Recent applications of biotechnological approaches to elucidate the biology of plant-nematode interactions

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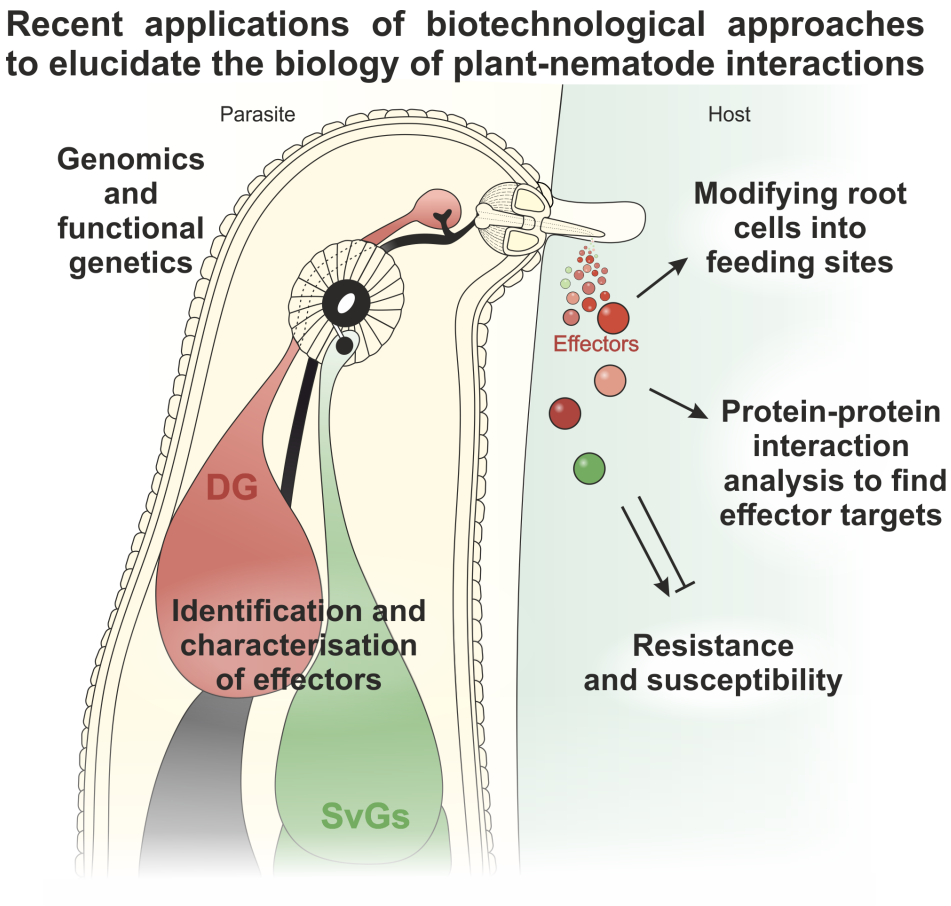
Highlights

- Genome and transcriptome sequencing of plant-parasitic nematodes has been a driver of recent progress.
- Recent progress towards transformation of plant-parasitic nematodes (by transient expression of exogenous mRNAs) may allow exploration of gene function "*In Nematoda*".
- In planta approaches have been, and will continue to be, important avenues to elucidate the biology of plant-nematode interactions in particular, and plant biology in general.

Graphical abstract



Abstract

Plant-parasitic nematodes are a major threat to food security. The most economically important species have remarkable abilities to manipulate host physiology and immunity. This review highlights recent applications of biotechnological approaches to elucidate the underlying biology on both sides of the interaction. Their obligate biotrophic nature has hindered the development of simple nematode transformation protocols. Instead, transient or stable expression of the effector (native or tagged) *in planta* has been instrumental in elucidating the biology of plant-nematode interactions. Recent progress in the development of functional genetics tools "*in nematoda*" promises further advances. Finally, we discuss how effector research has uncovered novel protein translocation routes in plant cells and may reveal additional unknown biological processes in the future.

Introduction

Plant-parasitic nematodes are major, and in some cases dominant, threats to crop security in the developed and developing worlds. While the plant-parasitic nematodes encompass several distinct lifestyles (Figure 1), parasitic strategies, and phylogenetically distinct groups [1], they are often considered together due to similar sets of challenges they overcome. Plant-parasitic nematodes must subvert, suppress, or avoid the plant immune system, extract and metabolise nutrients from their host, and in many cases manipulate plant physiology and/or development to form a permanent "feeding site". This review will focus on recent research that deploys biotechnological approaches to elucidate how plant-parasitic nematodes (PPN) have evolved/are evolving to overcome these challenges.

