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-Omics fields of study related to plant-parasitic nematodes

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ABSTRACT

Plant-parasitic nematodes (PPN) cause significant losses and these pathogens must be addressed amid the growing demand for food, global warming, and the discarded use of inorganic pesticides. For these reasons, acquiring deeper knowledge about PPN and devising new management strategies are important in order to meet future food demand. This review focuses on PPN and their applicable and diverse –omics fields of study. While most efforts have been centered on transcriptomics, other –omics studies have recently begun to expand. The few genomes sequenced (Meloidogyne incognita, M. hapla, and Bursaphelenchus xylophilus) have shown high diversity in PPN. This review also discusses the future prospects and uses of –omics relative to PPN.

Keywords: Plant-parasitic nematodes; Disease; Control; Management; -Omics .

Abbreviations:

PPN, Plant-Parasitic Nematodes; **PCN**, Potato Cyst Nematodes; **NGS**, Next Generation Sequencing; **RNAi**, RNA interference; **dsRNA**, Double-stranded RNA; **EST**, Expressed Sequenced Tag; **HGT**, Horizontal Gene Transfer.

1. Importance of plant-parasitic nematodes in agriculture

Plant-parasitic nematodes (PPN) cause an estimated crop yield loss of 14.6% in tropical and sub-tropical climates and losses of 8.8% in developing countries (1). These losses are estimated to exceed 100 billion US dollars (2; 3). Thus, PPN have a significant impact on food security and our ability to feed a growing human population (35% increase by 2050, 4) in the coming years. Other estimates project a 75% increase in food demand between 2010 and 2050, including changes in diet (toward consuming more protein) and steady population growth (5).

Plant-parasitic nematodes are considered the "unseen enemies" of plants because the symptoms seen in the aerial parts of plants are generally associated with forms of abiotic stress (e.g., lack of nitrogen, water stress). Diseased plants are usually found in patched patterns in the field (Fig. 1). Aside from the lack of specific symptoms, it is difficult to detect PPN, which are small, soil dwelling organisms. Nematodes can affect crops by directly feeding on plants through the stylet (a protrusible, syringe-needle-like structure), disrupting plant physiology through the growth of plant-specific structures, enabling secondary infection by opportunistic pathogens (bacteria and fungi) or, in some cases, transmitting plant viruses. The damage caused by PPN largely depends on the type of crop, its stage of development, and edaphic/climatic conditions. Table 1 lists the specific damage caused to crops in detail. Some genera are of major importance as plant parasites (e.g. *Meloidogyne*, *Globodera*, *Heterodera*, *Pratylenchus*, *Ditylenchus*, *Aphelenchoides*, *Bursaphelenchus*, *Xiphinema*, *Trichodorus*), while others are of minor importance (given their limited crop damage or low numbers in the soil). Some PPN are highly polyphagous (e.g.. *Meloidogyne* spp., *Pratylenchus* spp.) and can infect many species of plants, while others are species or genus specific (e.g. *Globodera* spp., *Heterodera* spp.).

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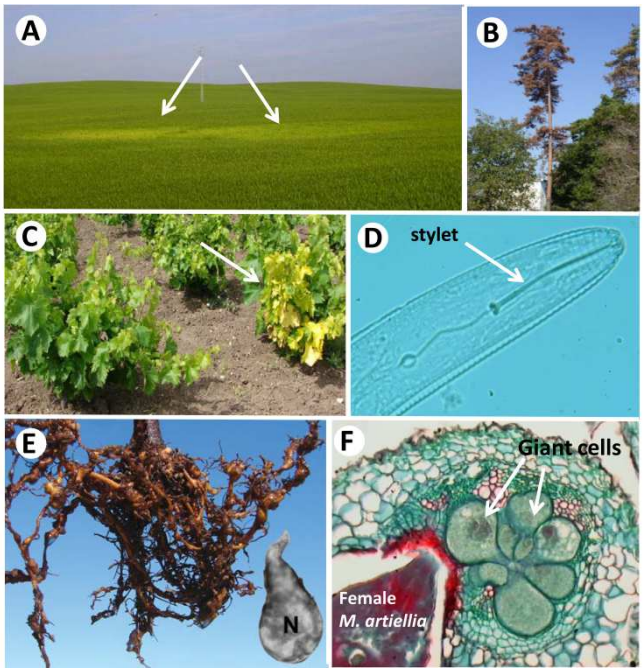

Fig. 1. Plant-parasitic nematodes and their damage to plants. A) Patchy distribution of symptomatic plants (yellow and reduced growth) in a wheat field caused by Heterodera avenae; B) pine tree death by Bursaphelenchus xylophilus; C) Grapevine Fan Leaf virus in grapevine transmited by Xiphinema index; D) Anterior part of Hemicycliophora sp. showing the stylet; E) Grapevine rootstock (41 B Millardet et De Grasset) infected by the root-knot nematodes Meloidogyne incognita race 1, in detail: a female extracted from roots; F) Cross section of M. artiellia chickpea infected root showing the typical feeding site with giant cells.

