application process. Guidance on limiting environmental side effects from nematicides is increasingly important and should be part of any product stewardship scheme. For example, switching off the application equipment 1 m from the end of the row, before the applicator is lifted out of the soil, easily reduces the risk to birds from eating uncovered granules at the ends of rows.

16.6. Naturally Occurring Nematicides

There is continuing research into new nematicidal compounds and much of this has centred on ‘naturally occurring’ active substances. Such compounds are thought to be more acceptable to both legislators and the increasingly aware public. Examples of such compounds are given in Table 16.3. To be widely accepted, such compounds need to be reliable and have significant benefits over existing conventional nematicides, or find market niches where conventional products are unacceptable, e.g. in organic agriculture. Whilst the efficacy of many ‘natural nematicides’ is unproven, azadirachtin (the active substance contained in neem tree products), DiTera® (containing fermentation extracts from the bacterium *Myrothecium verrucaria*) and garlic extracts (Danquah *et al.*, 2011) have an increasing body of published data to confirm their efficacy. There are also green manures and soil amendments that affect both nematode populations and plant growth (Widmer *et al.*, 2002). These effects are probably as a result of beneficial effects on nutrient and water status of treated soils and upon the populations of nematode antagonistic organisms. Some green manures have been shown to have direct nematicidal activity, e.g. butyric acid formed by the decomposition of the grasses timothy and rye, and isothiocyanate formed by the decomposition of oil-seed rape and other brassicas (Zasada and Ferris, 2004). Soil amendments are covered in more detail in Chapter 14.

Table 16.3. Examples of commercially available 'natural' nematicides.

Tradename	Manufacturer	Source	Active substance(s) and mode of action (if known)	Comments
Dragonfire-CPP	Poulenger USA Inc www.poulengerusa.com	Sesame seed oil	Aldehydes, ketones, linolenic acids	Marketed for control of pathogenic nematodes in turf, horticultural and agricultural situations
Ontrol		Sesame seed meal		
Neo-trol	Barmac Industries, Australia www.barmac.com.au	Sesame stalk	Aldehydes, ketones, linolenic acids	A pelletized product for use on golf greens
CropGuard	Illovo Sugar Ltd, South Africa www.cropguard.co.za	Woody biomass	2-furfuraldehyde, a pentose sugar derivative	A solvent produced by acid hydrolysis of the pentosan contained in woody biomass such as maize cobs
Clandosan 618	Igene Biotechnology Inc., USA www.igene.com	Crab and shrimp shells	Chitin and urea increase soil microorganisms that feed on chitin	Recovered as by-products of seafood processing; the ground shells, along with urea, are formed into granules
DiTera	Valent BioSciences Corporation, USA www.valentbiosciences.com	<i>Myrothecium verrucaria</i>	Fermentation extracts from the fungus	Approved for commercial use in USA in 1997 by EPA
Neem	www.agriinfotech.com	Neem plant extract or cake	Azadirachtin	Residue from neem oil extraction process
Nemate 10G (or liquid formulations)	Many suppliers, particularly in India	Neem plant extract	Azadirachtin	

The development of alternative ‘natural’ nematicides and research to increase efficiency and reduce environmental impact of existing nematicides both aim to get as close as possible to the ‘ideal nematicide’, whose characteristics are indicated in Box 16.2.

Box 16.2. Characteristics of the ‘ideal nematicide’.

Cost: Inexpensive to develop, register, manufacture and market. Inexpensive to purchase. Economic to apply; gives reliable increase in economic gross margin for the grower.

Efficacy: Very effective at low rates of application.

Toxicity: Low acute and chronic (skin) toxicity to humans. Low toxicity to non-target organisms. Highly toxic to target nematode(s). Non-phytotoxic with no adverse effects on plant growth and vigour.

Persistence: Persistent enough to control fully the target nematode, then rapid degradation to harmless molecules.

Source: Naturally occurring or produced from living organisms.

Compatibility: Compatible with other control components, e.g. biocontrol agents.

Application: Chemically suited to range of formulations and application systems.

Mobility in plant: Good mobility to roots; can be applied to foliage and stems of perennials.

Solubility: Water soluble but not subject to leaching or run-off.

Residues: Leaves no detectable residues in harvested plant material.

Note: The authors do not accept liability for any error or omission in the content, or for any loss, damage or other accident arising from the use of techniques, active substances or products mentioned in this chapter. Always consult the manufacturer and competent advisors before using any product.

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Glossary

Alae: thickened wings of cuticle located laterally or sublaterally on the body.

