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Review

Top 10 plant-parasitic nematodes in molecular plant pathology

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SUMMARY

The aim of this review was to undertake a survey of researchers working with plant-parasitic nematodes in order to determine a 'top 10' list of these pathogens based on scientific and economic importance. Any such list will not be definitive as economic importance will vary depending on the region of the world in which a researcher is based. However, care was taken to include researchers from as many parts of the world as possible when carrying out the survey. The top 10 list emerging from the survey is composed of: (1) root-knot nematodes (*Meloidogyne* spp.); (2) cyst nematodes (*Heterodera* and *Globodera* spp.); (3) root lesion nematodes (*Pratylenchus* spp.); (4) the burrowing nematode *Radopholus similis*; (5) *Ditylenchus dipsaci*; (6) the pine wilt nematode *Bursaphelenchus xylophilus*; (7) the reniform nematode *Rotylenchulus reniformis*; (8) *Xiphinema index* (the only virus vector nematode to make the list); (9) *Nacobbus aberrans*; and (10) *Aphelenchoides besseyi*. The biology of each nematode (or nematode group) is reviewed briefly.

INTRODUCTION

Over the past 2 years, *Molecular Plant Pathology* has published reviews of the top 10 viruses (Scholthof *et al*., 2011), fungi (Dean *et al*., 2012) and bacteria (Mansfield *et al*., 2012). These surveys have prompted discussions within each community about what makes an important pathogen in terms of the economic damage caused and its contribution to the further development of the field of molecular plant pathology. These peer-reviewed articles have been produced on the basis of the results of large surveys to which the entire scientific community working with each pathogen group has been invited to respond, thus providing some measure of support for statements made in grant applications and publications about the importance of various pathogens. Each of the articles has been well received and has been made available free to download through the *Molecular Plant Pathology* website. A similar survey was therefore conducted for plant-parasitic nematodes, and this article, similar in layout to those for the other plant pathogens, is the result.

There are over 4100 species of plant-parasitic nematode described to date (Decraemer and Hunt, 2006) and, collectively, they represent an important constraint on the delivery of global food security. Damage caused by plant nematodes has been estimated at $US80 billion per year (Nicol *et al*., 2011). However, this is likely to be a significant underestimate of the true figure, as many growers, particularly in developing nations, are unaware of plant-parasitic nematodes. These nematodes are usually small, soil-borne pathogens and the symptoms they cause are often nonspecific.

Plant-parasitic nematodes display a wide variety of interactions with their hosts. All have hollow, protrusible stylets, or mouth spears, used to penetrate cells to allow feeding and, for endoparasitic forms, entry into the host (Fig. 1). Some nematodes are migratory ectoparasites that never enter the host, but simply migrate through the soil, using roots as an ephemeral food source as they encounter them. Migratory endoparasites enter the host and migrate through host tissues causing extensive damage. Semi-endoparasitic nematodes may have migratory stages, but also partially penetrate the host plant in order to feed at one stage of the life cycle. Such nematodes, including *Rotylenchulus reniformis* (below), induce a feeding structure within their host at the sedentary stage. However, the most economically important nematodes, the root-knot and cyst nematodes, are biotrophic and

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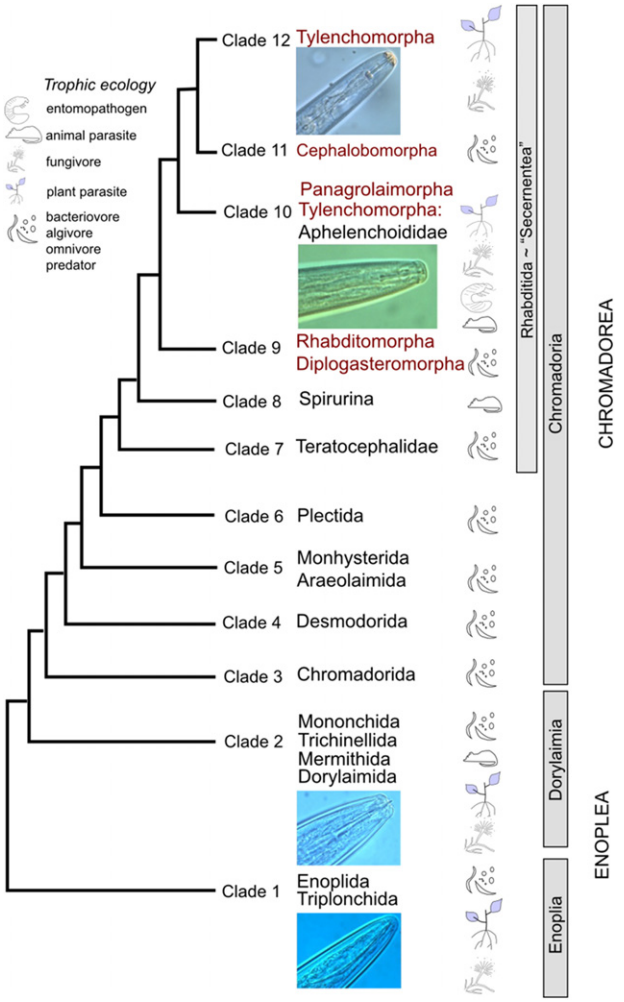

Fig. 1 Interrelationships of nematode clades (Van Megen *et al*., 2009) with phylogenetic positions and feeding apparatus of plant-parasitic nematodes indicated. 'Infraorders' (a less common taxonomic unit) are indicated in red type.

induce complex feeding structures in the roots of their hosts which supply the nematode with a rich and long-lasting food source. It is important to note that parasitism of plants by nematodes has evolved independently on several occasions, with biotrophy also having evolved independently in at least four different clades (Van Megen *et al*., 2009) (Fig. 1).

The small size of plant-parasitic nematodes, as well as the fact that many of the most important species are obligate biotrophs and cannot be cultured in large numbers, makes them extremely difficult experimental organisms. Studies on the molecular basis of plant parasitism by nematodes have therefore lagged behind other fields of plant pathology for many years. However, the development of genomics tools suitable for use with small quantities of starting material and the evolution of tools such as RNA interference (RNAi) for use with many plant nematodes (i.e. Chen *et al*., 2005) have had a significant impact on the field of plant nematology. Expressed sequence tag (EST) datasets are now available for a wide range of species (reviewed by Jacob and Mitreva, 2011) and the genome sequences of three of the species featured in this article have been published, with others in the pipeline (Abad *et al*., 2008; Kikuchi *et al*., 2011; Opperman *et al*., 2008). Of particular importance was the finding that representatives of the two most economically important biotrophic groups, the cyst nematodes and the root-knot nematodes, could infect *Arabidopsis thaliana* (Sijmons *et al*., 1991). For the first time, this made the resources developed for the study of this model plant available for the analysis of plant–nematode interactions, and allowed the mechanisms by which the complex feeding structures induced by these nematodes are produced to be probed using the full scope of genomic resources.

The survey was conducted at the end of 2012. Members of Nematology Societies across the world, as well as alumni from major postgraduate plant nematology courses, were asked to nominate their top five plant-parasitic nematodes. Over 225 responses, representing around 1100 individual votes, were received, from which the overall list of the top 10 species was obtained. In order to avoid repetition, all cyst nematode species have been grouped, as have all root-knot nematode species. This has also allowed some less familiar, but still economically important, nematodes to appear in this article. Even so, some important nematodes just missed out on being included. Honourable mention should go to several nematodes in this category, including *Helicotylenchus* spp. (Subbotin *et al*., 2011) and the ectoparasitic *Trichodorus* spp., the vector of *Tobacco rattle virus* (Decraemer and Geraert, 2006).

1. ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

Root-knot nematodes are obligate plant parasites that are distributed worldwide. The genus consists of 98 species (as of February 2013) and they parasitize almost every species of vascular plant. Their vernacular name comes from the galls (root-knots) induced by *Meloidogyne* on the roots of their host plant (Fig. 2). The most important species (sometimes referred to as the four major species—Moens *et al*., 2009) are the tropical *M. arenaria*, *M. incognita* and *M. javanica*, and the temperate *M. hapla*.

