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Review

Are roots special? Nematodes have their say

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Abstract

Nematodes are ubiquitous and cosmopolitan parasites of vascular plants, causing substantial crop damage. Although various species exploit all parts of the plant, roots are the major target. Nematodes deploy a broad spectrum of feeding strategies, ranging from simple grazing to the establishment of complex cellular structures (including galls) in host tissues. Various models of feeding site formation have been proposed, and a rôle for phytohormones has long been speculated. Based on recent molecular evidence we present several scenarios involving phytohormones in the induction of giant cells by root-knot nematodes. The origin of parasitism by nematodes, and the rôle of horizontal gene transfer from microbes is discussed. Throughout, parallels with aphid-plant interactions are emphasized.

Keywords: *Caenorhabditis elegans*; Genomics; Horizontal-gene transfer; *Macrosiphum euphorbiae*; *Meloidogyne* spp; Rhizobia

1. Introduction

Nematodes are the most successful and abundant metazoans [22] and they occupy a wide range of ecological niches. They are parasites of animals and plants [20]. The impact of nematodes on humans is felt through yield reductions in food and fiber crops, through debilitation of livestock and companion animals, and by direct infection; nematodes such as hookworm and Ascaris each infect more than a billion people world-wide, and nematodes are responsible for exotic diseases such as elephantiasis and river-blindness. Plant-parasitic nematodes are probably the single major uncontrollable biotic cause of plant stress and crop loss.

Although research on many of the parasitic forms of nematodes is made complicated by their lifestyle, the bacterivorous nematode *Caenorhabditis elegans* has become the best understood and experimentally tractable animal [10,79,98]. The sheer volume of information obtained for *C. elegans*, including a complete genomic sequence [23] and suite of research tools [35], serves as an essential resource to underpin the burgeoning deployment of genomics in studies of parasitic nematode biology [15,16,19,20,65,66,73]. For plant pathology, the integration of host and nematode genomics, in consort with genetic approaches and cell biology will undoubtedly reveal much about root biology, and the interactions of roots with the rhizosphere.

Surprisingly, the relationship of Nematoda to other animal phyla remains controversial. The traditional view is that the unsegmented round-worms (Fig. 1) that comprise this phylum are an ancient group that branched from the metazoan tree perhaps a billion years ago. However, molecular phylogenies place nematodes and insects together in a high-level taxon, named Ecdysozoa by Aguinaldo et al. [1]. Recent studies seem to support this grouping [61] although some data remain contradictory [18]. In light of this evolutionary closeness of nematodes with insects, similarities between insects and nematodes in their various interactions with plants become all the more intriguing. For example, certain plant-parasitic nematode species (including the root-knot nematode, *Meloidogyne* spp.) and insects (such as the phylloxera 'aphid' *Daktulosphaira vitifoliae*) induce cellular modifications in root tissues, leading to the formation of galls. Whether or not induction of galls by insects involves the same host pathways as feeding site induction by nematodes is unknown, but it is becoming increasingly apparent that host responses to a diverse range of rhizosphere organisms do involve common plant regulatory cascades [55]. It is a reasonable hypothesis that phytohormones play a rôle in many of these interactions.

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Fig. 1. Newly hatched *Meloidogyne incognita* L2 (J2) larva (juvenile). The feeding stylet (S) is fully retracted. A large number of lipid granules that provide energy reserves until the host root is located and feeding is initiated, are apparent (arrows). Scale bar: 50 μm. Reproduced with permission from *Journal of Plant Growth Regulation* **19:** 183–194.

Thus it seems likely that nematodes and insects have acquired the ability to manipulate fundamental aspects of their host's biology. Although the data are still sparse, a number of classes of enzymatic components appear to be shared by aphids and nematodes, suggesting that these parasites might also employ common toolboxes to attack their host. The most tantalizing evidence that this might be the case has come from studies of the tomato *Mi-1.2* gene, which was originally identified as conferring resistance to three important root-knot nematode species [93]. Strikingly, this same gene conditions resistance to the potato aphid, *Macrosiphum euphorbiae* [80]. Whether this indicates a common nematode/aphid signaling molecule(s), or merely a common host response mechanism is unclear, but either possibility is fascinating.