It is widely cited that increased knowledge of plant-parasitic nematode biology will lead to future routes to control, and thereby mitigate their threat to food security. It is similarly understood that, by default, plants are resistant to nematodes, and that nematode "parasitism genes" (including those that encode for "effectors") are required to make hosts susceptible [2,3]. Effectors, broadly defined as nematode-derived molecules (often, but not exclusively, proteins) secreted into the host, have evolved to manipulate various aspects of host metabolism, physiology, development, and immunity to render a host susceptible. Many effectors in PPNs are produced in two sets of gland cells (one dorsal and two subventral) and delivered into the host through a needle-like stylet. Research on plant-nematode interactions in general, and plant-nematode effectors in particular, has in some aspects lagged because of the lack of biotechnological tools available for their study. This review will highlight some of the ways biotechnology, in its broadest sense, can expedite research on plant-parasitic nematode biology, and thereby the pursuit of novel routes to control.

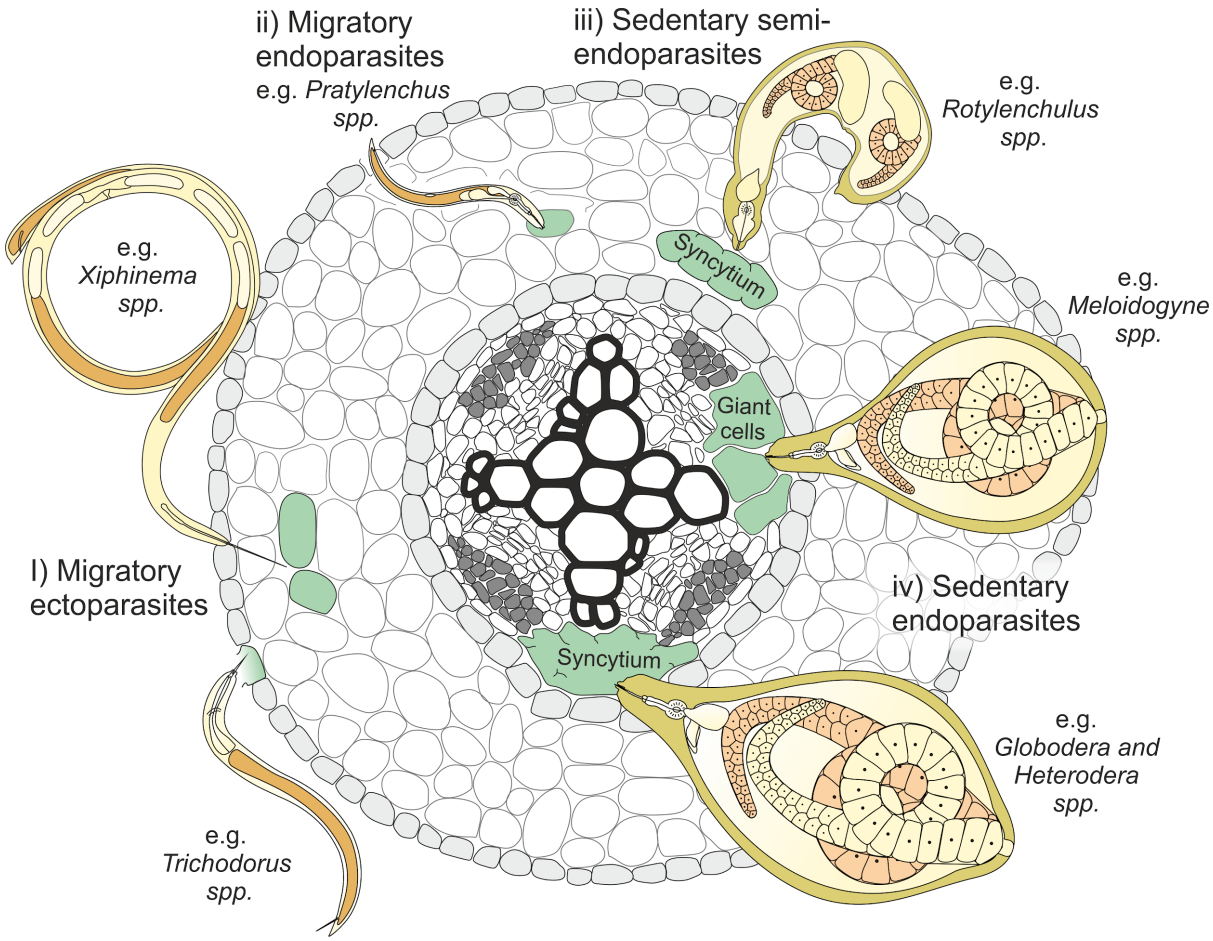


Figure 1. Some of the major plant-parasitic nematode lifestyles. Schematic diagram, not to scale, of various plant-parasitic nematode lifestyles depicted on a cross section of a root. The most prevalent plant-parasitic lifestyles in the phylum Nematoda are dichotomised into the ecto- or (semi)endoparasites. i) Ectoparasites migrate outside the plant for their entire life cycle and typically parasitise the outer layer of root cells (e.g. *Trichodorus spp.*), or feed from inner layers of the root (e.g. *Xiphinema spp*.). The endoparasites, however, spend at least part of their parasitic life cycle inside the host, and are further divided into migratory or sedentary. ii) Migratory endoparasites burrow inside the host and parasitise cells from within while migrating (e.g. *Pratylenchus spp.*). iii and iv) Sedentary (semi-)endoparasites induce the formation of "feeding sites" inside the host, and withdraw nutrition in a non-destructive manner, for a prolonged period of biotrophy (e.g. the reniform, cyst, and root-knot nematodes).

Genomics and functional genetics

Each technological advance in sequencing has permitted a surge in the application of genomics to the study of plant-parasitic nematodes. First iteration, largely representative, genome sequences are now available for most major plant-parasitic nematodes of agricultural importance (reviewed in [4]). The first iteration genomes were important milestones in the field. However, due to the nature of the sequencing technology/ies used, they contained incomplete genic complements and were largely fragmented (therefore lacking long-range information). A more faithful representation of the genome, and by extension the genes involved in parasitism, will help understand the evolution and biology of plant-parasitic nematodes. Second, and in some cases, third iteration genomes are either already available or in progress for several species. This wealth of resources provides a foundation for future innovation and comparative near-phylum-wide analyses (e.g. [5]). At the same time, the depth of individual genome study has elucidated cis-regulatory mechanisms of effector transcription (e.g. the DOG box [6,7]), STATAWAARS [8], MiDOG box and linked the origins of many effectors to duplication/neofunctionalization (e.g. SPRYSECs [9] and GS-like effectors [10], *de novo* gene birth [11], and/or acquisition by horizontal gene transfer (e.g. [12]). A perennial discovery is that many, indeed most, parasitism genes are so-called "pioneers" - unlike any other sequence in public databases and without characterised domains.