Mature females lay eggs in a protective gelatinous matrix which forms an egg mass. Egg masses are found on the root surface or may be embedded in galls or plant tissue (e.g. potato tubers), and can contain up to 1000 eggs. After embryogenesis, the first-stage juvenile (J1) moults within the egg to the infective second-stage juvenile (J2), which hatches from the egg. Many *Meloidogyne* species have a broad host range and, in general, hatching is dependent solely on suitable temperature and moisture conditions, with no stimulus from host plants being required. However, in some cases, root diffusates and generation number within a season can influence the hatching response (Curtis *et al*., 2009). J2s usually penetrate the roots directly behind the root cap, but can penetrate at any site. To facilitate penetration, J2s use a combination of physical damage through thrusting of the stylet and breakdown of the cell wall by cellulolytic and pectolytic enzymes. However, in contrast with cyst nematodes, the J2s migrate intercellularly within the root. J2s migrate to the root tip and make a U-turn when they reach the apical meristematic region. They then move upwards in the vascular cylinder towards the zone of differentiation. The J2 initiates the formation of a permanent feeding site, which consists of several giant cells. These cells function as specialized sinks, supplying nutrients to the now sedentary J2. The head of the J2 is embedded in the periphery of the vascular tissue. After

feeding, the J2 swells and moults a further three times to reach the reproductive adult stage. J3 and J4 stages lack a functional stylet and do not feed. Males are vermiform and leave the root. The adult females continue to feed and enlarge to become pear-shaped. Root-knot nematodes have unbalanced sex ratios. They show an exceptional variety of reproductive strategies, ranging from amphimixis to obligatory mitotic parthenogenesis (Chitwood and Perry, 2009). Most species are parthenogenetic and males are only formed under adverse conditions.

The reaction of a plant to parasitism by root-knot nematodes depends on the plant species and cultivar. Crop rotation, field period, season, initial population density and soil type also influence the severity of damage. Typical symptoms include stunted growth, wilting, leaf discoloration and deformation of the roots. The increased metabolic activity in giant cells mobilizes photosynthetic products from shoots to roots (Hofmann and Grundler, 2007). Reduced yield is manifested in changes in quantity and/or quality. Damage thresholds (the point at which yield losses make crop production uneconomic) can be as low as 1 egg/100 cm^3 soil (Greco and Di Vito, 2009). Although the impact of these nematodes is greater in tropical areas, *Meloidogyne* species are of major economic importance worldwide. An overview of the damage potential of some *Meloidogyne* species has been given by Wesemael *et al*. (2011). Disease complexes with other pathogens, such as *Fusarium* wilt, *Rhizoctonia solani* and *Thielaviopsis basicola*, have also been reported (Manzanilla-López and Starr, 2009). The quarantine status of some species causes indirect costs in addition to direct loss. For example, *M. chitwoodi* and *M. fallax* are increasingly regulated as they can be spread through seed potatoes.

Meloidogyne graminicola is the major species attacking both upland (rainfed) and lowland (irrigated) rice. It is well adapted to flooded conditions and yield losses of up to 87% have been reported (Lilley *et al*., 2011; Netscher and Erlan, 1993; Padgham *et al*., 2004; Soriano *et al*., 2000; Tandingan *et al*., 1996). Its short life cycle and wide host range, including many weeds that are common in rice fields, make this species difficult to control (De Waele and Elsen, 2007).

The genomes of two root-knot nematodes, the mitotically parthenogenetic *M. incognita* and the meiotic facultative parthenogenetic *M. hapla*, were published in 2008 (Abad *et al*., 2008; Opperman *et al*., 2008). These were the first genome sequences of animals able to parasitize plants. The exploration of the structure and gene content of these genomes has revealed unique adaptations to their peculiar biology, including plant parasitism. Compared with the model free-living nematode *Caenorhabditis elegans*, both root-knot nematode genomes have a relatively lower proportion of genes encoding G-protein-coupled receptors (GPCRs) and collagens (Bird *et al*., 2009). A comparison of the *M. incognita* genome with other nematode genomes has revealed that the set of genes putatively involved in immunity, detoxification and defence mechanisms is reduced (Abad *et al*., 2008). This has been interpreted as a consequence of the ability to survive inside plant tissue, an environment relatively depleted in nematode predators, such as fungi and bacteria. A feature common to both root-knot nematode genomes, which can be more directly linked to their plant-parasitic lifestyle, is the presence of a set of genes encoding plant cell wall-degrading enzymes, whose abundance is unprecedented to date in animals. Phylogenetic analyses have shown that, apart from other closely related plant-parasitic nematodes, these enzymes are generally absent from animals, and their closest homologues are found in bacteria and fungi (Danchin *et al*., 2010). The genes encoding these enzymes are therefore likely to have been acquired via horizontal gene transfer. Because several of these enzymes are found in other plant-parasitic nematodes of the Tylenchida group, it is thought that acquisition via horizontal gene transfer occurred early in an ancestor of these nematodes, and probably catalysed the development of their ability to parasitize plants (Haegeman *et al*., 2011a; Rybarczyk-Mydłowska *et al*., 2012a). Recently, a systematic analysis of both root-knot nematode genomes has revealed that more than 3% of their protein-coding genes may have been acquired via horizontal gene transfer of nonanimal origin (Paganini *et al*., 2012). Candidate donors are mainly bacteria and fungi, including known plant pathogens.

Meloidogyne spp. are obligate biotrophs and need to maintain their feeding structures alive in their hosts for several weeks. The suppression of host defences is therefore likely to be of key importance. It has recently been shown that an *M. incognita*-secreted calreticulin can suppress host defence

Fig. 2 Damage caused to roots of tomato (*Solanum lycopersicum*) by the root-knot nematode *Meloidogyne incognita* in Ethiopia. (Photograph courtesy of Seid Awol, Ghent University, Ghent.)

responses (Jaouannet *et al*., 2013), possibly by preventing calcium ion flux through sequestration of free calcium in a manner similar to that seen for some plant-pathogenic bacteria (Aslam *et al*., 2008). Effectors from *Meloidogyne* are also thought to be responsible for the induction of a feeding site, essential for the completion of their life cycle. This feeding site is typically made up of five to seven multinucleate giant cells that arise from repeated nuclear divisions in the absence of cytokinesis (Caillaud *et al*., 2008; Jones and Payne, 1978). Although *Meloidogyne* determinants of feeding site induction are as yet unknown, a gene resembling rhizobial NodL has been found in these nematodes. Rhizobial NodL encodes one of the proteins involved in the synthesis of nodulation factors, responsible for the formation of nodules in legumes. Mutants of leguminous plants that are unable to support normal nodulation are resistant to nematodes, and an unknown substance present in nematode exudates also induces changes in plants normally associated with nodulation signalling (Weerasinghe *et al*., 2005). However, the potential role of nematode NodL in the establishment of feeding sites remains uncertain (McCarter *et al*., 2003).

Some genome features appear to be markedly different in *M. incognita* and *M. hapla*. For example, whereas *M. incognita* has an 86-Mb genome and encodes 19 212 protein-coding genes, *M. hapla* has a compact 54-Mb genome and encodes only 14 420 genes. However, these apparent differences are essentially a result of the peculiar genome architecture observed in *M. incognita*. Most of the *M. incognita* genome is present as pairs of similar, yet divergent, regions with an average nucleotide divergence of 7% between regions. *Meloidogyne incognita* reproduces without meiosis or sex, and whether these pairs of regions represent former allelic regions, or are the result of a past hybridization event, is still being investigated. By contrast, *M. hapla* is able to undergo meiosis and to reproduce sexually, and its genome shows no indication of duplication. Its facultative sexual mode of reproduction turned out to be decisive in the establishment of a genetic map. Based on this initial genetic map, recent cross experiments between *M. hapla* strains have shown that the rate of genetic recombination is particularly high in this species, and might provide a mechanism for the generation of within-population genetic diversification (Thomas *et al*., 2012). Despite these features and the postulated advantages of sexual reproduction, *M. incognita* has a wider host range and geographical distribution than *M. hapla*. It has been suggested that part of this paradox could be linked to the duplicated genome structure of *M. incognita*, in which divergent genomic regions could favour the emergence of novel functions among gene copies (Abad *et al*., 2008).

2. CYST NEMATODES (*HETERODERA* AND *GLOBODERA* SPP.)

The cyst nematodes are obligate biotrophs and are of great economic importance throughout the world. The most damaging species include soybean cyst nematodes (SCNs) (*Heterodera glycines*), potato cyst nematodes (PCNs) (*Globodera pallida* and *G. rostochiensis*) and cereal cyst nematodes (CCNs) (including *Heterodera avenae* and *H. filipjevi*). Economic losses are difficult to establish, but SCNs have been calculated to be responsible for losses in excess of $US1.5 billion each year in the USA alone (Chen *et al*., 2001). Losses caused by CCNs are heavily dependent on environmental conditions, but can be in excess of 90% in some environments (Nicol *et al*., 2011). PCNs have been estimated to cause losses of 9% of total potato production worldwide (Turner and Rowe, 2006). This nematode originated in South America, but has subsequently spread to nearly all major potato-growing regions of the world, and is still a major quarantine pathogen (Hockland *et al*., 2012).