Alginate: a polysaccharide obtained from seaweed, frequently used to formulate microbial biological control agents.

Allelochemical: volatile or non-volatile chemical mediating interspecific responses (e.g. nematodes responding to host root diffusate).

Amixis: see Parthenogenesis.

Amphid: anterior sensillum; paired lateral structures, rarely shifted dorsad, located post-labially or on the lip region; main anterior chemosensilla.

Amphidelphic: reproductive system with two uteri extending in opposite directions.

Amphimixis: sexual reproduction where male gametes (sperm cells) and female gametes (oocytes) fuse to form the zygote.

Anastomoses: cross-connections: (i) common in criconematid taxa where the body annuli do not form continuous rings; the junction between the dorsal and ventral part of the annuli usually results in a lateral zigzag line and (ii) the points of fusion between compatible fungal hyphae.

Anhydrobiosis: a state of dormancy induced by desiccation.

Annuli: deep transverse grooves involving at least one of the two deeper main zones of the cuticle (median and basal zone) (cf. **striae**).

Antibiosis: inhibiting life.

Apomixis: mitotic parthenogenesis; the most common method of asexual reproduction, in which the only division that occurs is mitosis and the oocytes retain the diploid chromosome number (cf. **automixis**).

Appressorium: swelling of fungal hypha or germ tube that helps in attachment to, and penetration of, the host.

Areolation: occurs at the level of the lateral field when the transverse grooves (**annuli**) or transverse **striae** extend into the lateral field.

Automixis: meiotic parthenogenesis; there is a first meiotic division in the oocytes that permits some genetic reorganization, even though the diploid number is restored by self-fertilization (cf. **apomixis**).

Bacillary bands: modified epidermal gland cells opening through a complex cuticular pore.

Bar code: small sequence fragment of DNA sequence used as a unique identifier to separate organisms into species.

Basal spiral fibre layers: helically arranged fibre layers (angle 54°44' or more); they play a role in maintaining the internal turgor pressure.

Basal zone: innermost zone of the cuticle.

Biocontrol agent: parasitic, pathogenic, antagonistic or competitive organism that kills or damages another organism (e.g. the nematode host) and interferes with the establishment, function, growth, multiplication or survival of the host.

Biocontrol: see **Biological control**.

Biofumigation: use of organic amendments that release volatile breakdown products in soil that are toxic to pests and diseases.

Biological control: the total or partial destruction of pathogen and pest populations with the aid of other living organisms, excluding plant hosts and antagonistic plants.

Biotroph: an organism that obtains its nutrients only from another living organism.

Buccal cavity: stoma *sensu lato*, usually referring to cheilostome and pharyngostome.

Bursa: also referred to as caudal alae; wing-like subventral thickened body cuticle in the tail region of males.

Caudalids: anal-lumbar commissures that link the pre-anal and lumbar ganglia.

Cellulases: enzymes that break β 1–4 linkages between glucan sugar residues in cellulose.

Cephalic framework: sclerotized framework within the head.

Cephalic sensilla: papilliform, setiform or embedded (in parasitic nematodes) sensilla that form the third circlet of anterior sensilla; submedian in position (laterodorsal or lateroventral); derived from somatic sensilla.

Cheilarhabdia: sclerotized plates/rods in the cuticular lining of the cheilostome.

Cheilostome: stoma *sensu stricto* or lip cavity, with hexaradiate or triradiate symmetry, lined with body cuticle.

Chemical control: management strategies that utilize the application of synthetic or naturally derived nematicides.

Chlamydospore: type of resting spore with thick cell walls usually formed within fungal hyphae.

Circomyarian muscle cell: muscle cell in which the sarcoplasm is completely surrounded by contractile elements.

Coelommyarian muscle cell: spindle-shaped muscle cell laterally flattened so that the contractile elements are arranged not only along the epidermis but also along the sides of the flattened spindle; coelommyarian muscle cells bulge into the pseudocoel.

Conducive soil: a soil where multiplication of nematodes or other pathogens on a susceptible crop is favoured (i.e. the opposite of a **suppressive soil**).

Conidiophore: specialized fungal hypha on which conidia are produced.

Conidium (plural: conidia): any asexual fungal spore, formed on a conidiophore.

Corpus: anterior part of pharynx.

Cortical zone: zone of the cuticle beneath the epicuticle.

Cultural control: management strategies based on cultivation and cropping methods that lead to the reduction of pests.

Damage threshold: nematode population density above which crop growth and yield are suppressed (see also **Economic threshold**).