In light of the increasingly apparent rôle that horizontal transfer of microbial genes seems to have played in the evolution of plant-parasitic nematodes [14], it is perhaps not surprising that bacterial endosymbioses with nematodes and with insects seem to be widespread [37]. The most intimate of the bacterial-nematode associations involves rickettsia-like, alpha-proteobacteria found in obligate intracellular association with a wide variety of arthropods, and an increasing number of nematodes [32,84,92]. All aphids appear to have symbiotic bacteria, believed to have entered an aphid ancestor as a free-living *Buchnera* some 250 million years ago [3], and tephritid flies are hosts for an *Erwinia* endosymbiont [29]. The fact that similar associations exist between bacteria and gall-forming insects and nematodes may be coincidence, or it may reflect some underlying universal mechanism(s) involving host-plant modification.

In this review, we will expand upon some of our ideas on how nematodes interact with their plant hosts, and the root in particular. It is increasingly evident that there are recurring themes in the response of plants to invasion by a wide range of micro-organisms [74], presumably reflecting the exploitation of a common or overlapping set of host core components by the invader. We will attempt to draw parallels with other forms of root symbiosis and parasitism, and at times this will be speculative. We also will indicate other recent reviews on nematode-plant interactions, and endeavor not simply to reiterate what has already been said.

2. Parasitic nematodes and agriculture

Collectively, nematodes exploit all parts of vascular plants, but the most economically significant species infect the root. Partly because many of the effective control strategies (such as soil fumigants) also target other pathogens, the net impact of nematodes on yield is difficult to establish accurately. Based on extensive surveys [53,83], it has been estimated that the overall yield loss averages over 10%, with this figure approaching 20% for some crops. In monetary terms, worldwide losses certainly exceed $US100 billion annually. Most of the damage is caused by a relatively small number of the dozens of nematode genera that attack crops [72], principally the sedentary root-knot (*Meloidogyne* spp.) and cyst (*Globodera* and *Heterodera* spp.) nematodes, as well as several migratory nematodes (including *Pratylenchus* and *Radopholus* spp.).

Another way to consider the impact of plant-parasitic nematodes is through the management strategies employed in their control. In 1982, 109 million pounds of nematocide active ingredient were applied to crops in the U.S.A., at a cost exceeding $US1 billion [57]. However in recent decades, issues such as ground water contamination, mammalian and avian toxicity, and residues in food have caused much tighter restrictions on the use of agricultural chemicals, and in many countries effective nematocides have been, and continue to be, deregistered [89]. The literature also is replete with studies on organic means, such as green manures, to control nematodes, but assessing their effectiveness remains difficult. In one, four-year study in the Netherlands, researchers augmented organic approaches with chemicals, and found that nematocide application of more than three times the combined total of chemicals needed to combat insects, fungi and weeds was required for effective nematode control on experimental, sustainable farms [58].

Until environmentally-safe nematocides are developed, or practical bio-control agents identified, host resistance remains the most sound nematode management approach. Unfortunately, nematode resistance is yet to be identified for many crop plants, although several naturally occurring resistance genes have been cloned [95]. The potential use of these dominant loci to construct transgenic plants to circumvent breeding difficulties is an appealing approach. For example, transfer of cloned $Hs1^{pro-1}$ from a wild relative of sugar beet was shown to confer beet cyst nematode-resistance upon susceptible sugar beet roots [24]. However, experiments to transfer resistance from tomato into tobacco using the cloned *Mi-1.2* gene have so far been unsuccessful, for reasons that remain unclear [94]. Other approaches to make transgenic, nematode-resistant crop plants based on

an understanding of the host-parasite interaction have been proposed [13] and reviewed in detail [2].