Plant-parasitic nematodes can be separated based on their life strategy into several groups:

i) ectoparasites: nematodes remaining in the soil and not entering plant tissue; ii) endoparasites: nematodes that fully penetrate root tissue; and iii) semi-endoparasites: nematodes that penetrate roots with only their anterior part, with their posterior part remaining in the soil phase. This division becomes more complicated with the presence of migratory or sedentary life cycles. Nematodes also serve important roles in the flow of energy in the soil and are important in other ecological niches (such as bacterial and fungal feeders, predatory and omnivorous). Some PPN, primarily the sedentary endoparasites (*Meloidogyne* spp., *Heterodera* spp., *Globodera* spp.), have a complicated relationship with their host. They induce the re-differentiation or fusion of root cells into "specialized feeding sites" (Fig 1) from which they feed continuously. Feeding sites are thought to be produced as a result of the action of proteins and/or small molecules (*e.g.* hormones) secreted into the host via the stylet. Other secretions from the nematodes (via cuticles, amphids and rectal glands) may also play an important role in plant parasitism. The current trend toward discontinuing the use of inorganic pesticides due to environmental and human health concerns, and the effects of climate change which may increase the geographical range of some nematodes, are predicted to increase the damage caused by PPN (1). For these reasons, greater and more detailed knowledge about the causal agents of disease and the life cycle (e.g., development, survival, interaction with host, virulence, pest resistance) of PPN are essential for devising new management strategies. Omics studies will offer new ways of obtaining the knowledge necessary for achieving these objectives.

2. –Omics fields of study related to plant-parasitic nematodes

Plant-parasitic nematodes are intimately associated with their hosts and are highly adapted to this lifestyle. Studies conducted on the model nematode (*Cænorhabditis elegans*) have contributed enormously towards advancing our understanding of the basic biology of PPN. However, studies of PPN are far more difficult than those on *C. elegans* as several features of their life cycle make them extremely difficult to work with. These include: i) different specific development stages that exist only inside the roots (endoparasitic nematodes); ii) difficulty to culture the nematodes as most PPN are biotrophic organisms; and iii) high diversity in some species. Moreover, many PPN studies are dictated by the importance of certain groups of nematodes over the rest. Studies tend to be focused on the most damaging PPN (i.e., sedentary endoparasites *Meloidogyne* spp., *Globodera* spp., *Heterodera* spp.). However, studies on other nematode groups will expand as more genomes and sets of genes are deposited in public databases.

2.1. *Genomics*

Whole genome sequencing yields access to all the genes, avoiding the difficulty of finding genes expressed at low levels by using other strategies (such as ESTs). However, the gene catalogue from whole genome sequencing may still be incomplete, as some regions remain difficult to sequence and certain transcripts could be difficult to predict based on the genomic sequence alone (6). Second generation of sequencing technologies collectively known as "Next Generation Sequencing" (NGS) has emerged in recent years including: 454 sequencing (Roche, Branford, CT, USA), Illumina sequencing (Illumina Inc., San Diego, CA, USA), and sequencing oligonucleotide ligation and detection (SOLiD) (Applied Biosystems, Carlsbad, CA, USA) (7). These new technologies offer advantages over Sanger sequencing in terms of i) reduced cost per DNA base sequenced; ii) speed at which large volumes of data are generated; iii) an ability to work with less starting material, and iv) no need to go through a cloning vector or host organism (7). However, challenges remain when using NGS including issues with the assembly process and data storage. In addition, specific problems are associated with some technologies, such as the unreliable determination of homopolymer regions and large repeats in 454 sequencing technology (6). Nevertheless, several programs are available for the assembly process and data management (see reviews by 6 and 7).

Table 1. International survey of crop losses due to nematodes based on 371 responses (Source: 1).