Dauer: an alternative nematode developmental stage for surviving unfavourable conditions for long periods.

Deirids: cervical papillae or somatic sense organs in the form of protuberances that lack an opening, located at mid-lateral field.

Denticles: minute teeth-like structures.

Diapause: a physiological state of arrested growth and development, usually in response to unfavourable conditions, whereby development does not continue until specific requirements have been satisfied, even if favourable conditions return.

Didelphic: reproductive system with two uteri.

Differential host plant: host plant with the ability to distinguish between nematode pathotypes based on differential rates of nematode reproduction.

Diorchic: reproductive system with two testes.

Distal: furthest from a specified centre.

Dormancy: a suspension of growth and development, usually in response to unfavourable conditions, associated with a lowered metabolic rate; dormancy is usually subdivided into **quiescence** and **diapause**.

Ductus ejaculatorius: posterior part of *vas deferens* when differentiated and connected to the cloaca.

Economic threshold: nematode population density above which the extra cost of nematode control is exceeded by the increase in crop returns (see also **Damage threshold**).

Endoparasitic fungus: a fungus that penetrates its host and feeds from within it, and growth outside the host is limited.

Endophyte: a fungus or bacterium that lives inside a plant without causing disease.

Epicuticle: trilaminar outermost part of the cuticle, first layer to be laid down during moulting.

Epidermal glands: various glands that open through pores or sometimes through special setae on the cuticle; unicellular and often associated with somatic receptors; most important epidermal glands are the ventral and caudal glands.

Epidermis: a thin cellular or acellular layer beneath the cuticle arranged into four longitudinal chords (dorsal, ventral and two lateral); secretes the cuticle, contains proteins associated with nutrient and ion transport, participates in osmotic and volume regulation in nematodes and is functionally important during embryogenesis.

Expansins: enzymes that break non-covalent linkages between cellulose chains.

Expressed sequence tags (ESTs): sequences obtained from clones randomly selected from cDNA libraries.

Feeding sites: enlarged, metabolically active plant cells on which nematodes feed (see **Giant cell** and **Syncytium**).

Fenestration: the arrangement and form of the translucent cuticular areas around the vulva in cyst nematodes (circumfenestrate, bifenestrate, ambifenestrate).

Gene flow: incorporation of genes into the gene pool of one population from one or more other populations.

Genetic distance: measures of the degree of genetic differences between populations or species based on differences in allele frequencies.

Genital papillae: sensory organs located on the male tail.

Genotype: precise genetic constitution of an organism.

Giant cell: large multinucleate cell formed by cycles of mitosis uncoupled from cytokinesis induced by some nematodes, including root-knot nematodes, in plant roots.

Gonad: ovary (female), testis (male).

Gonoduct: oviduct and uterus (female); *vas deferens* (male).

Green manure: fresh plant material ploughed into soil to decrease nematode infestations, either directly or indirectly through stimulation of the activity of the soil microbial community.

Gubernaculum: sclerotized accessory guiding device for the spicules.

Harvest interval: period between application of control agent and crop harvest.

Hemizonid: a commissure that runs between the nerve ring (lateral ganglion) and the ventral nerve; it is located near the secretory-excretory pore, i.e. the ventral side of the body.

Hemizonion: a small commissure situated posterior to the hemizonid.

Hermaphroditism: a method of reproduction where both egg and sperm are produced by the same gonad in the same individual.

Homology: correspondence between structures that is attributable to their evolutionary descent from a common ancestor.

Horizontal gene transfer: acquisition of genetic material from a source other than the parents.

Intestine: mesenteron or mid-gut; may be subdivided into: (i) an anterior ventricular area; (ii) the mid-intestinal region; and (iii) a pre-rectum posteriorly.

Isozyme (or Isoenzyme): one of several forms of an enzyme, produced by different loci in the genome of an individual organism.

Kinesis: nematode movement resulting from undirected responses to changes in stimulus intensity.

Labial sensilla: anterior sensilla comprising two circlets, each with six sensilla (six inner labial sensilla and six outer labial sensilla) arranged in a hexaradiate pattern; mechano- and/or chemoreceptors.

Lateral lines: the borders of longitudinal ridges at the level of the lateral chords.

Lectin: a plant protein that binds to specific carbohydrates.

Longitudinal ridges (or **Lamellae**): usually restricted to the lateral region but may be present all around the body; they result from thickening of the body cuticle (e.g. of the median zone).

Median zone: zone of the cuticle, located immediately beneath the cortical zone. In some nematodes, the median zone may be absent.