Particular problems are caused by the ability of cyst nematodes to survive for prolonged periods in the soil in the absence of a host (up to 20 years—Grainger, 1964), making control by rotations difficult and eradication, once established, almost impossible. Cyst nematodes moult to the J2 in the egg. This is the dormant stage of the life cycle and, in host-specific species, hatching occurs in response to host-derived chemical cues present in root diffusates (Perry, 2002). The J2 then locates its host, invades and migrates destructively and intracellularly through the root until it reaches the inner cortex. Here, the behaviour of the nematode changes. The J2 inserts its stylet carefully into a cell and awaits the cell response. If the protoplast collapses or if the stylet becomes covered with a layer of callose-like material, the stylet is retracted. This behaviour is repeated until a suitable cell that does not respond adversely to J2 probing is found. This cell becomes the initial syncytial cell (ISC) (Golinowski *et al*., 1997). The syncytium, a large multinucleate feeding structure (Fig. 3), is formed by partial cell wall dissolution, a process which begins at the plasmodesmata, followed by fusion of the protoplasts of adjoining cells. Cells surrounding the ISC are incorporated into the syncytium, and this process is repeated, with successive layers of cells becoming part of the syncytium. In addition, DNA synthesis is induced and metabolism increases, providing a rich food source for the nematode (reviewed by Sobczak and Golinowski, 2011). The nematode remains at this feeding site for several weeks, going through a further three moults to the adult stage. Females grow until their spherical bodies burst through the root surface, whereas males revert to the vermiform body shape, leave the roots and follow sex pheromone gradients to find females. After fertilization, the female dies and her body wall tans to form a cyst which encloses the next generation of eggs.

The major economic importance of the cyst nematodes, and the remarkable changes that they induce in their hosts, have meant that significant efforts have been made to understand the molecular basis of the host–parasite interaction. Large numbers of effectors have been identified, mainly through transcriptome analyses (i.e. Gao *et al*., 2003; Jones *et al*., 2009), and genome projects are underway for several major species including PCNs, SCNs and CCNs. Expression profiles of effectors, and other genes, have been examined throughout the life cycle, and these studies have shown that different pools of effectors are deployed at different phases of parasitism (Elling *et al*., 2009; Palomares-Rius *et al*., 2012). In addition to the cell wall-modifying proteins present in many plant nematode species, effectors have been identified that suppress host defences (i.e. Postma *et al*., 2012), a process likely to be essential for a species that relies on biotrophy to such an extent. Effectors that target a range of host structures, including the nucleus (Jones *et al*., 2009—Fig. 4), have been identified.

Significant progress has been made in terms of the development of an understanding of how nematodes induce syncytia in their hosts. Cyst nematodes produce a peptide similar to plant CLAVATA3 peptides which can complement mutant plants lacking this peptide (Wang *et al*., 2005), and it is possible that manipulation of the Clavata signalling pathway is important in feeding site induction. However, an effector that interacts directly with an auxin influx protein has also been identified, which may change the patterns of auxin flow into feeding structures (Lee *et al*., 2011). A model for auxin manipulation underlying feeding site induction, based on studies of the early activation (18 h post-inoculation) of an artificial auxin-sensitive promoter by *H. schachtii* (Karczmarek *et al*., 2004), and of the expression profiles and localization of auxin transporter proteins, has been proposed (Grunewald *et al*., 2009). Although PIN-FORMED1 (PIN1) is required for the transport of auxin to the developing feeding structure from other parts of the root, PIN1 is down-regulated in the early feeding site, preventing the transport of auxin away from the feeding structure, and thus leading to a local increase in the level of this hormone. In addition, the localization of PIN3 in the feeding structure changes from a basal to lateral distribution, allowing for the radial expansion of the syncytium. On a larger scale, microarrays have been used to track the wholesale changes in gene expression that are induced within syncytia (i.e. Ithal *et al*., 2007).

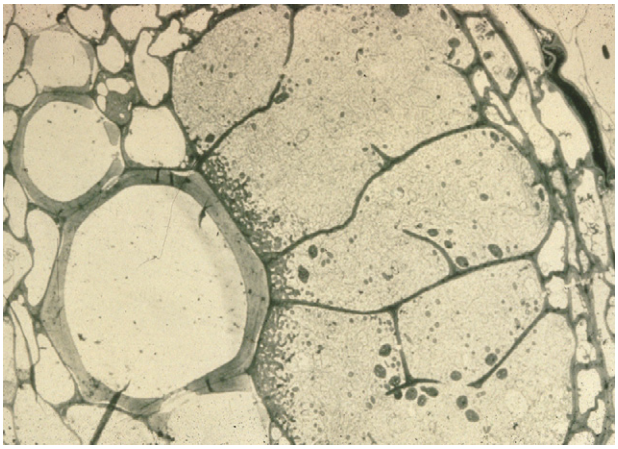

Fig. 3 Syncytium induced by the potato cyst nematode *Globodera rostochiensis* in the roots of potato.

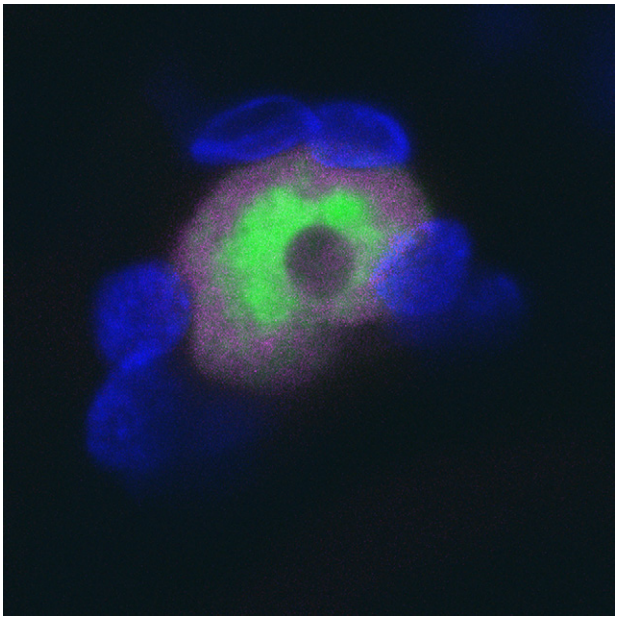

Fig. 4 Targeting of the nucleus and nucleolus of a *Nicotiana benthamiana* cell by a *Globodera pallida* effector of unknown function fused to green fluorescent protein. The nuclei are labelled with a monomeric red fluorescent protein (mRFP)–histone fusion (Martin *et al*., 2009). Autofluorescence from chloroplasts is shown in blue.

3. ROOT LESION NEMATODES (*PRATYLENCHUS* SPP.)

There are over 60 named species of root lesion nematode (*Pratylenchus* spp.) which are distributed worldwide. *Pratylenchus* species rank third only to root-knot and cyst nematodes as having the greatest impact on crops worldwide (Castillo and Volvas, 2007). The most important of these are species such as *P. penetrans*, *P. thornei*, *P. neglectus*, *P. zeae*, *P. vulnus* and *P. coffeae*. *Pratylenchus* spp. are polyphagous, migratory, intercellular root endoparasites with a life cycle that lasts 3–8 weeks depending on the species and conditions. After development within the egg to the J1, the nematode moults to the J2 which hatches from the egg. All subsequent juvenile and adult stages are worm-like and mobile, and can infect host plant roots or storage organs. Adults and juveniles can enter and leave roots and females lay eggs inside roots or in adjacent soil. Males are common in some species and absent in others, although reproduction is often by parthenogenesis. Longer term survival under adverse conditions can occur at the egg stage, or through anhydrobiosis, when the nematodes can survive in the soil for more than a year.

A reduction in root growth occurs on infection, accompanied by the formation of lesions, necrotic areas, browning and cell death, often followed by root rotting from secondary attack by soil fungi or bacteria. Root damage slows plant growth (Fig. 5), increases susceptibility to water stress and causes stunting and yellowing. Infected roots are usually discoloured and stubby. Infection can occur along the entire length of the root, with damage to the epidermis, cortex and root endodermis. *Pratylenchus* spp. are economically serious and often underestimated pests of many crops, such as cereals, causing yield losses of up to 30% in wheat in Australia (Vanstone *et al*., 2008); they also infect a range of other crops, including sugarcane, coffee, banana, maize, legumes, potato, many vegetables and fruit trees (Castillo and Volvas, 2007).