Life-sustaining Crops	Annual Loss (%)	Economically-important Crops	Annual Loss (%)
Banana	19.7	Cacao	10.5
Barley	6.3	Citrus	4.2
Cassava	8.4	Coffee	15.0
Chickpea	13.7	Cotton	10.7
Coconut	17.1	Cowpea	15.1
Corn	10.2	Eggplant	16.9
Field bean	10.9	Forages	8.2
Millet	11.8	Grape	12.5
Oat	4.2	Guava	10.8
Peanut	12.0	Melons	13.8
Pigeon pea	13.2	Misc. other	17.3
Potato	12.2	Okra	20.4
Rice	10.0	Ornamentals	11.1
Rye	3.3	Papaya	15.1
Sorghum	6.9	Pepper	12.2
Soybean	10.6	Pineapple	14.9
Sugar beet	10.9	Tea	8.2
Sugar cane	15.3	Tobacco	14.7
Sweet potato	10.2	Tomato	20.6
Wheat	7.0	Yam	17.7
Average	10.7%	**Average**	14.0%
	Overall Average 12.3%		

Caenorhabditis elegans was the first genome of a multicellular organism to be completely sequenced, thereby providing a platform for further nematode genomics (*C. elegans* Sequencing Consortium, 1998). Nematode genomes analysed to date range from 15 megabases (*Pratylenchus* spp.) up to 0.5 gigabase (in some estimates of the *Ascaris suum* genome), and were organized from as few as one chromosome (*Parascaris univalens*) to many tens (in certain *Meloidogyne* spp.) (6). Representative species across the diversity of the phylum Nematoda is very important for understanding the molecular and ecological aspects of nematodes. To date, three species of PPN including two from the genus *Meloidogyne* (sedentary endoparasites), *M. incognita* (8), *M. hapla* (9), and *Bursaphelenchus xylophilus* (migratory endoparasites) (10) have been sequenced and published. Further plant nematode genome sequencing programmes are in progress including, *Bursaphelenchus mucronatus*, *Ditylenchus destructor*, *Globodera pallida*, *Globodera rostochiensis*, *Meloidogyne arenaria*, *Heterodera glycines*, *Meloidogyne floridensis*, and *Meloidogyne javanica* (www.nematodes.or). Information on with the progress of some of these sequencing projects is now available on various websites. However, other important PPN not related to the order Rhabditida are not yet being sequenced. These include nematodes in the orders Enoplea (i.e., genera *Xiphinema*, *Longidorus*, *Paralongidorus*) and Triplonchida (i.e., genera *Trichodorus*, *Paratrichodorus*).

The genome sequences obtained to date have showed important differences between them, even in species from the same genus (e.g., *M. incognita*, *M. hapla*). These differences may reflect the important biological differences that exist between these species. *Meloidogyne incognita* is a parthenogenetic and polyphagous species, while *M. hapla* is capable of sexual reproduction and has a narrower host range (11). Many isolates of *M. incognita* are subject to extensive polyploidy and/or aneuploidy, while *M. hapla* has a meiotic reproduction lifestyle, which helps make controlled crossings possible (11). The main differences between both genomes are the very small size of *M. hapla*; in contrast, the *M. incognita* genome has shown the genetic consequences of reproduction by asexual mitosis, as demonstrated by the high sequence divergence between aligned regions (11). The num-

ber of proteins predicted in the two RKN genomes is also different (14,454 and 19,212 for *M. hapla* and *M. incognita*, respectively). This is due to the duplications that have occurred within the *M. incognita* genome (11).

The pine wood nematode, *B. xylophilus* is a migratory endoparasite that causes severe damage to forest ecosystems. Several species of pine trees (mainly outside the area of co-evolution with pines in North America) are susceptible to *B. xylophilus*. *Bursaphelenchus xylophilus* has a complex ecology that combines fungal feeding and the plant-parasitic/insect-associated stages. The genome of this species contains 18,074 genes distributed across six chromosomes. The assembled genome showed a G+ C content of 40.4%, which is higher than that of both *M. incognita* (31.4%) and *M. hapla* (27.4%). This nematode showed great expansion in terms of digestive and detoxification proteins, which may reflect an unusual diversity in the foods consumed and the environments encountered during its life cycle (10). In addition, *B. xylophilus* has the largest number of digestive proteases known for any nematode, and shows expanded families of lysosome pathway genes, ABC transporters, and cytochrome P450 pathway genes (10). *Bursaphelenchus xylophilus* sequences that matched those of parasitism genes (except cell wall degrading enzymes) either did not have the predicted signal peptide or, if one was predicted, homologues were also present in a wide range of other species including *C. elegans* and animal PPN (10). Two exceptions were found—venom allergen proteins and a putative cystein protease inhibitor. These findings are consistent with the independent evolution of plant parasitism within *Bursaphelenchus* compared to other nematodes (e.g., *Meloidogyne* and *Heterodera*, *Globodera* spp.,). In addition, *B. xylophilus* is a migratory endoparasite that is not biotrophic (10).