Therefore, the wealth of information therefore comes with a major challenge: understanding the function/s of parasitism genes is a bottleneck. Functional studies generally include ectopic expression and knock-down/out experiments, but these rely on effective genetic transformation protocols for the nematode. Genetic modification has eluded the plant-parasitic nematode community for decades. Generally, plant-parasitic nematode biology is not conducive to genetic modification (long life cycles, obligate biotrophy, inaccessible immature germlines, etc.) and as a result, genetic modification techniques that are readily deployed in other nematode species are prohibitively difficult [13]. As a result, reverse genetics in PPN is almost exclusively reliant on gene silencing by RNA interference (RNAi). RNAi is technically facile, soaking the juveniles in dsRNA can suffice, however the efficacy and reproducibility vary widely depending on the nematode species [14] and the target gene. More critically, silencing of a gene in PPN typically results in the inability of the nematode to successfully infect the plant. This phenotype may be very similar for housekeeping genes or genes involved in development or parasitism. In other words, RNAi proves the importance of a gene but reveals little or nothing about the particular function. Nevertheless, RNAi is a valuable complementary tool to check the significance of a specific gene and elucidate its function in combination with appropriate assays. For instance, RNAi was crucial to prove the role of a cathepsin gene for reproduction of the pinewood nematode *Bursaphelenchus xylophilus* in *Pinus massoniana* [15]. Pine trees produce phytoalexins such as carvone as biochemical defense upon infection. RNAi induced gene silencing of *Bx-cathepsin W* reduced the survival of *B. xylophilus* after carvone treatment *in vitro* and after inoculation of pine trees, supporting the role of *Bx-cathepsin W* in phytoalexin detoxification.

A recent advance provides the first example of reporter gene expression in any plant-parasitic nematode [13]. This was achieved by bathing *Heterodera schachtii* juveniles in a solution containing octopamine (to stimulate uptake) and mRNA encoding a reporter protein encapsulated in a lipid bilayer (termed a liposome). The result was low-level expression of eGFP throughout the body of second-stage juveniles (Figure 2), using a technique as technically facile as RNAi. Like RNAi, it is not yet clear how liposomes are

taken up into the cell (most likely by either fusion with the plasma membrane and/or endocytosis and subsequent release) nor how they aid the spread of the signal [16]. While in need of substantial optimisation to provide widespread utility, this technique is extremely promising for two reasons: i) even transient expression of arbitrary proteins "*in nematoda*" would permit reverse genetic approaches that are at present technically intractable (e.g. gain of function experiments, protein-protein or protein-DNA interactions, etc.), and ii) the systemic spread may pave the way for stable editing of the germline by transient expression of genome editing components.