When feeding and migrating, secretions from the pharyngeal glands are injected into cells, and these are thought to contain various effectors and cell wall-degrading enzymes. The feeding behaviour involves phases of cell probing, stylet penetration, salivation and ingestion. However, in spite of their economic importance, they have been less well studied than some other root endoparasitic nematodes.

As for most species, the most numerous molecular studies involving *Pratylenchus* spp. are those resolving species determination and phylogenetic relationships by the sequencing of ribosomal DNA (Al-Banna *et al*., 1997, 2004; Bert *et al*., 2008; Holterman *et al*., 2009; Subbotin *et al*., 2008). Subbotin *et al*. (2008) showed that *P. scribneri* could be a paraphyletic species. However, the topology of the relevant part of the large subunit ribosomal DNA tree could also be explained by a synonymization of *P. agilis* with *P. scribneri* (Subbotin *et al*., 2008). A similar explanation could be given for the presumed paraphyletic nature of *P. penetrans*; most probably *P. convallariae* and *P. fallax* should be synonymized with *P. penetrans* (J. Helder, Laboratory of Nematology, Wageningen University, The Netherlands, personal communication). Sequence information from ribosomal DNA has also been used to develop species-specific primers for quantitative polymerase chain reaction (qPCR) to identify *P. zeae* (Berry *et al*., 2008) and *P. thornei* (Yan *et al*., 2012) in the field. Recently, the sequencing of the complete mitochondrial genome of *P. vulnus* has shown that it is the largest mitochondrial genome among Chromadorean nematodes to date because of the abnormally lengthy noncoding regions that harbour tandemly repeated sequences (Sultana *et al*., 2013).

In order to gain an insight into the molecular basis of the host–parasite interaction, three *Pratylenchus* species have been subjected to EST analysis: *P. penetrans* (Mitreva *et al*., 2004), *P. coffeae* (Haegeman *et al*., 2011b) and *P. thornei* (Nicol *et al*., 2012). The last two EST projects were among the first to use 454 sequencing technology to investigate the transcriptomes of plant-parasitic nematodes. These projects revealed the presence of putative new parasitism genes or effectors, including genes encoding plant cell wall-degrading enzymes potentially acquired by horizontal gene transfer (Nicol *et al*., 2012) and a gene encoding chorismate mutase, previously thought to be present only in sedentary nematodes (Haegeman *et al*., 2011b). The transcriptome projects also provided sequence information on various genes which can be used for further studies, for example for RNAi knockout experiments. *Pratylenchus coffeae*, *P. thornei* and *P. zeae* have proved to be susceptible to gene silencing by applying double-stranded (ds)RNA targeting the *pat-10* and *unc-87* genes, both of which are involved in movement (Joseph *et al*., 2012; Tan *et al*., 2013). All species showed the expected phenotype, demonstrating that gene silencing by RNAi is possible in root lesion nematodes (Fig. 6), and could be an interesting future strategy for control, especially as cross-species activity of the dsRNA and a reduction in reproduction of up to 81% were observed (Tan *et al*., 2013).

In terms of management, in addition to crop rotation with a poor host, the two main aspects are tolerance and genetic resistance. Tolerance is manifested in plants that can still grow and yield well in soil with many nematodes, whereas resistance determines the level of reproduction in a plant. Host resistance to *Pratylenchus* spp. is usually only partial, and breeders aim to pyramid different resistance genes to confer multiple and durable resistance (Thompson *et al*., 2008). Molecular marker studies have identified loci linked to resistance to root lesion nematodes in wheat (i.e. Williams *et al*., 2002) and barley (Sharma *et al*., 2011), whereas resistant alfalfa plants show higher expression of the phenylpropanoid pathway (Baldridge *et al*., 1998). Transgenic alfalfa plants expressing a rice phytocystatin conferred higher resistance against *P. penetrans* (Samac and Smigocki, 2003).

Fig. 5 Patches of stunted wheat plants infected with root lesion nematodes *Pratylenchus* spp. [Photograph courtesy of Sarah Collins, Department of Agriculture and Food, Government of Western Australia (DAFWA)].

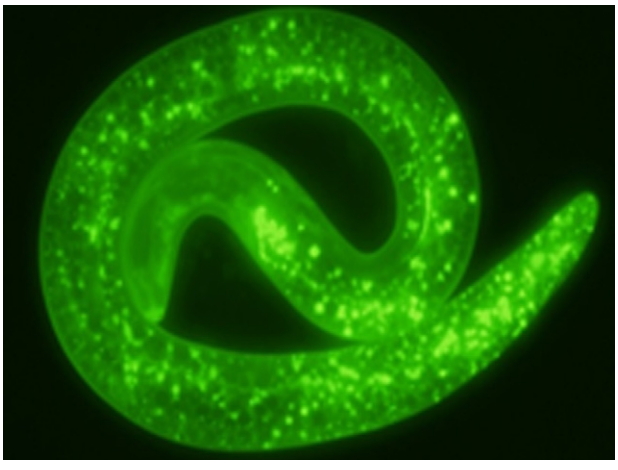

Fig. 6 Uptake of fluorescein isothiocyanate (FITC) (green) in a solution of double-stranded RNA (dsRNA) by *Pratylenchus* (Tan *et al*., 2013).

4. THE BURROWING NEMATODE *RADOPHOLUS SIMILIS*

Of the more than 30 species in the genus *Radopholus*, the burrowing nematode, *Radopholus similis*, is the only pathogen of widespread economic importance (Duncan and Moens, 2006). *Radopholus similis* is a migratory endoparasitic nematode (Fig. 7) that is known to be a destructive pest of citrus crops, pepper and, most importantly, banana, on which it causes toppling disease. The nematode causes economic problems throughout the world, most notably in warmer regions, including South America, the Caribbean, Africa, Asia and the Pacific. It has also been introduced into more temperate regions, where it may cause problems on glasshouse crops and when populations appear to have become adapted to cause disease at lower temperatures (reviewed by Haegeman *et al*., 2010). Infection by *R. similis* results in extensive damage to root systems, which show dark lesions caused as the nematodes destructively migrate through host tissues. Tissue rot occurs as a result of secondary bacterial and/or fungal infections, weakening the root system and leaving the plant susceptible to toppling, especially in windy conditions (Duncan and Moens, 2006).

All life stages of *R. similis* occur inside the roots. Nematodes infect at or near the root tip and burrow through the root system, showing a preference for younger roots rather than those that are hardened or suberized, and periodically feed on cell contents. They are found mainly in the root cortex, although they also damage the stele in banana. Females can lay eggs within the roots, but may also lay eggs in soil when the nematodes leave an infected root system as a result of overcrowding. Males are produced, but do not feed. Sexual reproduction can occur, but, in the absence of males or if the females have not mated within 50 days of the moult to adult, the females become hermaphrodites (Kaplan and Opperman, 2000). This reproductive strategy allows the rapid build-up of populations after colonization of a new host.

Molecular analysis of *R. similis* has been greatly simplified by the availability of a culture system on which the nematode can be grown in large numbers, highly unusual for a plant-parasitic nematode. This has allowed a detailed transcriptomic analysis to be undertaken (Jacob *et al*., 2008) and a genome project for this nematode is currently in progress (C. Opperman, North Carolina State University, Raleigh, NC, USA, personal communication). These analyses have allowed the identification of effectors from *R. similis*, most notably several cell wall-degrading enzymes, including cellulases (Haegeman *et al*., 2008), xylanases (Haegeman *et al*., 2009a) and pectate lyases (Maier *et al*., 2013). The importance of these enzymes for a migratory nematode is clear, and this is supported by RNAi studies which have shown that they are important for pathogenicity (Haegeman *et al*., 2009a; Zhang *et al*., 2012). Some *R. similis* orthologues of effectors from cyst nematodes have also been identified (Jacob *et al*., 2008), in line with the relatively close phylogenetic relationship between *Radopholus* and *Heterodera/Globodera* (Holterman *et al*., 2009). A detailed comparison of the effectors present in these groups offers the prospect of identifying effectors with roles specific to cyst nematodes and with roles in processes such as migration and defence suppression, likely to be common to both. Work in this area will be helped enormously by the recent development of a method that allows the extraction of mRNA from purified gland cells of *R. similis* and subsequent sequencing (Maier *et al*., 2013).

