1 *Meloidogyne* Species – a Diverse Group of Novel and Important Plant Parasites

Maurice Moens[1], Roland N. Perry[2] and James L. Starr[3]
[1]*Institute for Agricultural and Fisheries Research, Merelbeke, Belgium and Ghent University, Ghent, Belgium;* [2]*Rothamsted Research, Harpenden, Hertfordshire, UK and Ghent University, Ghent, Belgium;* [3]*Texas A&M University, College Station,Texas, USA*

1.1 Introduction

Root-knot nematodes are members of the genus *Meloidogyne* (Göldi, 1892), *Meloidogyne* is of Greek origin and means 'apple-shaped female'. They are an economically important polyphagous group of highly adapted obligate plant parasites, are distributed worldwide and parasitize nearly every species of higher plant. Typically they reproduce and feed on modified living plant cells within plant roots, where they induce small to large galls or root-knots, hence their vernacular name. The above-ground symptoms are not readily apparent and may be similar to those produced on any plants having a damaged and malfunctioning root system. Hosts may be heavily infected without showing external symptoms on the harvested products, e.g. symptomless potato tubers. The rapid rate of development and reproduction on good hosts results, in the majority of species, in several generations during one cropping season, leading to severe crop damage. Damage may consist of various degrees of stunting, lack of vigour, and wilting under moisture stress. Secondary infection by other pathogens often results in extensive decay of nematode-infected tissues. The common explanation for these above-ground symptoms is that *Meloidogyne* infection affects water and nutrient uptake and upward translocation by the root system. By disrupting the host plant physiology, root-knot nematodes may not only reduce crop yield but also product quality

 Root-knot Nematodes (eds R.N. Perry, M. Moens and J.L. Starr)

(e.g. of potatoes and carrots) and therefore are of great economic and social importance (see Greco and Di Vito, Chapter 11, this volume).

1.2 Impact

Damage and yield losses caused by plant pathogens, including plant-parasitic nematodes, are, on average, greater in tropical than in temperate regions because of greater pathogen diversity, more favourable environmental conditions for pathogen colonization, development, reproduction and dispersal, and lack of human, technical and financial resources to combat infections (De Waele and Elsen, 2007). Severity of damage caused by *Meloidogyne* can be species-specific and also vary by host, crop rotation, season and soil type (Greco *et al.*, 1992; Potter and Olthof, 1993). Similarly, economic thresholds vary, primarily depending on these same factors. Damage thresholds have been established for several crops, where the average is approximately 0.5–2 juveniles/g of soil (or from the lower limits of detection, over 1000 individuals/500 cm^3 of soil; see Greco and Di Vito, Chapter 11, this volume). In addition to direct costs, root-knot nematodes cause indirect costs because of the quarantine status of some species of *Meloidogyne* in several countries or regions. For example, *M. chitwoodi* is increasingly regulated because it is a serious pest of potato and other economically important crops such as carrot, and the known geographical distribution is still relatively limited. It is on the list of prohibited pests of many countries (Canada, the EU, Mexico and other countries in Latin America, and the Far East) (Hockland *et al.*, 2006). *Meloidogyne fallax*, another pest of potato, is recognized as an important pest by fewer countries (e.g. the EU), although it poses a similar economic risk. In the future, additional species of root-knot nematodes that might be added to the list of quarantine species include recently described species such as *M. minor* in Europe and *M. citri* in the USA.

1.3 History of the Genus

A more detailed account of the background literature, progress in descriptions and classification, and authorities for the species of the genus is given in Hunt and Handoo, Chapter 3, this volume; a brief summary is included here as part of the introduction to the genus and its basic biology. The first illustrated report of root-knot disease appeared during the middle of the 19th century when the clergyman Miles Joseph Berkeley (1855) first attributed galls detected on glasshouse cucumber roots to nematodes. The first description of a root-knot nematode was made by Cornu (1879); it was based on nematodes found in root galls that were detected on sainfoin (*Onobrychis sativus* Lam.) in the Loire valley, France. In 1887, Göldi briefly described and illustrated a root-knot nematode from coffee plants in Brazil and named it *M. exigua*. Although the 1887 publication was an advance copy or preprint of the full article subsequently published by Göldi in 1892, the 1887 article meets the requirements to establish the actual publication date for the genus and type species as 1887 (see Hunt and Handoo, Chapter 3, this volume for a full account of this decision).

The name *Heterodera marioni* was widely used for root-knot nematodes until 1949, when Chitwood removed the root-knot nematodes from the genus *Heterodera* because they differed from cyst nematodes. Since the oldest name for the genus was Göldi's *Meloidogyne*, that name had precedence. Chitwood redescribed *M. arenaria*, *M. exigua*, *M. incognita* and *M. javanica*, and described *M. hapla* and a variety of *M. incognita* he termed *M. incognita* var. *acrita*. The species were separated from each other on the basis of perineal pattern morphology, stylet knob shape, and length of stylet and dorsal gland orifice. Since Chitwood's publication, many more *Meloidogyne* species have been described (see Hunt and Handoo, Chapter 3, this volume, for a full list). Species descriptions gradually included increasing numbers of features (observed by light microscopy and/or electron microscopy) of females, males and second-stage juveniles (J2) (see Jepson, 1987, for a review, and Eisenback and Hunt, Chapter 2, this volume).