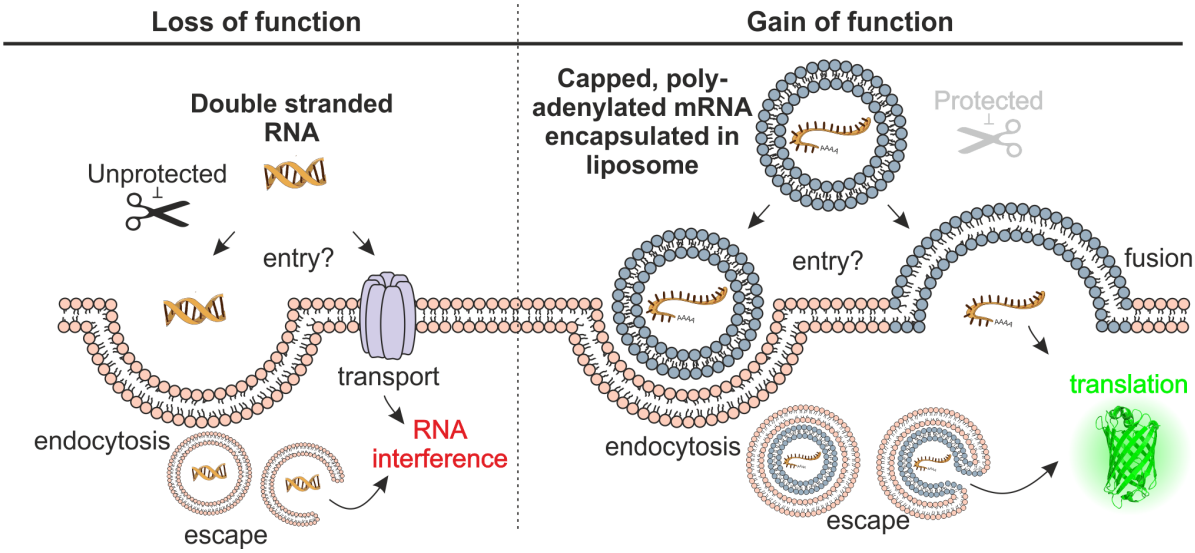


Figure 2. Reverse genetic approaches *in nematoda.* Left, loss-of-function is achieved by delivery of double stranded RNA to parasitic nematodes. Mode of entry into cells is unclear, but the result is targeted knockdown of nematode mRNA. Right, gain-of-function is achieved by delivery of capped, poly-adenylated mRNA encapsulated in liposomes to parasitic nematodes. Mode of entry is also unclear, but the result is expression of exogenous genes *in nematoda*.

While PPN-transformation is not yet an option for functional analysis of effectors, plant transformation is routinely used for this purpose (Table 1). Plants that produce dsRNA against a nematode effector are usually less susceptible to infection by the nematode. This host-delivered RNAi could also be useful for crop protection. This host-delivered RNAi could also be useful for crop protection. Joshi et al. 2020 transformed hairpin constructs -targeting 4 effector genes of *Meloidogyne incognita*- into Arabidopsis and the resulting lines showed a 70 to >300-fold reduction in nematode reproduction [17]. On the other hand, plants expressing nematode effectors are often more susceptible (sometimes also to other pathogens [18,19]). For instance, Arabidopsis plants expressing the *H. schachtii* effector 4E02 were more susceptible to that nematode and to the fungus *Botrytis cinerea*. Plants that express a nematode effector are also instrumental for exploring the molecular changes evoked by that effector e.g. by RNA-seq [18,20,21]. According to the essence of the infection process, the secreted proteins of sedentary PPN can be divided into three groups: cell wall modifying proteins that facilitate migration through the root, suppressors of plant defense, and factors that manipulate root cells into feeding sites.

Identification and characterisation of effectors

Nematode produced cell wall modifying proteins have been identified by their resemblance to plant, bacterial, and fungal proteins with similar functions [2]. Sequence similarity can

sometimes also suggest that particular proteins might interfere with plant defense. Fatty acid and retinol-binding (FAR) proteins have been discovered in PPN based on their counterparts in animal parasitic nematodes. FARs have been proposed to hinder lipid signalling involved in host immunity. Adding to the list of FARs in PPN, *B. xylophilus Bx-FAR-1* has recently been shown to suppress plant defense and facilitate nematode infection of pine trees [22]. In a similar vein, fungal effectors were models for finding chorismate mutase (CM) and isochorismatase (ICM) in PPN. Both enzymes can interfere in the production of the defense molecules salicylic acid (SA) and/or phenylpropanoids by the plant host. Production of the chorismate mutase Mi-CM-3 of *M. incognita* in *Nicotiana benthamiana* decreased SA levels upon pathogen infection [23]. The CM and ICM of *Hirschmanniella oryzae*, on the other hand, appeared to have no effect on SA levels in rice but reduced phenylpropanoid metabolites [24].