Metacorpus: posterior part of corpus (anterior part of pharynx), may be strongly muscular and provided with a valve.

Micro-arrays: glass slides bearing short stretches of oligonucleotide probes or cDNA clones used to measure expression levels of many thousands of genes simultaneously.

Microsatellite: short sequences of 1–6 base nucleotide repeats with highly variable length distributed widely throughout the genome.

Migration: movement of an individual or population in a direction oriented with respect to a stimulus field (can be accomplished by **Taxis** or **Kinesis**).

Minimum yield: the yield obtained irrespective of nematode population density.

Molecular probe: a molecule that is labelled and used to detect another molecule (target) through its specific affinity.

Monodelphic: reproductive system with one uterus.

Monorchic: reproductive system with a single, usually anterior, testis.

Mulch: a protective covering of various substances, usually organic, but can be inorganic (e.g. plastic) placed on the ground around plants to retain moisture, prevent frost, retard weeds, suppress disease and provide nutrients (organic).

Multiple sequence alignment: a matrix of sequence data, in which the individual columns represent homologous characters.

Mycorrhiza: fungi that live in mutualistic symbiosis within or on the roots of a plant.

Nematicide: a general term used to describe a synthetic chemical that is used to control plant-parasitic nematodes.

- Nematistat or Nematostatic:** a non-fumigant nematicide, which paralyses rather than kills plant-parasitic nematodes in the soil.
- Nematophagous fungus:** fungus that is able to obtain nutrition from nematodes by parasitizing them.
- Ocelli:** eyespots or photoreceptors; pigmented areas located laterally or dorsolaterally along, or partly inside, the pharynx.
- Odontophore:** supporting structure of the odontostyle, formed by a modification of the anterior pharyngeal region.
- Odontostyle:** hollow protrusible anterior part of a dorylaimid spear/stylet with a dorsal opening and supported by the odontophore.
- Onchiostyle:** stylet of Trichodoridae; a stylettiform dorsal tooth with a solid tip, consists of an onchium or tooth and an onchiophore or pharyngeal supporting structure.
- Opisthodelphic:** reproductive system where the uterus or uteri are directed posteriad.
- Orthologous gene:** a gene derived from the same copy of a gene in the most recent common ancestor.
- Ovejector:** common region of the two uteri and connected to the vagina.
- Oviduct:** part of gonoduct next to the ovary, uniform or differentiated into a narrow distal and a wider proximal part that may act as spermatheca.
- Papilla** (plural: papillae): sensillum, 1 µm or smaller in size.
- Paralogous gene:** a gene derived from different copies of a gene in the most recent common ancestor.
- Parasite:** an organism living within or on another organism (the host) from which it obtains food or other requirements, usually to the detriment of the host.
- Parthenogenesis:** asexual reproduction, or amixis, where males are not involved.
- Pathogen:** a disease-causing organism.
- Pathogenicity:** relative ability of a nematode taxon or population to damage a given plant.
- Partial resistance:** any resistance that is less than 100% inhibition of nematode reproduction in comparison to a chosen standard.
- Pathotype:** a group of individual nematodes with common gene(s) for (a)virulence and differing from gene or gene combinations found in other groups, which can be distinguished from others of the species by their pathogenicity on a specific host.
- Pectate lyases:** enzymes that break galacturon sugar polymers.
- Perineal pattern:** the cuticular lines and associated structures surrounding the vulval region of root-knot and cyst nematodes.
- Pharyngeal gland cells:** large cells in the pharynx of nematodes that produce secretions thought to be important in host-parasite interactions.
- Pharyngostome:** triradially symmetrical specialized anterior part of the pharynx lined with pharyngeal cuticle (i.e. lacking the median zone); together with cheilostome forms the buccal cavity.
- Pharynx** (also called oesophagus): a complex muscular pumping organ with a triradiate lumen lined with cuticle, and unicellular glands; main part of stomodeum; variable in shape from simple cylinder to complex with differentiation in corpus (with or without a further subdivision into pro- and metacorpus), isthmus and post-corpus.