1.4 Current Trends in Species Identification

Research on cytogenetics, isozymes and the genome of root-knot nematodes, mostly since the

1970s, provided further evidence for the large diversity of species within the genus. Importantly, several new technologies have provided tools to assist in species identification. One of the most important has been the use of isozyme phenotypes, using PAGE of crude protein extracts and histochemical stains for non-specific esterases, superoxide dismutase, malate dehydrogenase, and glutamate oxaloacetate transaminase (Esbenshade and Triantaphyllou, 1985), with the esterases and malate dehydrogenase being the most useful for discriminating the four common *Meloidogyne* species: *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*. These enzymes have also been used successfully with less common species such as *M. enterolobii* (= *M. mayaguensis*) (Brito *et al.*, 2004), *M. partityla* (Starr *et al.*, 1996), and *M. trifoliophila* (Mercer *et al.*, 1997). With the appropriate equipment it is possible to make the species identification on individual females. Ibrahim and Perry (1992) showed that PAGE combined with staining for esterases could be used with *Meloidogyne* females in galled roots, thus obviating the need to separate nematodes from the host tissue. It remains to be determined if these procedures will be able to discriminate all of the currently recognized species of this genus.

More recently, much effort has been given to the development of species-specific markers using variation in the genomes of the different species coupled with PCR techniques (see Blok and Powers, Chapter 4, this volume). There are now many reports that describe protocols for distinguishing numerous species using species-specific primers derived from the nuclear genome (Adam *et al.*, 2007) and the mitochondrial genome (Powers and Harris, 1993). A major benefit of these systems is that they can be used on an individual J2, the most common stage of the nematode found in the soil, thus eliminating the need to use mature females from roots of field samples (which is not possible if the host of interest is not actually growing at the time of sample collection) or to establish a culture in the glasshouse and wait for the development of the females. At present, there are no primer pairs available for many species, and multiple assays with different primer pairs are often needed to complete the identification. For some species the PCR amplification product must be digested with a restriction enzyme to complete the analysis (Powers and Harris, 1993). Finally, this approach requires some expensive equipment (the PCR thermocycler) and supplies, and a clean laboratory environment. None the less, these approaches are likely to play an ever-increasing role in species identification (especially by those not well trained or experienced in classical morphometrics) and will be used extensively by regulatory agencies.

1.5 Life Cycle

The root-knot nematode life cycle is summarized in Fig. 1.1 and Plate 36. Females lay eggs into gelatinous masses composed of a glycoprotein matrix, which is produced by rectal glands in the female, keeps the eggs together and protects them against environmental extremes and predation. The egg masses are usually found on the surface of galled roots, although they may also be embedded within the gall tissue. The egg mass is initially soft, sticky and hyaline but becomes firmer and dark brown with age. Surprisingly, there has been only limited analysis of the glycoproteins (Sharon and Spiegel, 1993) or other components of the gelatinous matrix, despite its obvious importance. In addition to providing some protection to the eggs from environmental extremes, it has been demonstrated that the matrix has antimicrobial properties (Orion and Kritzman, 1991).

Within the egg, embryogenesis proceeds to the first-stage juvenile, which moults to the infective J2. Hatch of the J2 is primarily dependent on temperature and sufficient moisture, although other factors, including root diffusate and generation, modify the hatching response (see Curtis *et al.*, Chapter 6, this volume) so that the J2 hatch when conditions are favourable for movement and host location. The ability of *Meloidogyne* to survive is enhanced by several physiological and biochemical adaptations, including delayed embryogenesis, quiescence and diapause, and lipid reserves that prolong viability until the J2 reaches and invades a host; these aspects are discussed in detail by Evans and Perry, Chapter 9, this volume. In the soil, the J2 is vulnerable and needs to locate a host as rapidly as possible. J2 are attracted to roots, and there is evidence that when both resistant and susceptible plant roots are present the susceptible ones are

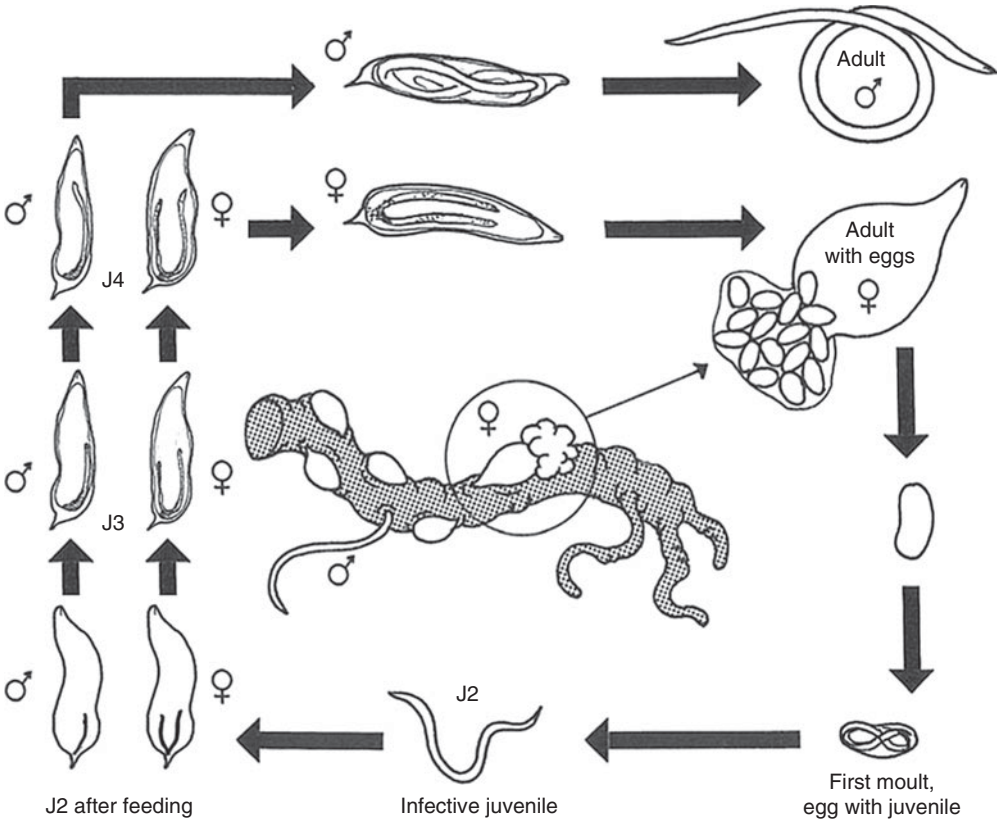

Fig. 1.1. Diagram of the life cycle of the root-knot nematode, *Meloidogyne*. J2: second-stage juvenile; J3: third-stage juvenile; J4: fourth-stage juvenile. (Adapted from Karssen and Moens, 2006.)

more attractive (see Curtis *et al.*, Chapter 6, this volume).