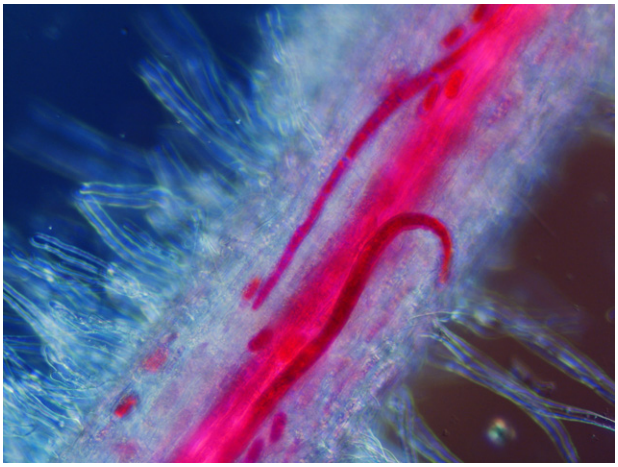

Fig. 7 Acid fuschin-stained *Radopholus similis* in roots of *Medicago sativa*.

5. *DITYLENCHUS DIPSACI*

The genus *Ditylenchus* has over 60 described species, but only a few are parasites of higher plants (Duncan and Moens, 2006). The rice-stem nematode, *Ditylenchus angustus*, causes a disease in rice, called Ufra, by feeding ectoparasitically on meristematic tissues of rice stems and leaves of deep-water and lowland rice in India and South-East Asia. The potato rot nematode, *D. destructor*, and the peanut pod nematode, *D. africanus*, are pests of potato tubers mainly in Europe and groundnuts in South Africa, respectively; both species are also fungivorous, as is *D. myceliophagus*, which is a major pest of commercial mushroom (*Agaricus bisporus*) production throughout the world. However, the best known species of the genus is *D. dipsaci*, partly because of its wide host range and partly because of its amazing ability to survive severe desiccation.

Ditylenchus dipsaci is a migratory endoparasite, commonly known as the 'stem' or 'stem and bulb' nematode, and is distributed worldwide, especially in temperate regions. It is on the list of quarantine organisms of many countries. Although *D. dipsaci* primarily infects onion and garlic, it has a wide host range of about 450 plant species, including peas, celery, strawberries, beetroot, vegetable marrow, pumpkin, rhubarb, ornamental bulbs (hyacinth, narcissus and tulip), oats, rye and some weeds, and is economically detrimental because infected crops are unmarketable. More than 30 physiological races of the nematode are known, some being host specific and others polyphagous; many races are named after a major host crop (i.e. the 'oat race'). The races of *D. dipsaci* probably represent a species complex and the 'giant race' multiplying on Fabaceae has recently been described as *D. gigas* (Vovlas *et al*., 2011).

All stages of *D. dipsaci* are infective and enter plants through stoma or wounds, releasing enzymes that soften cell walls and facilitate feeding on the parenchymatous cells of the cortex. The feeding nematode withdraws cell contents through its stylet and surrounding cells begin to divide and enlarge, resulting in the malformation of plant tissue. Reproduction is by amphimixis, and population growth can be very rapid; a female *D. dipsaci* lays approximately 250 eggs during a season, and six generations may develop under optimum conditions when the temperature is in the range 15–20 °C. J2s hatch within 2 days and, within 4–5 days, they have developed into females, which live for more than 10 weeks (Duncan and Moens, 2006). The rapid population growth can result in severe crop damage, even when the initial population density is low. As the population of nematodes increases, symptoms, including stunted growth, swollen and twisted stems, and discoloration of bulbs, become visible, and secondary pathogens, such as bacteria and fungi, can enter.

Ditylenchus dipsaci and *D. gigas* are classic examples of nematodes able to survive severe desiccation in a dormant state. In infected narcissus bulbs set out to dry or infected senescing field beans, development stops at the fourth-stage juvenile (J4), and hundreds of desiccated J4s coil and clump together in masses termed 'eelworm wool', usually around the basal scales of bulbs (*D. dipsaci*—Fig. 8) or inside bean pods (*D. gigas*), but also in dried plant debris in the field, where they can overwinter. Research on the mechanisms of survival has centred on *D. dipsaci* (Perry and Moens, 2011a, b). Coiling and clumping aid desiccation survival, but the intrinsic property of the cuticle of J4, involving an outer lipid layer, is also involved in controlling the rate of water loss. Control of the rate of drying is the first phase in the drying process; successful entry into long-term anhydrobiosis depends on subsequent biochemical and molecular adaptations that are part of the armoury of species such as *D. dipsaci*, which have been termed 'innate dehydration strategists' (Perry and Moens, 2011b). J4s have been recorded as surviving for more than 20 years in the dry state, and as dry individuals they can withstand other adverse conditions, such as extremes of temperature and nematicides. This capacity for desiccation survival and freeze tolerance facilitates dispersal in seeds and bulbs, plant debris or contaminated equipment, and also by wind.

The management of *D. dipsaci*-induced disease is achieved through the use of clean planting material (including seed sanitation), heat treatment, crop rotation and fumigation of fields; cleaning of agricultural equipment is also important. The use of fumigants and nematicides is increasingly problematic because of environmental concerns, and is anyway usually uneconomical for the management of *D. dipsaci* in most crops, although they can be used in nurseries to reduce infected planting material. Seed and bulb disinfestation by hot water or formaldehyde treatment is also practised. 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 (Duncan and Moens, 2006). The success of rotation depends on the host range of the race of *D. dipsaci*, the availability of suitable nonhost crops and effective weed control. Sources with good resistance to several of the races are available, with commercial resistant cultivars for some crops, such as lucerne, clover, oat, garlic, strawberry and sweet potato.

Few studies on the molecular basis of the interactions between *Ditylenchus* spp. and host plants have been published to date. Cellulases have been identified from *D. destructor* (Huan *et al*., 2009) and a small-scale transcriptomic analysis of *Ditylenchus africanus* (Haegeman *et al*., 2009b) has identified candidate effectors, including expansins, as well as novel secreted proteins expressed in the pharyngeal gland cells. A detailed comparison of the domain structure of the cellulases from *Ditylenchus* and other plant-parasitic nematodes has allowed a model for the evolution of these genes within a part of the phylum Nematoda (the Tylenchida) to be proposed (Kyndt *et al*., 2008). In addition, genes encoding proteins that may be important in the survival ability of this nematode have been identified, including late embryogenesis abundant (LEA) proteins and proteins involved in the synthesis of trehalose.

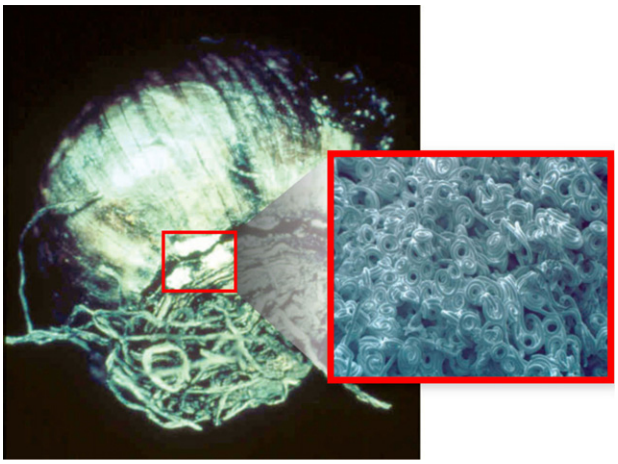

Fig. 8 Narcissus bulb with accumulation of *Ditylenchus dipsaci* fourth-stage juveniles as dry eelworm wool, and an inset showing a transmission electron microscope image of the eelworm wool with coiled, clumped individuals. (Photographs courtesy of Roland N. Perry, Rothamsted Research, Harpenden, UK; Perry and Moens, 2011b.)