- Phasmid:** somatic sensillum, located laterally, usually on the tail, but may also be found in a pre-anal position.
- Phenotype:** observed morphological, physiological, biochemical, behavioural and other properties of an organism.
- Pheromone:** volatile and non-volatile chemical(s) mediating intraspecific responses (e.g. sex pheromones).
- Phylogeny:** the evolutionary history of a group of taxa.
- Phylogenetic tree:** diagrammatic representation of the relatedness of a group of taxa.
- Physical control:** management strategies that utilize physical means, such as heat and drying, to control pests.
- Phytoalexins:** antimicrobial compounds produced by plants in response to pathogen attack.
- Platymyarian muscle cell:** the whole contractile part of the muscle cell that is flat and broad and borders the epidermis.
- Population:** a group of individuals of the same species at a given time and space.
- Population dynamics:** (i) changes in population size over time and space and; (ii) the forces that regulate populations over time and space.
- Postcorpus:** posterior bulb of pharynx, with or without valve, glandular and muscular.
- Postvulvar (postvulval) uterine sac:** also called postuterine sac, a rudimentary part of the reduced genital branch.
- Primer:** single-stranded synthetic oligonucleotide designed to hybridize to a known sequence of DNA as a starting point for DNA amplification.
- Probe:** see Molecular probe.
- Procorpus (or Precorpus):** anterior part of corpus (anterior part of pharynx).
- Proctodaeum:** see Rectum.
- Prodelphic:** reproductive system where the uterus or uteri are directed forward.
- Proximal:** closest to a certain centre that has to be specified.
- Pseudogene:** a non-functional member of a gene family.
- Quarantine:** official confinement of regulated articles, such as plant-parasitic nematodes, for observation and research or for further inspection, testing and/or treatment.
- Quarantine pest:** a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not yet widely distributed and being officially controlled.
- Quiescence:** a state of arrested development in response to unfavourable conditions, which is reversible when favourable conditions return.
- Race:** a subspecies group of nematodes that infects a given set of plant varieties.
- Rachis:** a central protoplasmic core to which developing oocytes are connected by cytoplasmic bridges.
- Radial filaments:** filaments of condensed material present in the cortical zone of the cuticle.
- Radial striae:** either in cortical or basal zone of the cuticle, consisting of longitudinal and transverse circumferential interwoven laminae, at high magnification appearing as rods separated by electron-light material.
- Rectum (or Proctodaeum):** posterior ectodermal part connected to the intestine through a valve surrounded by a sphincter muscle; may possess associated rectal glands.

Resistance: the ability of a plant to inhibit reproduction of a given nematode relative to a susceptible plant that supports high levels of reproduction.

Restriction enzyme: an enzyme that cuts double-stranded DNA at specific short nucleotide sequences.

Rhizosphere: the space around the root that is influenced by the root and its diffusates.

RNAi (RNA interference): degradation of an RNA due to the presence of a double-stranded RNA molecule of the same, or very similar, sequence. A naturally occurring phenomenon used to protect against viruses and transposable elements that replicate via a double-stranded RNA intermediate, and also to control expression of endogenous genes.

Root diffusate (also called root exudate): wide range of mucilages and metabolites released by the plant into the rhizosphere that sustains 50–100 times the microorganism population found in the bulk soil.

Rugae: a series of cuticular ridges forming part of cuticular ornamentation.

Sampling: the collection of soil or plant tissue for the purpose of estimating a given attribute (e.g. the density of nematodes or crop damage) by examining a defined portion of the collected material.

Saprophyte: organism that obtains its nutrition from dead organic material.

Scutellum: a plasmid with a large plug, probably secreted by the glandular sheath cell.

Secretory-excretory pore: ventrally located pore, usually in the pharyngeal region, through which the secretory-excretory system opens to the exterior.

Semiochemicals: volatile and non-volatile chemicals that cause interactions between organisms (see Allelochemical and Pheromone).

Sensillum (plural: sensilla): sense organ consisting of: (i) a socket cell; (ii) a sheath cell; and (iii) a neural part of bipolar neurons whose dendrites end in dendritic processes or receptors.

Seta (plural: setae): sensillum, 2 µm in size or longer.

Setiform papillae: sensilla with a length intermediate between a papilla and a seta, i.e. 1.5 µm.

Sex pheromone: see Pheromone.

Sibling species: species that are difficult or impossible to distinguish by morphological characters.

Soil solarization: using solar energy to increase the temperature of soil, usually covered with a polythene sheet, to levels that are lethal to nematodes and some soil microbes.

Somatic muscle cell: mainly with spindle-like form, but with a complex, irregular shape in those cells with a special function, e.g. in area of vulva; consists of three parts: (i) a contractile portion of the cell towards the epidermis; (ii) a non-contractile portion towards the body cavity; and (iii) an arm or process extending from the non-contractile portion of the cell towards the dorsal or ventral nerve; muscle cells anterior to the nerve ring send processes directly into the nerve ring.