The invasive J2 commences feeding after it has invaded the root, usually behind the root tip, and moved through the root to initiate and develop a permanent feeding site. The feeding of the J2 on protoxylem and protophloem cells induces these cells to differentiate into specialized nurse cells, which are called giant cells. Once a giant cell is initiated, the nematode becomes sedentary and enlarges greatly to assume a 'sausage' shape. Under favourable conditions, the J2 stage moults to the third-stage juvenile (J3) after *c.*14 days, then to the fourth-stage juvenile (J4), and finally to the adult stage. The combined time for the J3 and J4 stages is much shorter than for the J2 or the adult, typically 4–6 days. J3 and J4 lack a functional stylet and do not feed. Males, when present, are vermiform and there is no evidence that they feed. Males may be found in parthenogenetic species when conditions are unfavourable for female development, such as when population densities are very high and presumably there is a limitation of food supply.

The initiation, development and maintenance of the giant cell is the subject of continuing investigation, facilitated by molecular techniques with the impetus of developing novel control strategies based on preventing giant cell formation or, more likely, development. These specialized feeding sites are remarkable for their complexity. They are greatly enlarged from typical phloem and xylem parenchyma, or cortical cells, with final cell volumes nearly 100-fold greater than normal root cells. The giant cells are functionally similar to syncytia induced by other plant-parasitic nematodes that have sedentary adult females, but are distinct in their development. Like syncytia, they are functional transfer cells, based on morphology (Jones and

Northcote, 1972) and because photosynthates pass through the giant cells before being ingested by the nematodes (Bird and Loveys, 1975). Unlike syncytia, each giant cell develops from a single initial cell rather than by coalescence of several adjacent cells. The giant cells are not only multinucleate, containing as many as 80 nuclei each, but individual nuclei within each giant cell are polyploid, some with *c*. eight-fold increase in chromosome number (Huang and Maggenti, 1969; Wiggers *et al*., 1990). Thus, each giant cell may have up to a 600-fold increase in copy number of each plant gene. Several studies have documented the effects of nematode infection on gene expression, with a variety of genes being upregulated (Gheysen and Jones, 2006; Schaff *et al*., 2007) and probably a greater number downregulated (Schaff *et al*., 2007). A few studies on gene expression in the giant cells have reported that the mRNA for some genes can be present in giant cells at levels that are many fold greater than in non-infected root cells (Ramsey *et al*., 2004; He *et al*., 2005). These data demonstrate that the giant cells induced by all *Meloidogyn*e spp. are unique examples of how parasites can affect normal host development. Most recently (Huang *et al*., 2006), some progress has been made in the characterization of parasitism genes in the nematode that enable it fundamentally to alter plant growth and development for the benefit of the parasite (see Abad *et al*., Chapter 7, this volume).

On the bases of cytogenetic studies on about 600 populations (representing 24 species) and in collaboration with the International *Meloidogyne* Project, Triantaphyllou (1985) was able to demonstrate that root-knot nematodes have undergone extensive cytogenetic diversification, probably unparalleled by that of any other animal group. Triantaphyllou concluded that characteristic features are the establishment of meiotic and mitotic parthenogenesis in association with various degrees of polyploidy and aneuploidy. Obligatory cross-fertilization also occurs in some diploid and polyploid forms (e.g. *M. kikuyensis* and *M. megatyla*), whereas facultative meiotic (automixis) (e.g. *M. exigua*, *M. chitwoodi* and *M. graminicola*) and obligatory mitotic parthenogenesis (apomixis) (e.g. *M. incognita*, *M. enterolobii* and *M. oryzae*) prevail in most polyploid and aneuploid forms (see Chitwood and Perry, Chapter 8, this volume).

The trend from amphimictic reproduction to apomixis is generally associated with shorter life cycles, higher reproductive rates and increasing importance as crop pathogens. Only a small number of species reproduce by amphimixis, i.e. with the obligatory fusion of a male and female gamete (e.g. *M. carolinensis*, *M. microtyla*, *M. pini*). These species are considered as minor root-knot nematode species because of their very restricted distribution, host range and economic impact (Jepson, 1987). Automictic root-knot nematode species are facultatively parthenogenetic; apomictic species are obligatory parthenogenetic. The apomictic mode of reproduction is found in the most important species in terms of geographic distribution and agronomic impact. There are two possible explanations for the worldwide distribution of the apomictic root-knot nematodes. Either they are indigenous in much or all of their current range, and therefore are very ancient species, or they are recently evolved and have been widely spread by agriculture (Trudgill and Blok, 2001). The former is widely viewed as unlikely, partly because parthenogenetic species are considered an evolutionary dead end. 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 ($2n$ = 30–55 chromosomes) (Karssen and Moens, 2006; see Chitwood and Perry, Chapter 8, this volume). Root-knot nematode species further differ in their male-to-female ratio. Cross-fertilizing species such as *M. carolinensis* and *M. spartinae* usually have a 1:1 ratio. Species that reproduce by facultative or obligatory parthenogenesis such as *M. hapla* and *M. incognita* have variable sex ratios.