These analysis of genomes from PPN have led to the identification of novel secreted proteins that could be important parasitism genes (e.g., cell wall-degrading enzymes, those involved in modulation of the plant's defense system, those important for establishment of nematode feeding sites, and those required for synthesis or processing of nutrients). Some of these identified parasitism genes were acquired by horizontal gene transfer (HGT) from bacteria or fungi (12). In addition, these genomes have given researchers important tools for understanding parasitism and have led to the identification of genes that could be good targets for nematode control and basic biological research (reviewed by 13).

The sequenced genomes of other nematodes having different ways of life (e.g., free moving bacterial feeder, bacterial-fungus-nematode feeder, necromenic and animal parasitic) and/or different life-related vectors take different genomic approaches to deal with their environments. In some cases, such unexpected results as cellulases or diapausin have been found in the genome of *Pristionchus pacificus*. These cellulases exhibited a probable and different functional role (probably used for biofilm degradation of certain bacteria), and origin by HGT (amoebozoa) than cellulases in PPN (14). Moreover, diapausin and other genes (509 genes in total) have insect-like codon usage more akin to insects than nematodes (15). For this reason, they are considered to be acquired via HGT from insects (14; 15). *Ascaris suum* (an animal parasite) presents a large genome with a similar number and size of genes. However, it has a low repeat content (4.4%) and a greater intron size compared to the other species sequenced (Table 2; 16). Conversely, the genome or *Dirofilaria immitis* (an animal parasite) harbors neither DNA transposons nor active retrotransposons (17). Some animal-parasitic nematodes with sequenced genomes (e.g., *B. malayi*, *D. immitis*) harbor intracellular symbiotic bacteria of the genus *Wolbachia* (17), and the genomes reveal the genetic basis of this interrelationship. As explained before, the genomes of nematodes are very diverse between and within the same genus employing the same parasite strategy; thus, new genomes will show more diversity in this sense.

Phylogenomics, which uses genomes (or large portions thereof) to reconstruct evolutionary relationships between species, is dependent on the development of genomic resources. This approach is not yet applicable to PPN as only three species have been sequenced to date. However, Kikuchi *et al.* (2011) conducted this analysis on the seven nematodes studied. The study of PPN families with difficult evolutionary relationships (such as Meloidogynidae or Pratylenchidae) will become more defined as more genomes become available.

2.2. *Transcriptomics*

The most rapid and cost-effective approach to gene discovery in eukaryotic genomes (including PPN) has been the generation of expressed sequence tags (ESTs) (18). However, EST data has such shortcomings as: i) base-calling errors when dataset redundancy is not sufficiently considered; ii) ESTs are generally not complete and do not cover the gene's entire coding sequence; and iii) short inserts tending to clone more efficiently than longer ones (18). Nevertheless, ESTs are considered among the most important tools for studying PPN at the molecular level. Analysis of the GenBank division housing ESTs (http://www.ncbi.nlm.nih.gov/dbEST) showed 73 datasets for nematodes, 21 of which were derived from PPN (28.8%). However, PPN only accounted for 210,422 (17.7%) of the total ESTs (1,190,246), with sedentary endoparasites accounting for the majority of these sequences. Some web tools provide more useful data for researchers, such as wormbase (www.wormbase.or; 19), Nembase4 (http://www.nematodes.org/nembase4; 20), and Nematode.net (www.nematode.ne; 20). The use of such new techniques as NGS or microarrays (based on EST datasets, complete nematode genomes, or joined nematode/plant genes) will expand as the techniques become less expensive and more available at a greater number of research centers.

Sedentary endoparasites account for the majority of transcriptomic data at all levels. These species include *M. incognita*, *M. hapla*, *M. javanica*, *M. paranaensis*, *M. chitwoodi*., *Globodera pallida*, *G. rostochiensis*, *G. mexicana*, *Heterodera*

Table 2. General features of nematode genomes sequenced to date (adapted 10).