Effectors from other pathogens are known to affect gene expression by binding to the host DNA, most notably the TALE (transcription activator like-like effectors) of *Xanthomonas* bacteria. Hence, the *H. schachtii* effector GLAND4 was selected for further study based on sequence similarity to known transcription regulators [18]. One DNA-fragment that binds with high affinity to the GLAND4 protein was found near two lipid transfer protein (LTP) genes involved in plant defense. When GLAND4 was expressed in *A. thaliana*, the *LTP*-genes were downregulated and upon *LTP* overexpression the plants became less susceptible to *H. schachtii.*

Many pioneer effectors have been implicated in defense suppression. In contrast, relatively few effectors have been connected to non-immunity related parasitism processes (e.g. feeding site formation) despite the clear necessity. This is at least in part due to the wealth and ease of biotechnological approaches to screen for, and characterise, effectors that interfere with the plant immune system (Table 1). These approaches generally involve triggering a defense response in *N. benthamiana*, and examining whether expression of the effector, in isolation, can suppress that response [25–27]. Screening 51 uncharacterised putative effectors from *Heterodera glycines* revealed 10 with some ability to suppress plant immunity [28]. While technically facile, both negative and positive results of the screen come with caveats: it is not clear whether similar responses would be achieved in roots, cognate hosts, or during infection when effectors are delivered in much lower quantities and in combination. Developing biotechnological approaches that would allow us to account for some of these caveats during screening would improve the validity of the approach, and presumably thereby progress in the field.

Understanding how effectors function is an additional challenge. There is no single method that can answer this, but the field is equipped with a range of techniques (Table 1). The subcellular localisation of an effector can provide clues as to how it functions. This is frequently explored through expressing an effector-GFP translational fusion in *N. benthamiana* leaf cells [19,26]. This artificial situation suffers from many of the same caveats as defense suppression assays, and has additional complications. It comes with a major assumption that the "mature" effector is intracellular. Moreover, expressing the full coding sequence of the effector in plant cells will generally direct the protein to the apoplast, because it includes the signal peptide that is needed to secrete the effector from the nematode glands. This gives no insight into the actual cellular location during nematode infection of the root as the signal peptide is cleaved off before the effector reaches the plant tissue. A superior but more technically challenging technique is immunolocalization. Antibodies generated against the effector reveal the actual location of

the effector in the nematode gland and the plant root [10,20,29]. This location can even vary depending on the infection stage, for instance, first in the apoplast and later in the giant cell nuclei [26]. We suggest that expression of tagged effectors (transient or stable), *in nematoda*, may provide a technically facile route to address this common question while avoiding the challenges associated with generating specific antibodies against individual effectors.

Protein-protein interaction analysis to find effector targets

While not all effectors will carry out their function by binding to other proteins, many will. To understand the role of a nematode effector in the plant, it can therefore be informative to identify the plant proteins it interacts with [30]. The diversity of techniques to discover or confirm such interactions is expanding, but the traditional Yeast-two-Hybrid (Y2H) is still popular (Table 1) although it requires additional validation [31]. Y2H identified the *A. thaliana* defense protease RD21A as a target of the cyst nematode (*H. schachtii*) effector 4E02 [19]. Expression of 4E02 in *A. thaliana* mediates a re-localization of RD21A from the vacuole to the cytoplasm and nucleus, which enhances the susceptibility of the plant to *H. schachtii* and the fungus *B. cinerea.* The mislocalization of the host target protein by the effector prevents the protease from realising its defense function. Another effector involved in defense suppression, MiMIF-2 of *M. incognita,* interacts with two plant annexins (revealed by immunoprecipitation [27]). These annexins are engaged in the transport of calcium ions and stress response. Expression of MiMIF-2 or mutation of one of the annexins in *A. thaliana* plants increased susceptibility to *M. incognita* and decreased Ca^{2+} signalling upon H_2O_2 stress. This indicates a possible role of MiMIF-2 in protecting the nematodes from oxidative stress during the plant defense response via interaction with annexins. The effector GpSPRY-414-2 from the potato cyst nematode suppresses plant defense and interacts with the microtubule-associated StCLASP potato protein [25]. The two proteins colocalize at the microtubules but how this is linked to plant defense remains unknown.