The root galling upon which the nematode's common name is based is quite variable among the different species of the genus and plant hosts (Plates 1–12). Some differences in galling among the different species of this genus are well known. *Meloidogyne hapla* is particularly known for the high incidence of adventitious roots that develop from root galls (Sasser, 1954; Plate 4). *Meloidogyne trifoliophila* on clover produces galls that are distinctly elongated, and the egg masses are more typically embedded within the galls than found erupting from the gall surface (Mercer *et al*., 1997; Plates 6, 7). Other species have a tendency to produce galls at the root terminus. Root galls

can be quite small or indistinct on many hosts, which often results in failure to recognize that the plant is being parasitized. Graminaceous hosts rarely form galls. Most plants with fibrous or woody roots will have small or indistinct galls, especially early in a growing season or when nematode population densities are low. Infection sites of *M. partityla* on pecan that contain a single nematode do not form galls; rather, both the mature female and the egg mass are exposed on the root surface (Plate 8). Cotton and groundnut are examples of two highly susceptible crops in which root galls can be difficult to detect early in the growing season but massive galls can be evident at crop maturity. Plants with succulent roots, especially the cucurbits and tomato, develop the readily detectable galls for which the species is named, even with low infection incidence (Plates 2, 3, 9). Under extreme conditions, a plant's root system may be entirely gall tissue with no remaining fibrous roots.

1.5.1 Incompatible host reactions

The induction and maintenance of giant cells by *Meloidogyne* spp. and the associated physiological and molecular changes in a compatible host–parasite interaction are discussed by Bleve-Zacheo and Melillo (1997) and Abad *et al.* (Chapter 7, this volume). Resistance to *Meloidogyne* spp. is a much-researched topic and Veech (1981) provides an excellent summary of the older literature. Williamson and Roberts (Chapter 13, this volume) discuss resistance genes and the genetics of plant resistance to root-knot nematodes. The analysis of histochemical changes and signal transduction pathways using the resistant tomato plant–*Meloidogyne* model system has provided useful information on the changes associated with the incompatible response (Bleve-Zacheo *et al.*, 2007). Many plant defences against pathogens are regulated by signalling pathways in which jasmonic acid, for example, plays a key role. Soriano *et al.* (2004) showed that application of exogenous methyl jasmonate to roots of spinach and oats induced nematode resistance. Jasmonates induce *de novo* ecdysteroid synthesis in roots but Soriano *et al.* (2004) found that the invasion of spinach by *M. javanica* was impaired by the induction of ecdysteroid, indicating movement of ecdysteroid into the rhizosphere and sensitivity of the nematode to its presence; expression of resistance prior to invasion is uncommon. Cooper *et al.* (2005) demonstrated that jasmonic acid induced a systemic defence response that reduced the reproduction of avirulent *Meloidogyne* on susceptible tomato plants. Another resistant response associated with signal transduction during the hypersensitive reaction to pathogen invasion is the stress-induced oxidative burst. This response is complex and involves antioxidants, such as ascorbic acid. Application of ascorbic acid to susceptible tomato plants has been shown to inhibit invasion by *M. incognita*, and resistance has been associated with the ability to synthesize large amounts of ascorbic acid following J2 invasion (Arrigoni *et al.*, 1979). The oxidative burst occurs rapidly after nematode invasion, with a second burst associated with the hypersensitive reaction only detectable in the incompatible tomato–nematode interaction (Melillo *et al.*, 2006).

The responses associated with the resistance genes *Me1* and *Me3* in pepper differed (Bleve-Zacheo *et al.*, 1998). Many fewer J2 of *M. incognita* were able to invade the pepper line HDA149, carrying the *Me3* gene, compared with the line HDA330, carrying the *Me1* gene. The line HDA149 exhibited the typical early hypersensitive response to nematode invasion, while the resistance mechanism in HDA330 involved a delayed plant response after the J2 had set up several imperfect giant cells.

Antioxidant enzymes secreted by root-knot nematodes may be important to overcome the hypersensitive response of resistant roots and the associated generation of reactive oxygen species (Molinari *et al.*, 2008; Molinari, 2009). For example, a selected virulent isolate of *M. incognita* had greater activity of antioxidant enzymes, including catalase, superoxide dismutase and peroxidase, when compared with a near isogenic avirulent isolate and an avirulent field population (Molinari, 2009). However, it is not clear whether enhanced antioxidant activities contribute to the virulent phenotype, or whether they are a side effect.

1.6 Diversity in Biology

Species of root-knot nematodes demonstrate a large diversity in various aspects of their life cycles. With respect to their temperature requirements,

root-knot nematodes can be divided into two distinct groups of species, thermophils and cryophils, which can be separated by their ability to survive lipid-phase transitions that occur at 10 °C (Lyons *et al.*, 1975; see Evans and Perry, Chapter 9, this volume). *Meloidogyne chitwoodi*, *M. hapla* and probably *M. naasi* are cryophils and able to survive soil temperatures below 10 °C; *M. arenaria*, *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 (see Curtis *et al.*, Chapter 6, this volume). Thermotypes exist within species (e.g. Daulton and Nusbaum, 1961). Root-knot nematode species also differ in the number of generations they can produce per year; this number varies according to species and food availability. Usually there are many generations per year, but in some species (e.g. *M. naasi*) there is only one (Rivoal and Cook, 1993).

Root-knot nematodes demonstrate various degrees of specialization with respect to their host preference. Crops are usually better hosts than weeds (e.g. Mandulu and Trudgill, 1993; Hillocks *et al.*, 1995). Either man, when selecting crop plants, has inadvertently selected for increased susceptibility to root-knot nematode species, or the root-knot nematodes have been selected by repeated exposure to crop plants (Trudgill and Blok, 2001). Most amphimictic species have host ranges confined to a single subclass of plants, on either woody or perennial herbaceous hosts. *Meloidogyne spartinae* seems to be restricted to cordgrass (*Spartina* spp.), both *M. pini* and *M. megatyla* to *Pinus* spp. and *M. subartica* is confined to the Commelinidae (Jepson, 1987). Apart from *M. hapla*, which has a wide host range that does not include graminaceous species, the automictic species tend to have a narrow host range, while the mitotic species have a potential host range containing the majority of the higher plants (Trudgill and Blok, 2001). However, there are exceptions to these generalizations; for example, the apomictic species *M. quericiana* and *M. enterolobii* have restricted host ranges. The majority of apomictic species of root-knot nematodes appear to have a survival strategy based on a wide host range, which enables them to persist whatever the vegetation. They lack specific triggers for hatching, and hatch occurs as soon as the J2 has developed. On good hosts, where generation times are short and fecundity is high, several generations and rapid population increase occur. Consequently, as the growing season progresses, small populations of apomictic root-knot nematodes can become large and very damaging (Trudgill and Blok, 2001).