6. THE PINE WILT NEMATODE *BURSAPHELENCHUS XYLOPHILUS*

The pine wilt nematode, *Bursaphelenchus xylophilus*, is a migratory endoparasitic nematode. This nematode is native to North America and here it causes little damage to native trees, although non-native species are profoundly affected. However, it was introduced into East Asia (Japan, China, Korea) at the start of the 20th century, presumably as a result of increasing trade and transport. Here, where trees have not evolved resistance or tolerance to *B. xylophilus*, it causes enormous damage. Financial losses are significant, with losses of 2×10^6 m^3 of timber under some environmental conditions (Nose and Shiraishi, 2007), but the nematode can also cause devastation on an ecosystem scale, as all infected trees in an area can die when conditions are suitable for the disease (Fig. 9). In spite of quarantine efforts, *B. xylophilus* has recently been introduced into Portugal (Mota *et al*., 1999) and has now also spread to Spain (Robertson *et al*., 2011). The prevention of the spread of *B. xylophilus* is particularly difficult as the nematode is vectored to new hosts by the adult (flying) stage of *Monochamus* beetles. The biology of *B. xylophilus* has been reviewed in detail recently (Jones *et al*., 2008) and the life cycle is summarized in Fig. 10. Using the terminology of Van Megen *et al*. (2009), *B. xylophilus* is a member of the Parasitaphelenchidae and belongs to clade 10, whereas most other major plant parasites belong to clade 12. *Bursaphelenchus xylophilus* may therefore represent an independent and recently evolved plant parasite, making it an attractive model for comparative studies.

There are over 70 species described within the *Bursaphelenchus* genus and most, including the most closely related species *B. mucronatus*, are solely fungal feeders. These nematodes feed on fungi colonizing dead or dying trees and are vectored to such trees during oviposition by adult beetles. By contrast, *B. xylophilus* is also vectored to living trees during maturity feeding of the beetles and can invade the live trees before migrating through host tissues feeding and multiplying. Once the host is killed, the nematodes gain access to a new supply of fungus as the dead plant becomes decayed.

The life cycle of *B. xylophilus*, which includes both fungal feeding and plant feeding stages, means that it can be cultured on fungal mats in the laboratory, and this has facilitated molecular studies, including a genome sequence (Kikuchi *et al*., 2011). The genome was found to contain about 18 000 genes, a similar number to *C. elegans*, but included expanded numbers of digestive and detoxification genes. This expansion may reflect the diversity of foods that it exploits (plant tissues and fungi) and the range of environments that the nematode encounters during its life cycle (resin canals of its host trees, where it is exposed to a cocktail of secondary metabolites, and the tracheae of insect vectors). Genes involved in a range of crucial biological processes have also been identified in the genome, many of which, such as neuropeptides, GPCRs and developmental genes, could be viable control targets.

Bursaphelenchus xylophilus possesses a unique complement of plant cell wall-modifying proteins. One of the most intriguing findings is the presence of glycoside hydrolase family (GHF) 45 cellulases in *B. xylophilus*, which appear to have been acquired by horizontal gene transfer from fungi (Kikuchi *et al*., 2004). This is remarkable given that the (GHF5) cellulases present in other plant nematodes have been acquired from bacteria, and provides the strongest evidence supporting the idea that multiple independent horizontal gene transfer events have punctuated the evolution of plant parasitism by nematodes (Haegeman *et al*., 2011a). The unique complement of genes involved in cellulose degradation and other catabolic enzymes, and the absence of effectors similar to those known to function at the host–parasite interface in other nematodes, suggest that *B. xylophilus* has a mode of parasitism that is distinct from that of other plant-parasitic nematodes. The availability of a genome sequence for *B. xylophilus*, coupled to the relative ease with which this nematode can be cultured, mean that it presents an excellent opportunity for the future study of tree parasitism and for the analysis of tree responses to a nonbiotrophic pathogen, and the suppression of such responses.

Fig. 9 Damage to pine trees caused by *Bursaphelenchus xylophilus*. (Photograph courtesy of Takuya Aikawa, Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan.)

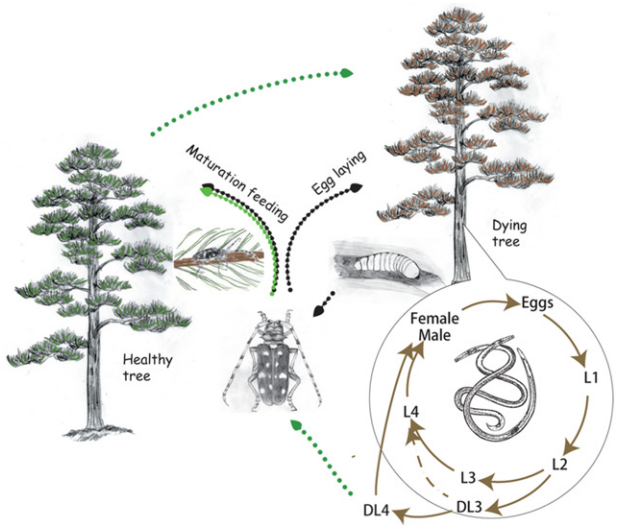

Fig. 10 Diagram summarizing the life cycle of the pine wilt nematode *Bursaphelenchus xylophilus*.

7. THE RENIFORM NEMATODE *ROTYLENCHULUS RENIFORMIS*

The reniform nematode, *Rotylenchulus reniformis*, is a sedentary semi-endoparasite of a large number of annual and perennial plant species. Of the nine other species of *Rotylenchulus*, only *R. parvus* is a significant pathogen, whereas the other eight have a limited distribution and are of minor economic importance (Gaur and Perry, 1991a; Robinson *et al*., 1997). Variability has been observed in the biology of *R. renifomis*. Dasgupta and Seshadri (1971) reported the occurrence of two races (Race-A and Race-B) in India. Most subsequent surveys have shown the presence of the amphimictic Race-A, which can reproduce on cowpea, cotton and castor. Recently, genetic variability has been demonstrated in different *R. reniformis* populations using microsatellite markers (Arias *et al*., 2009; Leach *et al*., 2012), suggesting that the parthenogenetic populations of *R. reniformis* could be another species.

Crop damage caused by *R. reniformis* is widespread in tropical and subtropical regions and it is a major pest in the southern USA. The wide host range involves more than 350 plant species, including many economically important vegetables, fruits, ornamentals and fibre crops, as well as weeds that support nematode populations in the absence of host crops (Gaur *et al*., 2011; Robinson *et al*., 1997). Very few commercially acceptable crop varieties with resistance to reniform nematodes have been reported, except for the soybean varieties Peking, Dyer, Custer and Pickett, which display a hypersensitive response on nematode infection. Resistance in *Gossypium* cotton appears to be controlled by two or more pairs of genes (McCarty *et al*., 2012). Efforts are being made to use molecular markers to aid the breeding of resistance genes. Pyramiding of resistance genes may also provide more durable control of reniform nematodes; it has been shown that combining *ren-1* and *ren-2* in cotton imparts greater resistance than either gene alone (Fang and Stetina, 2011).

The above-ground symptoms of *R. reniformis* infection, like those of other nematodes, resemble those of moisture and nutrient deficiencies. Although root growth is reduced with limited secondary root development, few or no symptoms are seen on the roots. However, many gelatinous egg sacs may be seen on the root surface. Root necrosis is seen in some plants, such as pineapple and banana. Infected plants may be severely stunted and chlorotic. Crop loss depends on the population density, temperature and crop growth conditions. Usually, one to two females per cubic centimetre of soil can cause significant yield reduction. Losses of 40%–60% have been reported. Disease complexes with opportunistic root pathogens, such as *Fusarium solani*, *Verticillium* spp., *Rhizoctonia solani*, etc., result in root rots and wilts, causing further damage.

The reniform nematodes have a unique life cycle. The J2s hatch from 8–10-day-old eggs and undergo three successive moults without feeding, and develop into vermiform adult males or females within 7–9 days. These adult stages are smaller in size than the J2s. The cuticles of the previous stages are retained and have been shown to reduce the rate of water loss, and thus improve the chances of anhydrobiotic survival in drying soil (Gaur and Perry, 1991b). When the nematodes move in moist soil, the friction with soil particles assists in the shedding of the cuticular sheaths. The young females are the infective stage. They penetrate the roots of plants, inserting about one-third of the anterior body, and establish feeding sites on endodermal and pericycle cells, similar to the syncytia of cyst nematodes, to which *Rotylenchulus* are closely related (Van Megen *et al*., 2009). Cells within the syncytia undergo hypertrophy and show increased cytoplasmic density and nuclei with enlarged nucleoli. As in other biotrophic nematodes, a feeding tube is produced through which all ingested materials must pass. Controlled breakdown of cell walls results in the formation of a syncytium which may be two cells deep. After feeding for 2–3 days, the posterior portion of the female starts to swell near the vulval region and, in about 1 week, the body of the female outside the root assumes a bean or kidney shape (Fig. 11—hence the species name). The uterine glands produce a gelatinous matrix into which 40–100 eggs are laid within 7–9 days under optimal weather conditions. Males are numerous in most populations; they do not feed and remain in the soil, but are attracted to gravid females and many are found entrapped in the gelatinous matrix. In some populations, males are rare or absent, and these populations may have parthenogenetic reproduction.