3. Plant-parasitic nematode niches

The first recorded observation of a plant parasitic nematode was of *Anguina tritici* [71], a flower and seed pathogen of wheat and other small grains, but in fact nematodes occupy all parts of vascular plants including leaves (*Aphelenchoides* spp.), stems (*Bursaphelenchus xylophilus*), tubers (*Globodera rostochiensis*), bulbs (*Ditylenchus dipsaci*), corms (*Radopholus similis*) and, of course, roots (*Heterodera* and *Meloidogyne* spp.). Various classification schemes based on motility during feeding (migratory or sedentary) and the site of feeding within the root (endo- or ecto-) have been proposed for the root-parasitic species [30,47]. Root-knot nematodes are considered to be sedentary endo-parasites. But, however convenient these schemata are, they provide no information on mechanisms of the host-parasite interaction, nor do they give any clues to the evolution of parasitism. We contend that differences between parasitic strategies reflect adaptations to exploit different ecological niches within the host, and this is particularly true of feeding behavior and the nature of the feeding site induced. Largely because of the great economic import of the nematodes that induce them, the feeding cells induced by root-knot and cyst nematodes, termed giant cells and syncytia, respectively, have been the subject of numerous studies. These large feeding cells within the root have been scrutinized using both the light and electron microscope [4,27,50,51], and their anatomy and cytology is well established. Further, the ontogeny of syncytia [43] and giant cells [21] and the physiology of both types [44] are the subject of comprehensive reviews.

4. Root-knot nematode biology

Mature female root-knot nematodes release hundreds of eggs into a proteinaceous matrix on the surface of the root. Following a first moult in the egg, motile, second-stage (L2) larvae hatch in the soil and typically re-infect the same plant (nematode larvae are synonymously termed ‘juveniles,’ but cogent arguments [10] favour the former term). The *Meloidogyne* L2 (Fig. 1) is a non-feeding, developmentally arrested, long-lived dispersal stage, and can survive in the soil for weeks or even months on stored lipid reserves. Based on these criteria, root-knot nematode L2s have been compared to the *C. elegans* dauer larva [15], which serves as a model for the dispersal stage of many nematode species [78]. The *C. elegans* dauer is a facultative developmental stage. Entry to, and exit from this stage is controlled by the environmental cues of ‘food signal’ and nematode population density, which is established based on a secreted pheromone; the latter process is often termed ‘quorum sensing’. *Meloidogyne* L2 dauers destructively penetrate the root, preferentially in the zone of elongation or at the site of a lateral root emergence, and migrate intercellularly into the vascular cylinder, causing little or no injury. Once in the vascular cylinder, the nematode makes a commitment to establish a feeding site. Although the basis for this decision is unknown, the events that immediately ensue are central to the host-parasite interaction and involve dramatic changes both in plant and nematode.

The migration phase within the root is accompanied by extensive secretion of proteins by the larvae. Nematodes have a number of secretory systems, and there is little doubt that secretions play numerous rôles in the host–parasite interaction [20]. All plant parasitic nematodes have an extensible stylet (Fig. 1) connected to a muscular pharynx with three or five associated gland cells. Significantly, changes in morphology of the pharyngeal glands appear to correlate with the establishment of the parasitic interaction. In root-knot nematodes, the subventral glands are more active prior to host penetration, with a reduction of secretory activity coordinated with the induction of giant cells [33,34], at which time activity of the dorsal gland increases [9]. Various enzymatic functions for the secretions have been proposed, and convincing biochemical evidence obtained at least for the secretion of root-knot nematode-encoded cellulase [11]. However, it has not been until genes encoding gland proteins have been sequenced that the nature of the secretion products has been discerned with confidence. Lambert et al. [56] demonstrated expression of a gene in the pharyngeal glands of *M. javanica* postulated to encode chorismate mutase, but this was not formally shown to be secreted. Using monoclonal antibodies directed to subventral gland antigens truly demonstrated to be secreted [28], genes defining a small family of endoglucanases were isolated from cyst [86,99] and also root-knot nematodes [81]. Transcripts for these, and also for other enzymes including pectinases, polygalacturonase, and phenol oxidase have subsequently been identified in EST sequencing projects [66,76]. Expression of the *eng* genes, which encode cellulases used during migration and perhaps also host penetration, recapitulates subventral pharyngeal gland activity; their expression ceases, and expression of host cellulases begins at some point during feeding-site induction [38]. Two additional points are worth noting about these enzymes. Firstly, they are not found in any other animals, and have been postulated to have been acquired *via* horizontal gene transfer from prokaryotes ([14,56,86,99], Bird and Scholl; unpublished data). Secondly, it has been proposed that the potential oligosaccharide products of these enzymes function as a class of plant growth regulators [25], possibly implicating a rôle in feeding-site induction (see below).