For those *Meloidogyne* spp. that have host ranges that are large (total number of hosts) and broad (large number of plant families with species susceptible to the nematode), it is somewhat amazing that there are quite distinct differences in their overlapping host ranges. Among the traditional four major species (which were four of the five original species described by Chitwood, 1949) there are some distinct and now classic differences. These four species (*M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*) have overlapping host ranges as they all infect many common vegetable crops, including tomato, which is often (but inappropriately) considered a universal host for *Meloidogyne* spp. *Meloidogyne hapla* reproduces only poorly or not at all on most grasses and grain crops. Of these four species, only some populations of *M. incognita* parasitize cotton. Groundnut is a good host for *M. hapla* and most *M. arenaria* populations but not for *M. incognita*. Most populations of *M. javanica* in the USA do not reproduce well on groundnut but populations of this species from India and northern Africa generally reproduce well on groundnut. Thus, even though they each have large, broad and overlapping host ranges, there are distinct differences in the host range of each of these species. Perhaps, despite the claims made above, some greater caution is warranted regarding statements of large host ranges. Given the continually increasing number of recognized species, how many of the previous reports on host ranges were based on incorrect species identification and/or having test populations that contained more than one species?

1.6.1 Concept of host races

Sasser (1952) was among the first to report the variation in host range within the original four major species. He later proposed the formal recognition of 'host races' within these species and proposed a standardized set of differential hosts for distinguishing these host races (Hartman and Sasser, 1985). Further, he proposed that the host

differential test could be used as an aid to species identification. Here it should be emphasized that Sasser never recommended the use of the host differentials as the sole basis for species identification. Since the 1970s, additional variation among the four major species has been recognized, especially among populations of *M. javanica*, which is now recognized as variable with respect to reproduction on pepper and groundnut, two important differentials for the host race test. This greatly complicates the use of the host race test as an aid to species identification. Perhaps more importantly, there has been a substantial number of new species described since the 1970s. Many of these recently described species have been found in mixed populations with one or more of the traditional major species and attack some of the same important crops. The North Carolina Host Race test does not clearly distinguish most of the newly described species from the original four. Similar variation in host range is reported for other species, with this variation being unrelated to the differential hosts used in the North Carolina Host Differential test.

Although the recognition of variation in host ranges is important, we suggest the formal recognition of the host races (often referred to simply as race) be discontinued. The host race concept has never been universally accepted, in part because it measured only a small portion of the potential variation in parasitic ability. Given the large number of hosts for many species, it is unlikely that the full extent of possible variation will ever be adequately characterized. Assigning each variant population a distinct number is likely to be very unwieldy with time. It had some value in the south-eastern USA because each of the differential hosts was an important and widely grown crop, but this is not always the case. Unfortunately, knowledge of host race rarely allowed one to predict accurately other behavioural characteristics of a population. Race 1 of *M. arenaria* is clearly not as aggressive on soybean as race 2 of that species (Ibrahim and Lewis, 1993) but this correlation of one aspect of behaviour with the host race status is the exception rather than the rule. Perhaps more importantly, the term race, when used in the context of plant disease, generally refers to populations of a pathogen that differ in virulence on host species that carry specific genes for resistance to other populations of that pathogenic species. This terminology is especially prevalent in the USA. The system also mixed variation in host range with variation in virulence. For example, the tobacco cv. NC95 carries specific resistance to *M. incognita*. Thus, variation in reproduction and galling among populations of *M. incognita* on tobacco actually indicates differences in virulence (the ability of some pathogen genotypes to have a compatible interaction with hosts carrying a specific gene for resistance to that pathogen species). Virulence within *M. arenaria*, *M. incognita* and *M. javanica* in relation to the *Mi* resistance gene in tomato is well documented (Semblat *et al.*, 2000). Similarly, variation among *M. incognita* populations with respect to different resistant cotton genotypes has been reported (Zhou *et al.*, 2000). Yet these examples of variation in virulence are unrelated to host race status. Ability to convey clearly information on the behaviour of a population via a proper and correct name is essential. It will be more useful when dealing with a population that has a variant host range to confirm that the population has been adequately identified and then to simply acknowledge the variant host range.

1.7 Major and Emerging Species

Discussion of *Meloidogyne* spp. frequently focuses on the major four species: the three tropical species, *M. incognita*, *M. arenaria* and *M. javanica*, and the temperate species, *M. hapla*. The recognition of these four species as being the 'major species' began with them being four of the five original species (Chitwood, 1949). That each has an extensive host range and that they are each globally distributed further contribute to their recognized importance. Finally, the oftencited publication by Taylor *et al.* (1982), that these four species comprised more than 99% of all species identified from a collection of 662 isolates from 65 countries, further strengthened their status as the major species. However, that survey had a couple of distinct biases that skewed the data. First, most samples were collected from warm temperate or tropical climates, with only 39 of the 662 samples from a climate where the coldest temperature was ≤5 °C. Additionally, although the samples were

collected from 121 different crop species, 33% of the samples were from just four crops (tomato, aubergine (= eggplant), tobacco and okra). Probably not surprisingly, tomato alone accounted for nearly 17% of all samples. None the less, the survey greatly strengthened the status of the four major species. Their widespread recognition has probably led to many cases of misidentification, e.g. once *M. hapla* was identified a few times parasitizing a given crop species in a particular region, most further finds of a root-knot nematode attacking that crop would probably be attributed to *M. hapla* without substantial scrutiny. The advent of isozyme phenotyping and species-specific DNA protocols has contributed greatly to our ability to determine rapidly, and with less equivocation than is possible with morphometric analysis, whether a given isolate belongs to one of the well-known species or is possibly a new species.