Few details of the molecular basis of the interaction between *R. reniformis* and host plants have been described. A small-scale EST project has shown that some effectors similar to those of cyst nematodes are present, including cellulases and orthologues of *H. glycines* effectors of unknown function (Wubben *et al*., 2010). Intriguingly, some genes are present in *R. reniformis*, which may have been acquired by horizontal gene transfer, which have not been reported from other plant-parasitic nematodes. These include an enzyme involved in thiamine biosynthesis and an isochorismate hydrolase. The latter may be significant as salicylic acid can be synthesized via isochorismate (Wildermuth *et al*., 2001); the ability to degrade this compound may therefore be a strategy used by *R. reniformis* to suppress host defences.

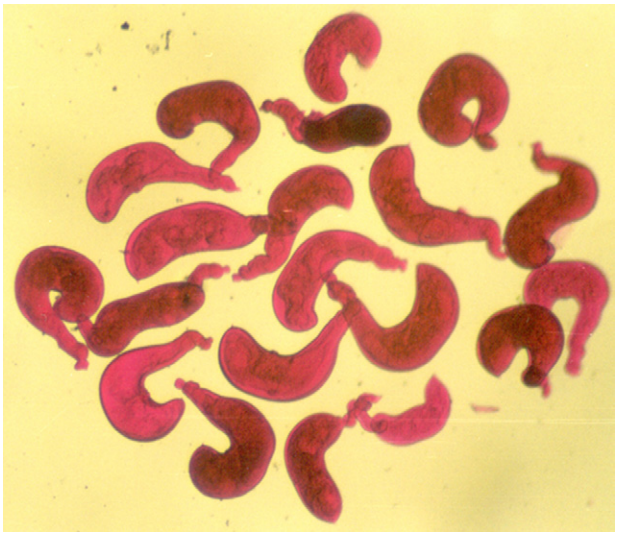

Fig. 11 Acid fuschin-stained adult females of *Rotylenchulus reniformis*, showing the characteristic body shape for which the species is named.

8. *XIPHINEMA INDEX*

Xiphinema index is a significant parasite of grapevine and has a worldwide distribution (Taylor and Brown, 1997). This nematode is significantly larger than most other plant-parasitic nematodes (up to 3 mm in length). Although all stages are biotrophic, and some induce complex multinucleate feeding structures in the roots of the host, it is part of the family Longidoridae within Clade 2 of the phylum Nematoda (Van Megen *et al*., 2009), and is entirely unrelated to other biotrophic species (root-knot and cyst nematodes; Clade 12). *Xiphinema index* reproduces by meiotic parthenogenesis, but sexual reproduction has been observed on the rare occasions when males are produced (Villate *et al*., 2010). The most economically important hosts are grapevine and fig, but it has been found to be associated with other plants, feeding ectoparasitically on root tips (Nicol *et al*., 1999). Feeding activity retards root extension, causing swelling and gall formation, reducing the growth of infected plants (Fig. 12A). Cells within the gall tissue are enlarged and multinucleate (Vovlas *et al*., 1978) (Fig. 12B). However, the most significant impact of *X. index* is a result of its activity as the vector of *Grapevine fanleaf virus* (GFLV) (Hewitt *et al*., 1958), one of the most important viruses of grapevine (Jelly *et al*., 2012). Virus particles are attached to the cuticular lining and lumen of the odontophore and the pharynx. The virus can be retained for up to 4 years (Demangeat *et al*., 2005), although it is not retained when the nematode moults or by nematodes in eggs.

There are few studies on the molecular basis of parasitism by *X. index*. Species-specific primers have been developed in order to distinguish it from other closely related species (*X. diversicaudatun*, *X. vuittenezi* and *X. italiae*) (Wang *et al*., 2003), making species identification easier for nonexpert nematologists. Molecular diversity and phylogeography between populations of *X. index* have been shown to be very low using ribosomal and mitochondrial genes, with a distribution matching the history of grapevine cultivation (Demangeat *et al*., 2010; Gutiérrez-Gutiérrez *et al*., 2011). By contrast, much more is known about the biology of GFLV, including an understanding of the interaction with the host, vector attachment and evolution (Mekuria *et al*., 2009; Schellenberger *et al*., 2011).

The feeding structures induced by *X. index* have some structural parallels with those induced by root-knot nematodes and, in both cases, are produced as a result of repeated rounds of nuclear division without cytokinesis (Griffiths *et al*., 1982). Given the huge evolutionary separation between *X. index* and root-knot nematodes, any similarities in the mechanisms by which these structures are induced are most probably a result of convergent evolution. The only molecular comparisons of the changes in gene expression in galls induced by *X. index* and in root-knot nematode giant cells used promoter-tagged lines of *A. thaliana*; this analysis showed that β-glucuronidase (GUS) expression was up-regulated in seven of eight promoter trap lines that showed GUS activation in root-knot nematode giant cells (Barthels *et al*., 1997). Given the relative ease with which it is possible to extract galls from plants infected by *X. index*, and the availability of extensive microarray datasets that have identified changes in gene expression in root-knot nematode giant cells (i.e. Barcala *et al*., 2010), a microarray analysis of the changes in gene expression induced by *X. index* would allow an interesting comparative analysis. On the nematode side, little is known about effectors from *X. index*. A small-scale EST project has been undertaken which included sequences of 1400 genes from dissected gland cells (Furlanetto *et al*., 2005). However, no further analysis of these sequences has been reported.

Opportunities for future research on *X. index* include the discovery of new resistance sources, exploiting the sequences of two grape genomes (Jaillon *et al*., 2007; Velasco *et al*., 2007). In addition, further genomic analysis of *X. index* will be useful from both a functional and phylogenetic perspective and, given the fact that *X. index* is entirely unrelated to any of the other plant-parasitic nematodes described here, will provide information about the basic requirements for plant parasitism by nematodes.

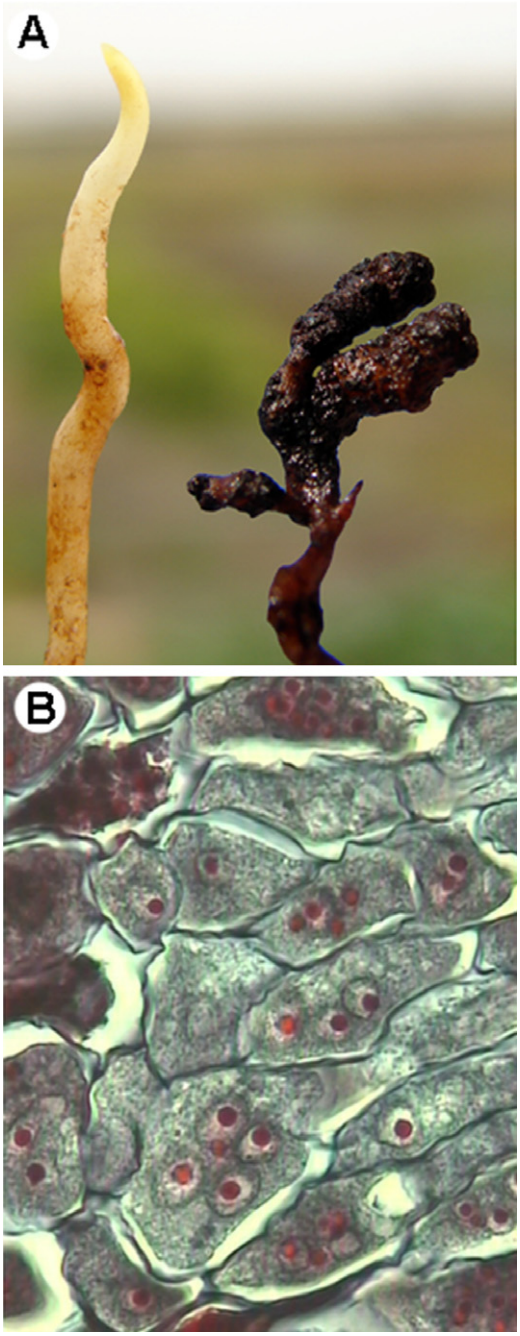

Fig. 12 Parasitism of *Xiphinema index* in roots of *Vitis* spp. (A) Typical root symptoms showing swellings of root tips in comparison with an uninfected root. (B) Multinucleate cells induced by *X. index* in *Vitis* rootstock Paulsen 1103 (*V. berlandieri* × *V. rupestris*). (Photographs courtesy of Dr P. Castillo and Dr N. Volvas, Institute for Sustainable Agriculture-CSIC, Córdoba, Spain, and Istituto per la Protezione delle Piante, Bari, Italy.)