Like *Meloidogyne*, the potato aphid *Macrosiphum euphorbiae* feeds via a stylet. The host is penetrated intercellularly until the stylet reaches the phloem sieve tubes, which are penetrated to permit ingestion of sap. Two

types of aphid secretion are involved in feeding: a secretion that solidifies to form the stylet sheath, and a watery saliva which does not gel, and is secreted both with, and independently of the sheath saliva [67]. The most important classes of enzymes present are pectinases and glucosidases and polyphenol oxidases [68,69]. It is necessary for aphids to penetrate the epidermal and mesophyl tissue to reach their feeding site, and pectinmethylesterase and polygalacturonase are present in the stylet sheath-forming saliva and are believed to be discharged into the phloem sap during feeding [60]. It would not surprise us to find a comprehensively overlapping complement of enzymes secreted into plants both by nematodes and aphids.

In *C. elegans*, recovery from the dauer stage (i.e. resumption of development and feeding) occurs rapidly (within 30 min) upon perception of 'food signal' [78]. Importantly, dauer recovery occurs prior to resumption of feeding, and we speculate that the same will be true for root-knot nematodes, with some host factor(s) defining the food signal. *C. elegans* dauer recovery is accompanied by dramatic biochemical, developmental, morphological and behavioural transitions [78], and this is the case for *Meloidogyne* too. Although the precise timing of these events is unknown, development resumes and the L2s begin to feed. The nematodes swell and the somatic musculature atrophies, rendering the nematodes sedentary. They continue to feed for several weeks before undergoing three superimposed moults to an adult female, or under environmental control, to a male.

Pari passu with changes in the nematode, striking changes also occur within the root, but the nature of these changes depends on the presence or absence of resistance loci. In a compatible host, stereotypical giant cells arise by expansion of individual parenchyma cells in the vascular cylinder close to the nematode's head. The developing cells undergo rounds of synchronous nuclear division uncoupled from cytokinesis, and individual nuclei become highly polyploid (Fig. 2). The cell wall is extensively remodeled, with the development of finger-like projections into the cell and a marked reduction in plasmodesmatal connections with cells other than neighboring giant cells. Giant cells function as carbon sinks, and have long been recognized as a type of transfer cell [50]. These events are tightly coupled to the developmental status of the nematode, and the giant cells (which serve as the sole nutritive source for the nematode) reach maximal size and activity at the onset of egg-laying [8]. Interestingly, the transition from a parenchyma cell to a fully differentiated giant cell occurs early in the parasitic association; once the giant cells have been initiated, their characteristics do not change appreciably throughout the period of nematode feeding (apart from getting bigger, having more nuclei, etc.). This is in marked contrast to determinant nitrogen-fixing nodules in which a programme of elaborate differentiation ensues [26]. In many hosts (but not all), cortical and pericycle cells around the giant cells expand and divide, resulting in the formation of a gall or knot which can lead to highly disfigured and functionally-compromised roots. Giant cells are central to the parasitic interaction, whereas the surrounding gall is presumably a secondary response.