Notwithstanding the increasing number of new *Meloidogyne* species, the four major ones are undoubtedly of immense economic importance and quite possibly still deserving of their status. They have been the subject of a considerable amount of research, which is reflected in the following chapters. Most knowledge of *Meloidogyne* spp. is based on studies of one or more of these four major species. Further, most of the more fundamental studies on these four species have been based on research with either tomato or tobacco as the host species. In the era of molecular genetics, future studies will depend on model systems for which the genetics of the host are well characterized. *Medicago truncatula* (Dhandaydham *et al.*, 2008) is an example of such a model system and has the advantage of being a legume; many legume crop species are very susceptible to one or more root-knot nematode species. Descriptions and hosts of 12 species, including the four major species, of economic importance in different geographical areas are given by Hunt and Handoo, Chapter 3, this volume, where naming authorities for all species of *Meloidogyne* are provided. Here we describe some species that we consider to be of 'emerging' importance. It is to be expected that changing selection pressures due to evolving cropping and management systems, especially the use of host resistance, will result in a dynamic landscape with regard to important *Meloidogyne* spp.

1.7.1 *Meloidogyne enterolobii* (= *Meloidogyne mayaguensis*)

This nematode is currently considered as one of the most important root-knot nematode species because of its wide geographical distribution, its wide host range, and its ability to overcome the resistance of important crop plants, such as genotypes of tomato, pepper, and some agronomic crops that carry the *Mi-1* gene, which confers resistance to *M. javanica*, *M. arenaria* and *M. incognita* (Fargette, 1987; Fargette *et al.*, 1994). This nematode was originally described from a population collected from aubergine (*Solanum melongena*) in Puerto Rico (Rammah and Hirshmann, 1988). Subsequently, it has been detected in Africa (Fargette *et al.*, 1994; Duponnois *et al.*, 1995; Willers, 1997; Trudgill *et al.*, 2000), the USA (Brito *et al.*, 2004), South and Central America (Decker and Rodriguez-Fuentes, 1989; Carneiro *et al.*, 2000, 2001; Trudgill *et al.*, 2000) and Europe (Blok *et al.*, 2002).

Other recorded hosts include vegetables, and other crops, such as bell pepper (*Capsicum annuum*), soybean (*Glycine max*), sweet potato (*Ipomoea batatas*), tobacco (*Nicotiana tabacum*) and watermelon (*Citrullus lanatus*). A tropical fruit tree, guava (*Psidium guajava*), is also a good host of this nematode (Plate 20). Spanish needle (*Bidens pilosa*), a weed host, has also been identified. In Cuba, reproduction was observed on coffee (*Coffea arabica* cv. Caturra), bean (*Phaseolus vulgaris* cv. Icapijao), beet (*Beta vulgaris*), broccoli (*Brassica oleracea* var. Botrytis), celery (*Apium graveolens* cv. Utah), horsebean (*Cannavalia ensiformis*), parsley (*Petroselynum crispum* cv. Plain), potato (*Solanum tuberosum*) and pumpkin (*Cucurbita* sp.). In Florida, this nematode has been found in roots of angel trumpet (*Brugmansia* cv. Sunray), basil (*Ocimum* sp.), cape honeysuckle (*Tecomaria capensis*), glory bush (*Tibouchina* cv. Compacta and *Tibouchina elegans*), carpet bugleweed (*Ajuga reptans*) and Uganda glorybower (*Clerodendrum ugandense*).

1.7.2 *Meloidogyne paranaensis*

This species was detected on coffee in Paraná state, Brazil, from which it is named (Carneiro *et al.*, 1996). Coffee (*C. arabica*) is the primary host of this species. However, the nematode has also been detected on soybean (Dinnys Roese *et al.*,

2004). In host studies, tobacco, tomato and watermelon were reported as suitable hosts (Carneiro *et al.*, 1996). Decline and dieback of coffee trees, and yield suppression of up to 50%, are associated with nematode infection in Brazil, where the damage occurring on coffee in Paraná state was initially erroneously attributed to *M. incognita* (Carneiro *et al.*, 1996). Currently the nematode has been detected only in the USA (including Hawaii), Central America, the Caribbean, Guatemala and South America.

Specific damage caused by *M. paranaensis* on roots of coffee typically does not involve gall formation. Instead, *M. paranaensis* causes the taproot of coffee to crack and split, as well as damaging other root tissue. Necrosis also occurs where females are embedded and near the giant cells where feeding occurs. Above-ground symptoms generally range from chlorosis and reduced plant growth to death (Carneiro *et al.*, 1996).

Meloidogyne paranaensis may occur by itself or in mixed populations with other *Meloidogyne* spp. (Carneiro *et al.*, 1996). Inserra *et al.* (2003) suggested that this nematode may lower yield potentials by 50%. This estimate is based on information provided by Carneiro *et al.* (1996), but the original authors only suggested that this particular species may '[account] for approximately 52% of all root-knot nematode infestations in Paraná'. Carneiro *et al.* (1996) do not comment on the magnitude of damage when these infestations occur.

1.7.3 *Meloidogyne fallax* and *Meloidogyne chitwoodi*

Meloidogyne chitwoodi and *M. fallax* are closely related species parasitizing monocotyledons and dicotyledons, including several major crop plants such as potatoes, carrots and tomatoes (Santo *et al.*, 1980; O'Bannon *et al.*, 1982; Brinkman *et al.*, 1996; Karssen, 2002 (Plates 10, 11, 22, 25–27)).