Modifying root cells into feeding sites

Understanding which nematode genes induce the formation of feeding sites has proven to be a grand challenge. There are very few precedents from other pathosystems in the literature and no large scale screening assays. Nematode-induced feeding sites are characterized by changes in the cell cycle, hormone levels, transcriptome, and proteome - while only a handful of effectors have been plausibly implicated (e.g. by increasing auxin import into the developing feeding site (reviewed in [32]). Gene expression changes in developing feeding sites are reasonably well documented, and some recent papers uncover how effectors could contribute. Verma et al. (2019) and Mejias et al. (2020) detected via Y2H that the cyst nematode effector 30D08 and the root knot nematode effector MiEFF18 both interact with a plant protein involved in splicing, SMU2 and SmD1 respectively [20,29]. RNA-seq of plants expressing those effectors revealed altered expression patterns, partially due to alternative splicing. The affected plant genes include those involved in cell cycle activities, development and hormone pathways. Effector 32E03 from *H. schachtii* interacts with and inhibits a histone deacetylase in *A. thaliana [33]*. Consequently, plants expressing this protein display higher acetylation, especially along the rRNA genes and allow more female nematodes to develop upon infection. The induced chromatin change derepresses a subset of rRNA genes leading to higher rRNA levels, which could be instrumental in the development of the highly metabolically active feeding sites.

Nematode feeding sites are typified not only by many large nuclei but also by changes in their cytoskeleton. Both actin filaments and cortical microtubules are more fragmented than in normal root cells. Leelarasamee et al. (2018) identified the effector MiPFN3 as a possible player in actin reorganization [34]. The profilin MiPFN3 disrupts actin polymerization *in vitro* and in plant cells and enhances susceptibility of *A. thaliana* to root-knot nematodes.

A subgroup of nematode effectors has become increasingly noticeable, namely the plant peptide mimics such as CLE, CEP and IDA [32]. The plant peptides are known to be involved mainly in plant development e.g. by controlling cell differentiation or lateral root development [32] and the nematode peptides can mimic that function, but their precise role in NFS development is not always clear. Nematode CLE research is a superb illustration of how understanding plant-nematode interactions can lead to new fundamental insights in plant biology. CLE peptides are delivered into the plant cytoplasm by the nematode's stylet, but from there, they travel to the apoplast by a formerly undiscovered mechanism of post-translational uptake into the ER [35]. Deletion analysis defined a 37 amino acid sequence from CLE to be sufficient for post-translational trafficking of CLE or of an unrelated peptide. In a similar vein, Bournaud et al. (2018) found a peptide (MiPM) from *M. incognita* to trigger an unknown endocytosis pathway for entering plant cells [36]. A recent addition to that group are the RALF peptides secreted by root-knot nematodes [37]. Plant and nematode RALF peptides possess similar activities through interaction with the plant receptor kinase FERONIA, thereby influencing plant defense and cell growth. This addition is important as it extends the functions of plant-peptide hormone mimics to include modulation of plant immunity.

Resistance and susceptibility

Evolution of effectors allows nematodes to adapt to new hosts [38] or to avoid recognition by plant resistance genes. Many nematode resistance genes have been genetically mapped and several have been cloned on the basis of their chromosomal location [39]. Research on canonical plant resistance genes against nematodes has somewhat dwindled, and examples of nematode effectors that are avirulence proteins are very scarce [39]. Conversely, the atypical soybean cyst nematode resistance *Rhg1* and *Rhg4* have lately drawn attention. The *Rhg1*-resistance involves three soybean genes, encoding a predicted amino acid transporter [40], a putative wound-induced protein and an α-SNAP involved in vesicle trafficking. Y2H found α-SNAP to interact with two syntaxins [41]. Despite soybean being a challenging system, the combination of CRISPR-Cas9 and hairy root transformation enabled the generation of quadruple knock-outs in those syntaxin genes. The knock-out roots were significantly more susceptible to cyst nematode infection than the controls, confirming a role for the syntaxins in the resistance.

To establish a successful interaction with a plant host, more is needed than merely the absence of resistance genes. Plant genes that enable infection have been named susceptibility genes, and they have been identified by various approaches. Warmerdam et al. (2018) performed a genome-wide association mapping on 340 genotypes of *A. thaliana* differing up to 10-fold in their susceptibility to distinguish allelic variants that influence this[42]. Radakovic et al. (2018) focused on genes that are highly upregulated in the feeding sites. Other prominent candidates are effector interaction partners such as those described above [43]. In this respect, CRISPR-Cas9 is clearly emerging as an important tool to understand plant-nematode interactions. In the past, researchers had to rely on available mutants, now knock-outs or different alleles can be generated as needed.