Features	*B. xylophilus*	*M. incognita*[a]	*M. hapla*[b]	*C. elegans*[b]	*P. pacificus*[b]	*B. malayi*[b]	*A. suum*[c]	*D. immitis*[d]
Overall/life-vector[e]	PPN, insect	PPN; -	PPN; -	BF; -	BF, FF, NP; insect	AP; insect	AP, -	AP, insect
Estimated size of genome (Mb)	61-73[f]	47-51	54	100*	169	90-95	309	84,2
Chromosome number	6	Variable	16	6	6	6	24	10
Total size of assembled sequence (Mb)	74,6	86	53	100	172,5	95,8	273	84,2
Number of scaffolds / chromosomes	1.231	2.817	1,523[g]	6 chr.	2,894[g]	8,180[g]	29.831	1.618
N50 of scaffolds (kb)	1.158	83	84[g]	-	1.244	94[g]	408	10.474
Maximum length of scaffold (kb)	3.612	593	360	-	5.268	6.534	-	168
G + C content (%)	40,4	31,4	27,4	35,4	42,8	30,5	37,9	28,3
Completeness[h]								
Cegma completeness (%): (complete/partial)	97/98	73/77	95/96	100/100	95/98	95/96	96/98	97/98
Average CEG gene number: (complete/partial)	1.08/1.09	1.53/1.61	1.07/1.12	1.05/1.06	1.20/1.23	1.07/1.11	1.18/1.22	1.18/1.22
Protein-coding regions								
Number of gene models	18.074	19.212	14.420	20.416	21.416	18.348	18.542	11.375
Number of proteins	18.074	20.365	13.072	24.890	24.217	21.252	18.542	12.344
Average protein length	345	354	309,7	439,6	331,5	311,9	327,7	-
Gene density (genes per Mb)	242,3	223,4	270	249	140,4	221,8	-	135
Mean exon size (bp)	288,9	169	171,5	201,6	96,7	159,8	153	-
Mean number of exons per gene	4,5	6,6	6,1	6,5	10,3	5,9	6,4	-
Mean intron size (bp)	153	230	154	320	309	280	1081	226

a 8, b WormBase release 221, c 16, d 17.

e PPN: Plant-parasitic nematode, BF: bacterial feeder, FF: fungal feeder, NP: nematode predator, AP: animal parasite.

f Genome size was estimated using real time PCR.

g These values are from 9, 80 and 81 respectively.

h Assembly completeness was estimated by CEGs (Core Eukaryotic Genes) with CEGMA software. Assemblies in WormBase release 221 was used for *M. incognita*, *M. hapla*, *C. elegans* and *P. pacificus* genomes. *Ascaris suum* and *D. immitis* were downloaded form http://www.wormbase.org/species/all#01--10 and http://nematodes.org/genomes/dirofilaria_immitis/, respectively.

The genome features of *B. xylophilus*, *M. incognita*, *M. hapla*, *P. pacificus*, *B. malayi*, *A. suum* and *D. immitis* are based on incomplete genome drafts and represent statistical estimates while *C. elegans* genome has been completely sequenced and well annotated.

glycines, *H. schachtii*, and *H. avenae* (6; 9, 22-31). The migratory endoparasite species studied are *Pratylenchus coffeae*, *P. thornei*, *P. penetrans*, *Radopholus similis*, *Ditylenchus africanus*, and *B. xylophilus* (30; 32-37). The only semi-endoparasitic species studied is *Rotylenchulus reniformis* (38), and the only ESTs from an ectoparasite are from *Xiphinema index* (39). The majority of these studies have been centered on understanding the mechanisms (mainly protein effectors) involved in plant-parasitism caused by PPN. Few studies have been directed at a comparison of different environmental studies, different specific nematode stages, or different nematodes strains/species (35; 40- 45). The study of avirulence factors has been achieved by employing a cDNA-amplification fragment length polymorphism (AFLP)-based strategy in *M. incognita* for the gene *Mi* (39; 46). However, the avirulence factor (*map-1*) (46) was not found for *M. javanica*, and when nematode juveniles of the *Mi-1*-avirulent strain were soaked in dsRNA from a different potential avirulence factor *Cg-1*, they produced progeny that were virulent on tomato carrying the *Mi-1* gene (41). Other example of avirulence factor is the cyst nematode SPRYSEC protein RBP-1 in *G. pallida*, which elicits Gpa2 and RanGAP2 dependent plant cell death (47). This recognition of Gp-RBP-1 correlated to a single amino acid polymorphism at position 187 in the Gp-RBP-1 SPRY domain (47). The effects of different plant resistant genotypes on the same nematode (*G. pallida*) have also been studied using microarrays in different breeding potato lines, revealing similarities in the mode of action against the nematode (45). Different *H. glycines* populations (virulent or avirulent) exposed to the resistant *Glycine max* genotype (Peking) were studied at pre-parasitic stages and at different times of post-infection (43), with numerous putative parasitism genes being found expressed differentially, as well as numerous genes (1668) being suppressed in the avirulent population, and induced in the virulent population (43).