Somatic muscles: body wall muscles arranged in a single layer of obliquely orientated and longitudinally aligned somatic muscle cells, divided into four sectors by the four epidermal chords. Meromyarian musculature: few (up to 5–6) rows of muscle cells are present per quadrant; common in small species. Polymyarian musculature: more than six rows of muscle cells per quadrant; spindle-shaped muscle cells laterally flattened.

- Spermatheca:** an enlarged portion either of the oviduct or of the uterus of the female genital system, used for storage of sperm.
- Sphincter muscle:** present at different sites (e.g. around the vagina).
- Spicular pouch:** formed from the spicular primordium, specialized cells of the dorsal wall of the cloaca.
- Spicule:** male copulatory apparatus, either simple or differentiated into a proximal head (capitulum), a shaft (calomus) and a blade that may be with or without a velum (a ventral membrane-like structure); usually paired.
- Stomatostyle:** hollow, protrusible needle-like structure of tylenches; consists of three parts: (i) a cone with a ventral aperture; (ii) a shaft; and (iii) a posterior part that swells to form three basal knobs.
- Strain:** a subspecies group of nematodes, bacteria and fungi distinguishable from the rest of the species by a heritable characteristic that the individuals in the group have in common (biotype).
- Striae:** shallow or superficial transverse grooves not penetrating to the deeper layers of the cuticle.
- Struts:** column-like supporting elements in the median zone.
- Stylet:** see **Stomatostyle**.
- Supplements:** pre-cloacal genital sensilla of different shape (e.g. papilliform or tubuliform), usually mid-ventral in position.
- Suppressive soil:** soil where multiplication of plant-parasitic nematodes on a susceptible crop is less than that normally observed on the same cultivar in another soil, in similar abiotic conditions, due to the presence of natural enemies to the nematodes (i.e. the opposite of a **Conducive soil**).
- Surface coat:** a stage-specific coating covering the cuticle.
- Susceptibility:** the counterpart of resistance; a measure of the ability of a plant to support reproduction of a nematode species.
- Sustainability:** the successful management of resources for agriculture to satisfy changing human needs, while maintaining or enhancing the natural resource base and avoiding environmental degradation.
- Syncytium:** large multinucleate cell produced by cell wall breakdown and fusion of protoplasts that are induced by nematodes, including cyst nematodes, in plants.
- Systematics:** the scientific study of the diversity of organisms and their interrelationships (cf. **Taxonomy**).
- Systemically acquired resistance (SAR):** increased resistance in a remote part of a plant due to detection of a pathogen in another region of the plant.
- Taxis:** locomotion resulting from orienting the body to the stimulus direction.
- Taxon** (plural: *taxa*): taxonomic category from subspecies to Kingdom.
- Taxonomy:** the theory and practice of identifying, naming and classifying organisms (cf. **Systematics**).
- Tolerance:** relative ability of a plant to sustain growth and yield when parasitized by a nematode; not related to resistance.
- Tolerance limit:** nematode population density below which there is no reduction in plant growth and, therefore, yield.
- Transcription factors:** proteins that activate expression of genes through interaction with promoters.
- Transformant:** modified organism obtained by changing (transforming) a piece of the genetic code of an organism.

Transverse striae: shallow transverse grooves restricted to the cortical zone.

Trapping fungus: fungus that captures its nematode prey in traps from specialized hyphae and/or adhesive structures.

Uterus: part of gonoduct connected to the vagina; either a simple tube but usually more complex and subdivided into a glandular part close to the oviduct, a muscular median part and a wider, or tubular, part towards the vagina.

Vagina: a canal lined with body cuticle that connects the gonoduct(s) with the vulva; may be divided into three parts: (i) *pars distalis* (distal section); (ii) *pars refringens* (intermediate section); and (iii) *pars proximalis* (proximal section).

Vas deferens: male gonoduct, uniform or subdivided.

Vector: an organism capable of carrying a plant-parasitic nematode from one host to another. Also, nematodes may act as carriers (vectors) for microorganisms (viruses, bacteria and fungi).

Vesicular seminalis: a part of each testis or the anterior part of the *vas deferens*.

Virulence: (i) a measure of the ability of a nematode to cause damage, with a highly virulent isolate causing more damage than a weakly virulent isolate and (ii) a measure of the ability of a nematode to reproduce on a plant, in particular when comparing populations of the same species for their ability to reproduce on resistant plants.

Zoospore: infective spore produced by fungal-like organisms (Oomycetes) that bears flagella(e) and swims in water.

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