Conclusions and future perspectives

This review has highlighted some of the ways biotechnology, in its broadest sense, can expedite research on plant-parasitic nematode biology in general. We focus on effector proteins for two reasons: i) there has been substantial progress in recent years and, ii) there is much more to explore in this area. For example, we expect that the further application of structural biology to the study of effectors is, at present, nearly absent and will be an area of considerable future growth and insight. It seems that the focus on plant-immunity altering functions is justified, but can only ever provide an incomplete picture of plant-nematode interactions. The same can be said for non-proteinaceous molecules involved in plant nematode interactions. Recent reports have drawn our attention to small RNAs [44], ascarosides [45] and electrical signals [46], but what about other nucleic acids, lipids, and carbohydrates? New biotechnological approaches will be needed to allow the identification of effectors (proteinaceous or otherwise) with non-immunity related function/s. Given the profound changes in host physiology, development, and metabolism, it may be that a majority of effectors fall in this category. With the CLE effectors as a precedent, we expect that understanding the role of non-immunity related effectors will reveal further unknown areas of plant biology. These intriguing new avenues promise an exciting future for the discipline.

Acknowledgements

Work on plant-parasitic nematodes at the University of Cambridge is supported by DEFRA licence 125034/359149/3, and was funded by biotechnology and biological sciences research council grants BB/R011311/1, BB/N021908/1, and BB/S006397/1. At Ghent University, research on nematode effectors and plant defense is supported by the UGent grant 01G01318 and the grant G.0087.18N from the Research Foundation Flanders (FWO).

Declaration of interest: none

Functional assays to study plant defense responses			
Method	***Outcome***	***Study objective***	***Reference***
ROS assays	Quantitative / Qualitative	Transient expression (*N. benthamiana, Arabidopsis*) / Stable transformation (*Arabidopsis*)	*[19,25,26,37]*
Cell death suppression	Qualitative	Transient expression (*N. benthamiana)*	*[25–27]*
Callose deposition	Quantitative / Qualitative	Stable transformation (*Arabidopsis*)	*[27]*
Ca^{2+} measurement	Quantitative	Stable transformation (*Arabidopsis*)	*[27]*

Protein-protein interactions					
Method	***Binary interactions***	***Throughput***	***Subcellular localisation***	***Study objective***	***Reference***
Y2H	Yes	Low / High	No	Transgenic yeast	*[19,25,47]*
BiFC	Yes	Low	Yes	Transient expression (*N. benthamiana*)	*[19,25,27,47]*
Co-IP	Yes	Low	No	Transgenic plants	*[27,37]*
Pull-Down	Yes	Low	No	*In vitro* and *E. coli*	*[19,37]*
IP-MS	No	Medium	No	Stable transformation (*Arabidopsis*)	*[27]*
APEX	No	High	Yes	Stable transformation (*N. tabacum)*	*[36]*

Expression analysis					
Method	***Sensitivity***	***Throughput***	***Level***	***Study objective***	***Reference***
RNA-seq	High	High	RNA	Stable transformation (*Arabidopsis*) / nematode stages	*[10,20,21,29]*
qRT-PCR	High	Medium / Low	RNA	Transgenic plants / nematode stages	*[18,19,27,34,37,47]*
*** ISH**	Low	Low	RNA	Nematodes	*[10,26,29,47]*
****LC-MS/MS**	High / Medium	High	PROTEIN	Nematode infected plants	*[48]*

Protein-DNA interactions and transcriptional analysis				
Method	***Detection***	***Throughput***	***Study objective***	***Reference***
EMSA	Chemiluminescence	Low	*In vitro*	*[18]*
ChIP-qPCR	qPCR	Medium / Low	Stable transformation (*Arabidopsis*)	*[33]*
Genomic SELEX	Sanger sequencing	Medium / Low	*In vitro*	*[18]*
GUS reporter	Reporter gene	Low	Stable transformation (*Arabidopsis*)	*[19,33]*

Subcellular localisation				
Method	***Tool***	***Level***	***Study objective***	***Reference***
Immunolocalisation	Specific antibody	PROTEIN	Plants and nematodes	*[10,26,27,29,47]*
Reporter proteins	Fluorescent proteins	PROTEIN	Transient expression (*N. benthamiana)*	*[19,26]*
Yeast signal sequence trap system	Qualitative cell survival assay	PROTEIN	Transgenic yeast	*[21,50]*

Table 1. Overview of the techniques and approaches used in nematode effector studies (2018-2020).

Activity assays, bioinformatical techniques, and metabolite analysis are not mentioned in the table. The table represents an overview of the most frequently used biotechnology techniques in the research field of nematode effectors during the past two years. The techniques are subdivided into categories, where their main disadvantages, advantages and crucial features are pointed out and compared to the other techniques within the same category.

*ISH has been used to study the effector's tissue-specific expression [10,26,29,47].

**LC-MS/MS approach has not been used for effector studies recently, but the field could benefit from applying it to investigate novel effectors [48].