Next Generation Sequencing has been used for several nematodes, the majority of which are migratory endoparasites. For example, *P. thornei* and *P. coffeae* transcriptomes have been studied using a 454 strategy (36; 37). Both *Pratylenchus* spp. showed similarities in terms of effectors relative to other species of PPN with different lifestyles. Illumina NGS has been used for a comparison between *B. xylophilus* (PPN) and *B. mucronatus* (only PPN under controlled conditions) that showed a similar adaptation to life on pine hosts (35). NGS has also been used in the genome annotation analysis of *B. xylophilus* (10). New web integration platforms (such as Nematode.net) enabling the use of NGS data and the development of new programs or web tools for large-scale genome analyses will help promote the development of these studies (48).

2.3. *Proteomics*

Proteomics is the study of proteins or proteomes. A specific proteome can be defined as all the proteins in an organism, organ, tissue or cell under specific environmental and temporal conditions. The use of proteomics in PPN has been hampered due to a lack of sequenced genomes or ESTs datasets for some species and due to the problems in obtaining the required sample amounts from species that have long life cycles or which are obligate endoparasites. The few studies using proteomics that have been conducted on PPN have been conducted with the aim of i) identifying effectors secreted by the nematode in order to understand their role in plant-nematode interaction; ii) map the entire proteome, and iii) confirm annotated genomes.

The use of proteomics in identifying effectors was made possible by the study of specific subproteomes. The secretome of *M. incognita* was investigated using 2-DE gels (49), followed by a more complete analysis using nano-LC-ESIMS/MS to find effectors (50). Rehman *et al*., (2009) used a monoclonal antibody to immunopurify the most abundant cellulases in the stylet secretions of pre-parasitic juveniles of *G. rostochiensis* (51). Different effectors have also been detected using these techniques. However, the detection capabilities are limited due to the amounts of protein required for their correct detection and identification.

Different proteomic maps using 2-DE gels have been made for *Heterodera glycines* (a soybean cyst nematode), *B. xylophilus*, *Ditylenchus dipsaci,* and different species of *Meloidogyne*. *Heterodera glycines* showed 803 proteins using 2-D gels, with 426 spots being identified by LC-MS/MS, indicating that those showing metabolic, developmental, and biological regulation processes were the most abundant (52). Navas *et al*. (2002) compared different isolates from the species of *Meloidogyne* (i.e., *M. incognita*, *M. javanica*, *M. arenaria*), in order to find proteomic markers for identification (53). The identification of several differential proteins in gel suggested their possible use as putative diagnostic markers for the species (54). A similar study was conducted on the discovery of possible protein biomarkers in *D. dipsaci* races (55). The peptides of galactose-binding lectin-1 of *B. xylophilus* were demonstrated as being the antigen target of MAb-D9-F10, as based several types of proteomic analyses (e.g., SDS-PAGE, 2 -DE, anion exchange chromatography, immunoprecipitation) (56), while other studies more centered on subproteomes showed important differences when the nematode is inside the host or at various stages of development (57; 58). On the other hand, the complete proteome of *M. hapla* (59) is the only one that has been used thus far to improve genome annotation and provide experimental confirmation of the computational predictions of intron/exon structures.