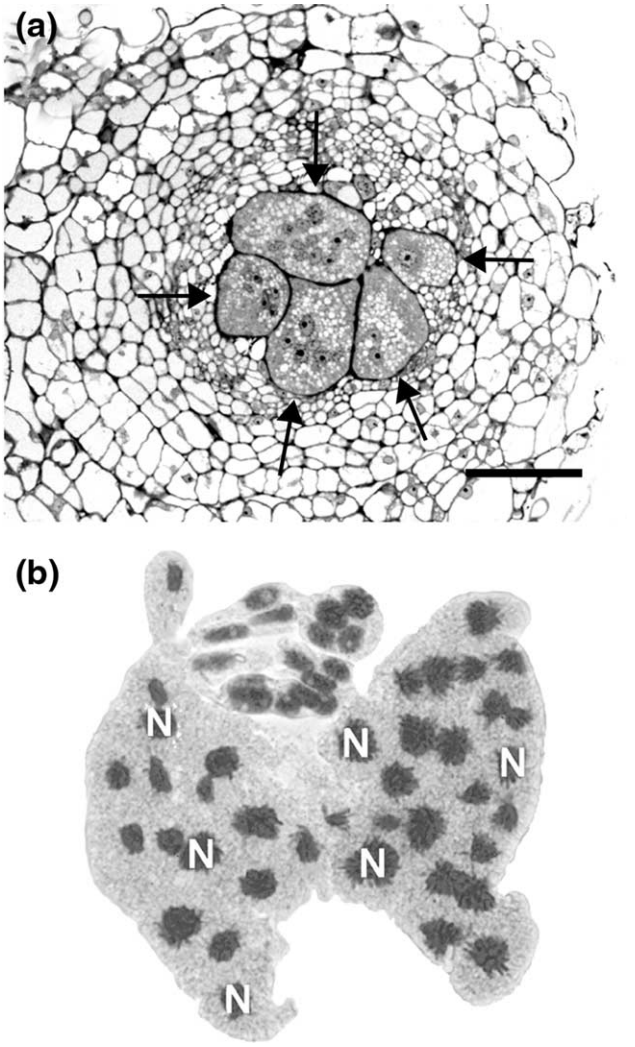

Fig. 2. Giant cells induced in tomato (*Lycopersicon esculentum*) roots by *Meloidogyne incognita*. (a) Toluidine blue stained, transverse paraffin section of a mature gall. Five giant cells are apparent (arrows). Scale bar: 100 μm. (b) A single, Feulgen-stained dissected giant cell with at least 51 mitotic nuclei (N) visible. Reproduced with permission from *Journal of Plant Growth Regulation* **19:** 183–194.

5. Giant cell induction

In 1937, Linford [59] speculated that feeding cells form in response to an inductive signal that emanates from the parasite. Although other models are possible, and in fact the most parsimonious alternative is that, as transfer cells, giant cells form simply in response to a unique sink function of the feeding nematode, most investigators support Linford's hypothesis [5,46]. However, the nature of the postulated inductive signal is subject to debate. Most researchers point

to secretory proteins originating in the pharyngeal glands [6,7,46,59], although the rôle of other secretory organs such as the amphids (chemosensory structures in the nematode's head) also has been formally discussed [12]. There is little doubt that proteinaceous secretory products play a critical role in the penetration and migratory phase of the root-knot nematode life-cycle. However, some models go as far as to postulate a physical interaction between pharyngeal gland proteins and host genes [47,96]. There is as yet, neither evidence to support nor refute a direct rôle for such proteins as being the inductive signal.

Induction of giant cells by root-knot nematodes is perhaps the most studied of the feeding sites and, although this process is still far from understood, a conceptual model in which giant cell formation is initiated via an incompletely executed host developmental programme has been proposed [13]. The temporal requirement for a specific inductive signal is unknown. In the developmental-switch model [13] a transient induction is sufficient, but it is clear that some ongoing interaction between parasite and giant cells is required as removal of the nematode leads to feeding site dissolution [5]. Whether this constitutive stimulus is simply a physiological effect caused by the metabolic sink of feeding [13,50] or something more specific, such as a nematode-synthesized ligand, remains unknown.

The ability of *Meloidogyne* to induce stereotypical giant cells in a vast range of vascular plants implies that the process must involve some fundamental and widely conserved aspect(s) of plant biology. Because of their central rôle in mediating developmental processes in plants, phytohormones are probably involved in feeding site formation, and indeed, may be the key factors in modulating this aspect of the host-parasite interaction. Although the older literature reports direct measurements of auxins and cytokinins during nematode infection these approaches were probably too insensitive and too low a resolution to be informative. More recently, transgenic reporter-gene constructs have been employed as a surrogate to map auxin levels in *Meloidogyne*-infected roots [45]. Induction of the *GH3* auxin-responsive promoter was observed in those parenchyma cells destined to become giant cells. These levels declined over several days, accompanied by apparent disruption of polar auxin flow at feeding sites. Auxin accumulated basipetal, and was reduced acropetal to the forming gall. Because flavonoids can affect auxin levels directly by interaction with auxin degrading enzymes [87] and indirectly by serving as auxin transport inhibitors [49], it was proposed that inhibition of auxin transport in the presence of the nematode is mediated through activation of the flavonoid pathway [45]. A similar induction of the flavonoid pathway has been observed in formation of lateral roots [75] and in *Rhizobium* nodules [64].