Some techniques that are not listed in the table:

- WB represents a widely applied technique and is still the golden standard to confirm protein expression in many methods mentioned in the table [19,29,37,47,49].
- The protein crystallography technique has been used to study the structure of the neofunctionalized housekeeping gene, e.g. glutathione synthetase [6], and serves as an example which could be used more often to understand the structure of the so-called "pioneer" nematode proteins.
- FRET-based techniques to analyze protein–DNA and/or protein-protein interactions span from the classical steady-state to the analysis of distances, conformational changes, and enzymatic reactions in individual complexes. The technique has not been used in its full potential, with some exceptions [41], especially not in the effector field.
- The approach starting from "hub" proteins to find effectors is rather unusual, but it is an extraordinary example of finding effector proteins by using the AP-MS approach [36].

APEX, a proximity labelling technique, represents a great example of how other techniques besides conventional Y2H and/or IP-MS can be used to find the interacting partners in the effector field [36]. The new development of proximity labelling variants of BirA, TurboID and miniTurboID, which have fast kinetics and are compatible with living organisms are excellent alternative approaches to broaden the spectrum of interacting partners *in vivo*. Furthermore, transient interactions can be captured, and at the same time, additional information of subcellular localisation and proteins in the proximity of the targeted protein (e.g. part of the protein complex) will be detected [51–53]. Doubtless, it is an upgrade of the similar techniques applied before from which the whole field can benefit.

The recently developed technique crY2H-seq [54] could be used to accelerate effector research since the technique allows screening a pool of baits against a pool of preys, to unravel the cross-kingdom interaction network involved in the complex biology of the feeding site formation, defense suppression and plant pathogen infection strategies. By applying and developing more techniques like this, the research will move from nematode effectors to nematode effectomes.

Nomenclature

Abbreviation	**Explanations**
APEX	Ascorbic acid peroxidase
AP-MS	Affinity purification–mass spectrometry
BiFC	Bimolecular fluorescence complementation
BirA	*E. coli* biotin ligase
Ca^{2+}	Calcium ion
CEP	C-Terminally Encoded Peptide
ChIP-qPCR	Chromatin Immunoprecipitation coupled with quantitative Polymerase chain reaction
CLE	CLAVATA3/Embryo Surrounding Region
CM	Chorismate mutase
Co-IP	Co-immunoprecipitation
CRISPR-Cas9	Clustered Regularly Interspaced Short Palindromic Repeats - CRISPR associated protein 9
crY2H-seq	Cre-reporter-mediated yeast two-hybrid coupled with next-generation sequencing
DAB	Diaminobenzidine tetrahydrochloride
DOG box	Dorsal Gland Box
dsRNA	Double stranded RNA

eGFP	Enhanced Green Fluorescent Protein
EMSA	Electrophoretic mobility shift assay
ER	Endoplasmic Reticulum
FAR	Fatty acid and retinol-binding
FRET	Fluorescence resonance energy transfer
GS-like effectors	Glutathione synthetase-like effectors
GUS	*β*-glucuronidase
ICM	Isochorismatase
IDA	INFLORESCENCE DEFICIENT IN ABSCISSION
IP-MS	Immunoprecipitation-Mass Spectrometry
ISH	*In situ* hybridization
LC-MS/MS	Liquid Chromatography with tandem mass spectrometry
MiDOG box	*Meloidogyne* Dorsal Gland Box
miniTurboID	The third-generation of proximity-dependent biotin labelling; 28kDa biotin ligase; N-terminal domain deleted and 13 mutations relative to wild-type BirA
MiPFN3	*Meloidogyne incognita* profilin 3
MiPM	*Meloidogyne incognita* Passe Muraille
NSF	N-ethylmaleimide-sensitive factors
PPN	Plant-parasitic nematode

qRT-PCR	Real-Time Quantitative Reverse Transcription Polymerase chain reaction
RALF	Rapid alkalinization factors
Rhg	Resistance Heterodera glycines
RNAi	RNA interference
RNA-seq	Ribonucleic acid sequencing
ROS	Reactive oxygen species
SA	Salicylic acid
SELEX	Systematic evolution of ligands by exponential enrichment
SMU2	Suppressor of mec-8 and unc-52 2
SNAP	Soluble NSF attachment proteins
SPRYSECseq effectors	Secreted SPRY (SPRY domain is named from SPla and the RYanodine Receptor) domain-containing proteins
StCLASP	Solanum tuberosum cytoplasmic linker protein (CLIP)-associated protein
TurboID	The third-generation of proximity-dependent biotin labelling; 35kDa biotin ligase with 15 mutations relative to wild-type BirA
WB	Western-blot
Y2H	Yeast two-hybrid

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Papers of particular interest, published within the period of review, have been highlighted as:

• of special interest

•• of outstanding interest

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