2.4. *Metabolomics and other –omics fields of study*

Technical advances made in high-resolution NMR spectroscopy and mass spectrometry, in an attempt to capture the complexity of metabolic networks (60), have increased the possibilities of metabolomics, which could be subdivided into other subdivisions such as lipidomics and glycomics. In a widest sense of the word, metabolomics has been applied

to *C. elegans* in order to study the regulation of signals and aggregating behavior (61), the effects of pesticides and heavy metals or a combination of both (62), changes in diet (63), and the detection of differences between mutants (64). Lipidomics characterizes the composition of intact lipid molecular species in biological systems (65). The discovery of new antiparasitic drug targets using membrane lipidomics and the changes in lipid balance in the membranes of parasites could provide clues to the dynamics of drugs and some mechanisms of drug resistance; work in this area has focusedmainly on vertebrate nematode parasites (66). However, few studies have been conducted using lipidomics in PPN. Only a few approaches to the variation in lipid reserves of second-stage juveniles of *Meloidogyne exigua* in a coffee field and its relation with infectivity have been studied (67). While in cyst nematodes, several studies showed the effect of long-term storage on the lipid reserves and fatty acid composition of cyst and hatched juveniles of *G. rostochiensis* and *G. pallida* (68); during rehydratation, exposure to the hatching stimulus and hatch in *G. rostochiensis* (69) or the influence of the host plant on lipid reserves of *G. rostochiensis* (70).Some studies attempted to identify fatty patterns in the soil for biodiversity assessment (71; 72). The glycome consists of all glycans (or carbohydrates) within a biological system, and modulates a wide range of important biological activities from protein folding to cellular communication (73). Arrays and mass spectrometry techniques are used to study the glycome. Glycomics is still in the development stage in nematodes, and only partial studies on certain glycans related to this matter have been reported (74; 75).

3. Use of –omics studies in plant-parasitic nematode population management

The best management of PNN is to avoid its introduction to the field, because once PPN is introduced and the population stabilized, they are almost impossible to eradicate given their long survival stages, deep soil penetration, and the ineffectiveness of most pesticides now permitted to be used in soil. The management of PPN in general, or that of a specific species, should be centered on maintaining population levels below the economic damage threshold for the crops being grown. The cost of control measures must also be adjusted relative to the cost of the expected yield reduction, as compared to the yield in a situation where there is no need for control (76). For these reasons, the nematodes in certain low -cash crops could significantly impact their management. The most efficient and economically viable strategy is usually host-plant resistance. However, "Integrated Pest Management", which integrates several control measures (agronomical, chemical, and genetic) is often the best solution for PPN management.

The impact of the several –omics studies discussed above is or will be significant in terms of PPN management. Correctly and quickly identifying species is the first point in the correct integrated management of PPN. Plant-parasitic nematodes have a conserved morphology and few characters are useful for experienced nematologists in delimitating species, but morphologically very similar species may have markedly different pathogenic features. As mentioned before, some species are highly host-specific and genomic or proteomic approaches could help to identify species. More importantly, both pathogenicity genes and survival genes play an important role in PPN management. –Omics studies offer a faster way to study these genes, but the sequences of interest will be pioneers that do not have orthologues in model organisms, such as *C. elegans*. Pathogenicity genes, specifically those implied in the formation of "feeding sites," are of great interest for their biological significance and putative use as control measures targeting their expression using RNAi. RNAi is a useful research tool for determining gene function and has potential applications in commercial nematode control through transgenic plant-derived dsRNA (11). This tool has been used extensively to knock down gene expression in *C. elegans* by introducing dsRNA using microinjection, soaking, and feeding (77). The soaking of second-stage juveniles (J2s) in dsRNA solution in combination with drugs (e.g., resorcinol) has been applied to several species of PPN (reviewed in 78), but more recent studies have highlighted the challenges of non-specific phenotypic effects from long dsRNAs (79).

4. Concluding Remarks

Plant-parasitic nematology will generally be assisted in finding solutions to minimize crop loss caused by PPN. In this sense, the next step in using these genome sequences will be comparative and functional analysis in order to find out genes involved in their pathogenicity/virulence which will be achieved by analyzing different strains or different species from the same genus. The future availability of "third-generation" sequencing approaches based on single DNA molecule sequencing, with longer read capabilities and greater parallel sequencing densities (i. e., Pacific Biosciences technology or Oxford Nanopore) may be helpful in this regard. Such new technologies will help reduce the costs of genome sequencing. On the other hand, the use of other –omics fields of study, such as metabolomics, lipidomics and glycomics in plant-parasitic nematology, will expand as more genomes become available to researchers. However, all these techniques should have an important applied role in crop protection as much as possible. Otherwise, we run the risk of creating science for scientists, and not for farmers.

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