Meloidogyne chitwoodi was first described from roots and tubers of potato in a field near Quincy, Washington state, USA. The species has been recorded from Argentina, Belgium, Germany, The Netherlands, Portugal, several states of the USA, Mexico and South Africa (EPPO, 2004). It is not clear whether this is its area of origin. In Europe, the nematode was first detected in the Netherlands, but a review of old illustrations and old specimens of *Meloidogyne* suggests that they may have occurred earlier (EPPO, 1991). The species can begin development when soil temperature rises above 5 °C and requires 600–800 degree-days to complete the first generation; subsequent generations require only 500–600 degree-days (Pinkerton *et al.*, 1991). Intraspecific variation in *M. chitwoodi* is manifest by the occurrence of three biotypes that can be distinguished based on reproduction on lucerne cv. Thor, carrot cv. Red Cored Chantenay, and *Solanum bulbocastanum* Dun. SB22 (Santo and Pinkerton, 1985; Mojtahedi *et al.*, 1988, 1994).

Symptoms caused by *M. chitwoodi* vary according to host, population density of the nematode and environmental conditions. Galls produced on potato tubers are often not easily detected. On carrots, galls appear mainly on the lenticels and reduce the commercial value of the crop (Wesemael and Moens, 2008).

Meloidogyne fallax was detected for the first time in 1992 in a field north of Baexem (The Netherlands) and initially identified as 'a deviating *M. chitwoodi* population' (Karssen, 1994). After this first report, it was recorded on potato at several locations in the southern and south-eastern part of the Netherlands and eventually described as a separate species (Karssen, 1996). Later it was also found in a plastic tunnel house in France (Daher *et al.*, 1996), and in Belgium (Waeyenberge and Moens, 2001) and Germany (Schmitz *et al.*, 1998). The species has been detected outside Europe, in New Zealand (Marshall *et al.*, 2001), Australia (Nobbs *et al.*, 2001) and South Africa (Fourie *et al.*, 2001). Above-ground symptoms of heavily infested plants include stunting and yellowing, while below-ground galling is typical. Variations in host ranges among different populations have not been described for *M. fallax*.

Successful hybridization was not obtained when *M. fallax* and *M. chitwoodi* were crossed; the F1 was viable, but the F2 second-stage juveniles were not viable and showed morphological distortions (van der Beek and Karssen, 1997).

The root galls produced by *M. chitwoodi* and *M. fallax* are comparable to those produced by several other root-knot species, relatively small galls in general, without secondary roots emerging from them (as found in *M. hapla*). On potato tubers, *M. chitwoodi* and *M. fallax* cause numerous small, pimple-like raised areas on the surface (with *M. hapla* these swellings are not evident).

There is a marked contrast in the hatching response of the two species (see Curtis *et al.*, Chapter 6, this volume). Hatching of J2 of *M. chitwoodi* produced on young plants does not require host root diffusate stimulus, whereas at the end of the plant growing season, egg masses contained a percentage of unhatched J2 that require host root diffusate to cause hatch. This form of obligate quiescence at the end of the host growing season was not found in *M. fallax*, which hatched well in water and did not require hatch stimulation from root diffusate, irrespective of the age of the plant on which the egg masses were produced (Wesemael *et al.*, 2006).

1.7.4 *Meloidogyne minor*

Karssen *et al.* (2004) described *M. minor*, which appeared to be the causal agent of yellow patch disease on several golf courses in the British Isles and root-knot symptoms in one potato field in The Netherlands in 2000 (Plates 12–15). A joint pest risk analysis (PRA) by nematologists in The Netherlands and the UK for the EU region established that *M. minor* was present mainly on coastal sand dunes, golf courses and sports grounds in the British Isles, and in The Netherlands *M. minor* was found on several golf courses, sports grounds and pasture fields. *Meloidogyne minor* has been reported on turfgrass in Belgium (Viaene *et al.*, 2007) but it is not known if it is indigenous to Europe and present in other EU countries. Additional surveys are required to determine its distribution and economic importance, but the PRA concluded that, with the current knowledge, *M. minor* was primarily a problem for golf courses, and it is not yet possible to determine whether quarantine measures are appropriate. It is presumed that its spread cannot readily be controlled since it can be carried on footwear and sports equipment. The PRA document will be available on the web sites of the Dutch and British NPPOs (National Plant Protection Organizations; www.minlnv.nl/pd and www.defra.gov.uk).

1.8 Interactions with Other Plant Pathogens

Meloidogyne spp. frequently play a role in disease interactions (Khan, 1993; see Manzanilla-López and Starr, Chapter 10, this volume), especially with other soil-borne pathogens. Plant pathologists often attribute these interactions to wounds made by the nematodes, which ignores the profound effects of parasitism by *Meloidogyne* spp. on plant physiology and gene expression. Regardless of the underlying mechanisms, the numerous interactions with other root and vascular pathogens only serve to exacerbate the ultimate damage to the crop and increase crop losses. Sometimes *Meloidogyne* spp. and ectoparasitic nematodes appear mutually antagonistic. However, interactions between these two groups may be beneficial for one or both of the species (Eisenback, 1993).

1.9 Management and Control

All methods for control of plant pathogens, including parasitic nematodes, can be categorized under one or more principles (Table 1.1). All of the various methods for control of nematodes fit within one of these principles. Management of nematodes (see Nyczepir and Thomas, Chapter 18, and Coyne *et al.*, Chapter 19, this volume) involves the manipulation of nematode densities to non-injurious or sub-economic threshold levels using several measures in relation to the whole production system, whereas control implies the use of a single measure to reduce or eliminate nematode pests, which in most cases is not possible (Thomason and Caswell, 1987). Maintenance of diversity is an objective of management but not of control, and of increasing importance is the additional need to take into consideration the impact of the pest management strategy on biodiversity and the ecological balance in the soil. Biological control (see Hallmann *et al.*, Chapter 17, this volume) is the management of plant diseases and pests with the aid of living organisms, including 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. Plant resistance (see Williamson and Roberts, Chapter 13, and Starr and Mercer, Chapter 14, this volume) is biologically based but is considered a distinct approach to control and management.