Evidence is accumulating to associate changes in phytohormone levels with transcriptional events inside giant cells and two tomato genes in particular, *Le-phan* and the *Tkn2 KNOX* gene, seem to be key players [14,54]. Members of the *KNOX* homeodomain gene are required for normal meristem function [42,91], and ectopic *KNOX* expression results in aberrant polar auxin transport [90]. A strict correlation between *KNOX* expression and elevated cytokinin levels has been observed, suggesting that cytokinins may either regulate *KNOX* expression or be a secondary signal regulated by *KNOX* [39,82]. *Le-phan* encodes a Myb transcription regulator [17,88] and it too is required for meristem function. Careful in situ analyses of *Le-phan* and *Tkn2* have shown these (and other) genes to be co-regulated both in giant cells and in the meristematic zones of nitrogen-fixing nodules [55]. It is appealing to speculate that a signal from the nematode directly (or indirectly) induces *PHAN/KNOX* expression which in turn activates the flavonoid pathway, resulting in altered auxin distribution. The rapid, but transient accumulation of auxin during the formation of giant cells is consistent with the developmental-switch model [13]. In this highly simplified model where signals are broadcast from the nematode, all cells in the vicinity might be expected to respond. Importantly, only that subset of cells competent to initiate a developmental programme is observed to respond further [21]. Pericycle cells, especially those that are outside the xylem poles and are the origin of lateral root meristems, were seen to divide, and vascular parenchyma cells began to develop into giant cells. In this sense, feeding site induction is typical of certain other hormonally-mediated, developmental events such as lateral root initiation and nodule growth [55].

Giant cells are a unique cell type, and presumably have a unique gene expression profile. Various strategies to identify these genes have been employed and have recently been extensively reviewed [36,41,44]. The most productive approach to identify transcripts that are expressed in giant cells and are not expressed in spatially or temporally equivalent healthy cells has been a subtractive cDNA cloning approach which defined hundreds of genes [17,97]. The sequences of these extensively annotated genes all are available from GenBank. Another productive experimental approach to understand giant cell formation has been to focus on the cell cycle events in feeding sites. Based on their cytogenetics (Fig. 2), it can be surmised that giant cells exhibit differences from typical mitotic cells in at least 3 points of the cell cycle: (1) giant cells re-enter the cycle (i.e. pass the G1 to S phase transition) without prior cell division; (2) the metaphase to anaphase transition is perturbed, resulting in endo-reduplication; and (3) the anaphase to telophase step is disrupted, leading to giant cells becoming multinucleate. Obviously, for any individual nucleus once mitosis is initiated the result will be either endoreduplication or nuclear division, but not both. Recent work in yeast has shown that these three points are major sites of cell cycle control. Because the cell cycle has been intensively studied in Arabidopsis, it has proven possible to probe giant cells by blocking various stages of the cycle using genetic and chemical inhibitors, and the results of

these experiments have been recently reviewed [40]. Importantly, it was found that blocking the cell cycle also arrests development of giant cells.

6. Host resistance

Dominant loci conferring resistance to root-knot nematodes have been identified in a number of plants, including tomato and tobacco. The best studied nematode-resistance gene is *Mi-1.2*, which has been cloned and found to be a member of the leucine zipper, nucleotide binding, leucine-rich repeat family of plant R genes [70]. This constitutively-expressed gene [63] confers resistance to *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, but not to *M. hapla*, even though these four species are present sympatrically. Resistance is accompanied by a hypersensitive response in the host, with a localized region of necrotic plant cells being visible around the head of the invading nematode within 12–24 h of inoculation of tomato roots with L2s. Through an elegant assay, a role for the leucine-rich repeat region and the amino-terminal domain of *Mi-1.2* in regulating localized cell death has been revealed [48]. If the nascent giant cells fail to develop or indeed, actually die, the now sedentary nematodes, robbed of their food supply, fail to develop.