9. *NACOBBUS ABERRANS*

The genus *Nacobbus*, the false root-knot nematode, is endemic to Argentina, Bolivia, Chile, Ecuador, México, Peru and the USA. The parasitic habit is complex (Manzanilla-López *et al*., 2002), with migratory, vermiform juveniles and immature adults moving through the root and feeding on cells, causing cavities and lesions inside the root tissues, similar to those caused by lesion nematodes, *Pratylenchus* spp.; the juveniles can also repeatedly leave and re-enter the root, causing additional damage. By contrast, the mature females are sedentary and induce partial dissolution of the cell walls and the fusion of cell protoplasts, resulting in specialized feeding sites, called syncytia, which are similar to those caused by cyst nematodes (Jones and Payne, 1977). The syncytium can grow to 8 mm and disrupts the stele (Manzanilla-López *et al*., 2002). Remarkably, the ability to induce these structures appears to have evolved independently in *Nacobbus* and in the cyst nematodes (Holterman *et al*., 2009). Root galls (Fig. 13) are formed around the feeding sites. Adult males may be found in the roots or the soil and several males may surround galls containing unfertilized females. Most eggs are laid into an egg sac, similar to those produced by *Meloidogyne* spp. The mode of reproduction of *Nacobbus* remains unclear, with a degree of uncertainty as to whether the nematode is obligately amphimictic or whether facultative parthenogenesis is possible.

Nacobbus aberrans sensu lato (EPPO, 2009) is polyphagous with 18 botanical families and 84 species reported as hosts, including tomato, potato, beans, chilli pepper and sugarbeet. Yield losses reported for *N. aberrans* average 65% for potato in Andean Latin America, 55% and 36% for tomato and bean, respectively, in Mexico and 10–20% for sugarbeet in the USA, but, for most crops, loss estimates are lacking (Manzanilla-López *et al*., 2002). Under field conditions, *N. aberrans* occurs together with other plant-parasitic nematodes and soil-borne pathogens. The interaction of *Phytophthora capsici* with *Nacobbus* can cause the breakdown of resistance in chilli (*Capsicum annuum* L.) against *Phytophthora infestans* (Hernández Anguiano *et al*., 1992; López-Martínez *et al*., 2011). As with other plant-parasitic nematodes, indirect costs, such as the establishment of control measures, degradation of land quality, cost of quarantine procedures and loss of nematode-free land for seed production, add to the direct losses caused by the impact on yield (Main *et al*., 1999).

Diagnostic criteria for *Nacobbus* species are mainly based on adult morphology, especially that of the vermiform immature female, and host range and biology. The level of morphological and morphometric variation within *N. aberrans sensu* Sher (1970) has been attributed to sibling or cryptic species (Manzanilla-López, 2010). Molecular studies have suggested that *N. aberrans sensu* Reid *et al*. (2003) is the major species, whereas *N. bolivianus* (regarded as a synonym of *N. aberrans* by some authors) is apparently restricted to Bolivia (Castillo *et al*., 2012). Subsequent studies (Anthoine and Mugniéry, 2005; Lax *et al*., 2007; Vovlas *et al*., 2007) have provided molecular evidence to support the existence of at least two major clades within South American *Nacobbus*. However, a consensus has yet to be achieved on the applicability of molecular diagnostics at species level, and hence most populations and quarantine interceptions will probably still be labelled as *N. aberrans sensu* Sher, despite mounting evidence to the contrary (Castillo *et al*., 2012). Within the assemblage known as *N. aberrans s.l*., three groups have been proposed based on host range: sugar beet, potato and bean (Manzanilla-López *et al*., 2002). These groups (also known as races) have been simplified, but are still used for quarantine purposes. The *Nacobbus aberrans s.l*. potato group deserves special attention as it is considered to be one of the most important plant pathogens in South America, and at least 40 countries have implemented quarantine measures to prevent its introduction (Manzanilla-López *et al*., 2002).

Nacobbus aberrans s.l. is adapted to a wide range of climatic conditions, the life cycle being strongly influenced by temperature. Quiescence and diapause in unhatched juveniles and other stages have been suggested to occur, with the J3s and J4s being considered as survival stages in soil. The life cycle is similar on all of its nontuber-forming hosts, but, on potato, it overwinters in the lenticels of stored crops or wild tubers in the ground (Costilla, 1985). The overwintering stages are juveniles, vermiform immature females and males, with mature females rarely found in lenticels. The life cycle resumes in the following spring on either planted crops or sprouting wild tubers. Infected seed tubers are an important means for dissemination (Manzanilla-López *et al*., 2002). As for other plant-parasitic nematodes, control methods and management strategies include the prevention of the introduction and dispersal of infected tubers, clean seedlings, crop rotation, resistant/tolerant varieties, trap crops, burning of stubble, weeding, cleaning agricultural tools and machinery, and chemical and biological control. Molecular tools can detect the nematode in soil and samples of tuber skin (Atkins *et al*., 2005). This method uses DNA extracted from soil or from tuber skin peels with primers targeting the internal transcribed spacer region of the ribosomal DNA. Accurate identification is vital, and the increasing number of deposited DNA sequences for *N. aberrans s.l*. will facilitate the design of specific primers for routine identification of these species/groups.

Fig. 13 *Nacobbus aberrans s.l*. on tomato, illustrating the origin of the common name of this nematode (the false root-knot nematode). Although the symptoms of *N. aberrans* infection resemble those of root-knot nematodes, biotrophic parasitism has evolved independently in *Nacobbus*. (Photograph courtesy of Carmen Triviño, INIAP, Ecuador.)

10. *APHELENCHOIDES BESSEYI*

Aphelenchoides besseyi is an important and widespread pathogen that causes 'white tip' disease on rice (Fig. 14). It makes a significant contribution to the estimated $US16 billion worth of damage caused by nematodes to rice crops (Lilley *et al*., 2011). The disease is seed borne, and infected plants are reduced in size. Symptoms of *A. besseyi* include the production of chlorotic tips on new leaves, which may subsequently necrotize. The flag leaf enclosing the panicle may also be distorted, leading to a reduction in the number and size of grains produced. Although rice is the most important host worldwide, this foliar nematode species can also feed on numerous other mono- and dicotyledonous plants (i.e. Hockland, 2004).

The extent of the interactions between plants and members of the genus *Aphelenchoides* is varied. *Aphelenchoides besseyi* and the strawberry and chrysanthemum foliar nematodes (*A. fragariae* and *A. ritzemabosi*, respectively) are predominantly plant parasites. These foliar nematodes feed as ecto- and endoparasites on above-ground plant parts, but can also feed on fungi. These nematodes may benefit from their ability to feed on fungi to survive in the absence of a plant host. Foliar nematodes are only active in above-ground plant parts under moist conditions. Certain life stages of most species can survive dehydration. In the case of *A. besseyi*, both larval and adult life stages are desiccation tolerant, whereas, for *A. ritzemabosi*, it is mainly the later developmental stages that can survive. It is notable that the great majority of the *Aphlenchoides* species are fungivorous, and molecular phylogenetic insight suggests that plant parasitism is a relatively recent evolutionary adaptation (Rybarczyk-Mydłowska *et al*., 2012b—Fig. 1). To date, little molecular information is available for any *Aphelenchoides* species. However, the isolated phylogenetic position of these facultative plant-parasitic nematodes (the only closely related nematode which is a plant parasite is *B. xylophilus*), coupled with their primitive mode of parasitism, means that they are potentially interesting organisms in terms of the evolution of plant parasitism. Primitive in this context implies the absence of an intimate interaction between the host plant and this nematode. Unlike many other nematode species, foliar nematodes do not induce re-differentiation of plant cells. Histological observations by Strumpel (1967) in epidermal cells and palisade parenchyma showed local cell damage, tissue disintegration and browning. With this in mind, a small-scale transcriptome project for *A. besseyi* is currently underway (Kikuchi *et al*., in press).

Fig. 14 Symptoms of *Aphelenchoides besseyi* on rice. (Photograph courtesy of Donald Groth, Louisiana State University AgCenter, Bugwood.org.)

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