As with most plant-parasitic nematodes, preventing the introduction and spread of species of

Table 1.1. Principles for control of plant pathogens.

Exclusion	Prohibiting, frequently by governmental regulations, the entry of the pathogen into a region or locale where it does not exist
Eradication	The complete or partial removal of the pathogen from a region, locale, or field (e.g. soil fumigation)
Avoidance	Avoiding environments and conditions that favour pathogen activity (e.g. altering planting date)
Protection	Usually the use of pesticides to inhibit pathogen activity (e.g. use of non-fumigant nematicides)
Resistance	Altering the genetic constitution of the host so it is able to inhibit pathogen activity (for nematodes, this is typically inhibition of reproduction)
Therapy	Action taken after infection has occurred to limit further development of the pathogen (e.g. hot water treatments of infected bulbs or corms)

Meloidogyne is a vital component of management strategies. *Meloidogyne* species may be spread on farm machinery and may be present in planting material, such as corms, bulbs or roots, but are not found in seeds. Cleaning machinery before use is recommended, and planting material can be discarded if infected, or treated with chemicals or hot water to reduce the numbers of *Meloidogyne*. Only seedlings produced in *Meloidogyne*-free seedbeds should be transplanted.

Listing species as quarantine organisms reduces the risks of spread through international trade. In general, root-knot nematodes are not regulated as a group because the major economically important species are already widely distributed (Hockland *et al.*, 2006). However, *M. chitwoodi* is of increasing importance, primarily because it is a serious pest of economically important crops such as potatoes and carrots, and is on the lists of prohibited pests of many countries (Canada, the EU, Mexico, and other countries in Latin America and the Far East). In the future, with the increase in the number of new species being described, initially with limited knowledge of distribution, more species are likely to be of regulatory concern.

Various cultural and physical control methods have been used with varying degrees of success, but often these methods are only of local or regional relevance. For example, soil solarization (Gaur and Perry, 1991) is only of use in regions where sufficient solar energy is available for long periods of time. Similarly, in some climates, ploughing at intervals of 2–4 weeks during the dry season exposes eggs and J2 to desiccation, killing many in the upper layers of soil. Population densities can be reduced by organic amendments, and flooding land to a depth of 10 cm or more for several months is also effective. The adverse effect of *Tagetes* species is highly variable, depending on the combination of *Tagetes* species and cultivar, and the species and population of *Meloidogyne*. It appears that reduction of *M. incognita* by marigold (*Tagetes patula*) is primarily due to an antagonistic or trap crop effect; J2 enter roots but there is neither giant cell formation nor a hypersensitive reaction.

The era of nematicides was the 1950s, 1960s, 1970s and 1980s. Their overall effectiveness is often cited as a reason why other alternative management systems did not receive greater attention for many years. Starting in the late 1970s, the use of some fumigants, initially 1,2-dibromo-3-chloropropane, was greatly restricted or forbidden entirely. That was followed by similar restrictions and eventual suspension (in some cases by corporate rather than governmental decision) of some of the granular nematicides. Currently, the list of available and effective nematicides is very short. Unfortunately, due in part to the small market share of nematicides relative to herbicides or insecticides, there is little prospect for new effective materials in the near future.

1.10 Conclusions and Future Directions

De Waele and Elsen (2007) attribute the general lack of awareness in tropical countries of even the

existence of plant-parasitic nematodes to the microscopic nature of nematodes, the lack of characteristic symptoms they cause, and the farmers' limited previous exposure to extension and community information. This is probably universally true. Knowledge of the pest involves identification of the species present and, clearly, the provision of better species descriptions, including all available information on morphology, morphometrics, genetics, phylogenetics, etc., is essential (see Eisenback and Hunt, Chapter 2, this volume). Future research in nematode systematics should comprise well-focused taxonomy based on a combination of classical and molecular methods (Coomans, 2002), and we consider that papers on *Meloidogyne* spp. should include a brief description of the method used to identify the population(s). A relevant example of the need for this was provided when a closer examination of *Meloidogyne* populations associated with coffee in Brazil, combining morphological observations with molecular diagnostics, led to the description of several new *Meloidogyne* species (Plate 17) and the suggestion that *Meloidogyne* spp. populations on coffee from Brazil and other Central and South American countries must frequently have been misidentified (Carneiro *et al*., 2004). That many of the recently described species have been found in association with more common species suggests that such misidentications may have occurred elsewhere.

There needs to be a uniform method to assess and disseminate information on damage caused by the nematode species. Costs of produce fluctuate greatly and are often not comparable between countries. Thus, economic losses are relevant data only for a particular year. Yield loss data should be in a form that can be easily converted to costs if comparison between years is required. But this presupposes a uniform estimation of damage, which may not be yield loss per se but is likely to include the amount of harvested crop that is unmarketable because of damage by *Meloidogyne* spp.

In this chapter we have recommended abandoning the host race system proposed by Hartman and Sasser (1985) that has been used by many scientists. This is not to dismiss the importance in variation in parasitic abilities but is a recognition that the system is no longer adequate for the greatly expanded genus. The variation in host ranges and parasitic fitness of the more than 90 currently recognized species on individual hosts now appears to be too great to be categorized by a numerical host race designation. With the increasing use of resistant hosts for control of root-knot nematodes, eliminating the use of the host race also avoids potential confusion with races (or pathotypes) that vary in virulence on resistant host genotypes.

This introductory chapter has set the scene for the subsequent chapters, where aspects of nematode biology, host–plant interactions and control will be discussed in depth. Information on the genomes of *M. hapla* and *M. incognita* will enable features of obligate parasitism to be defined, and the genomic information may aid in the identification of novel control targets and the refining of environmentally acceptable management options. Management of *Meloidogyne* in developed and resource-poor regions will be central to the provision of sufficient food for the ever-increasing global population.

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