The discovery that *Mi-1.2* also conditions resistance to the potato aphid, *Macrosiphum euphorbiae*, implies that a more expansive outlook might be productive in understanding the nematode-root interaction. Indeed, *bona fide* giant cells are induced under experimentally-contrived circumstances in which root-knot nematodes are injected into the tobacco-leaf mid-vein [77]. Thus, we suspect that nematode behavioural constraints restrict *Meloidogyne* to the roots, rather than any unique feature of root biology *per se*. Electronic monitoring studies of aphid feeding behaviour indicate that in the presence of *Mi-1.2* aphids no longer ingest vascular fluids, although they are able to reach the sieve elements. Although aphids die as early as 24 h after transfer to resistant plants there is apparently no hypersensitive response induced and, because aphids appear to recover fully when transferred from resistant to susceptible tomato [52], one can surmise that death is probably due to desiccation and/or starvation. Thus, *Mi-1.2* appears to modify aphid feeding behaviour, and it is an intriguing possibility that the same also may be true for nematodes. It may not be the cell death aspect of the hypersensitive response which deprives root-knot nematodes of nutrition, but an anti-feedant activity. Interestingly, although *Meloidogyne arenaria* induces a robust hypersensitive response on tobacco carrying *rk*, such plants are not resistant to this nematode species (although *rk* does condition resistance to other root-knot species). One explanation is that *M. arenaria* is not responsive to an *rk*-associated anti-feedant signal. Like resistance to root-knot nematodes, *Mi-1.2*-mediated aphid resistance is highly specific, and is limited to certain *Macrosiphum* biotypes [80]. *Mi-1.2* is presumed

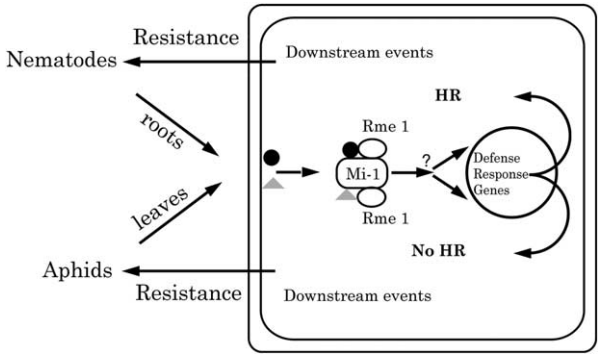

Fig. 3. A schematic model of the *Mi-1*-mediated resistance pathway.

to act in a classical gene-for-gene manner, but as the nematodes and aphids affected by *Mi-1.2* are parthenogenic, this hypothesis remains untested.

Mechanistically, it is possible that *Mi-1.2* encodes a shared trigger in the resistance pathway for aphids and nematodes, and that the pathways diverge downstream from *Mi-1.2* (Fig. 3). A genetic-suppressor screen for pathway components revealed the tomato *Rme1* locus [62], which is required for *Mi-1.2*-mediated resistance both to root-knot nematodes and the potato aphid. Importantly, *Rme1* function was found not to be required for expression of the tomato *I-2* gene. *I-2* is similar to *Mi-1.2* in that it encodes an NBS-LRR protein, but distinct in that it confers resistance to the fungal pathogen *Fusarium oxysporum* f.sp. *lycopersici* race 2 [85]. It is an intriguing possibility that *Rme1* might directly interact with *Mi-1.2*, but this is yet to be proven. Nevertheless, differences between the resistance to nematodes and the aphid have been noted. Resistance to nematodes is inherited in a dominant fashion while resistance to aphids is semi-dominant. Resistance to aphids, but not to nematodes, is developmentally regulated; seedlings up to four-week-old are susceptible to aphids, whereas six-week-old plants are resistant.

Mi-1.2-mediated resistance is lost at temperatures above 28°C, and in an elegant experiment, it was found that the temperature sensitive period is limited to the first 24–48 h after infection [31]. This suggests that determination of resistance occurs soon after the nematode reaches its feeding site, but whether *Mi-1.2* functions downstream of feeding initiation and/or giant cell induction is unknown. If resistance does supervene giant cell formation, there is presumably no *a priori* requirement for the *Mi-1.2* product to interact with any putative nematode avirulence factor. Dropkin et al. [31] also observed that *Mi-1.2* function can be experimentally over-ridden by exogenous cytokinin. Given the compelling data correlating phytohormones with the compatible interaction, it is possible that parasitism by those root-knot species not subject to *Mi-1.2* action (such as *M. hapla*) involve different host hormonal responses. Intriguingly, unlike the other common *Meloidogyne* species, *M. hapla* does not induce pronounced secondary galls.

So, are roots special? Of course, to many botanists, they are, but other organisms may not agree. Like rhizobia and aphids, root-knot nematodes interact with and exploit many general features of host biology. As our knowledge of these 'normal' processes grows, fueled at the moment by genomics, our understanding of interactions with other organisms will grow too. That knowledge will in turn serve to shed light on many aspects of plant biology, including roots.

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