

Chemical Hide and Seek: Nematode's Journey to Its Plant Host

Plant-parasitic nematodes are among the most destructive plant pathogens, causing estimated damage of more than US\$100 billion per year. An expert-based assessment of crop health listed nematodes as among the most damaging pests and pathogens in potato, soybean, and wheat crops (Savary et al., 2019). Most damage is caused by a small group of root-infecting sedentary endoparasitic nematodes including cyst nematodes and root-knot nematodes (RKNs). Second-stage juveniles (J2s) of plant-parasitic nematodes hatch from eggs into the soil and localize to the roots of host plants. The success of these non-feeding J2s depends on their ability to locate and infect a suitable host. The attraction of plant-parasitic nematodes to the host root has been a subject of study for the past 80 years following the pioneering research that demonstrated the attraction of RKN specifically toward root tips (Lindford, 1939). Both volatile and soluble components in the rhizosphere have been shown to influence nematode movement. Methyl salicylate, a volatile chemical root signal, has been demonstrated to be a strong root attractant for RKN toward several solanaceous plants (Kihika et al., 2017; Murungi et al., 2018). The non-volatile tomato root exudate quercetin was shown to elicit a concentration-dependent attraction or repulsion effect against *Meloidogyne incognita* to host root (Kirwa et al., 2018). A comprehensive list of water-soluble and volatile root-exuded signals implicated in nematode response to root is compiled in a recent review by Sikder and Vestergård (2020). Three recent studies have revealed that the recognition of and response to hosts by infective juveniles is a complex process with multiple stages during their interaction.

NEMATODE ATTRACTION TO HOSTS IS MEDIATED BY MULTIPLE FACTORS

While RKN are primarily attracted to the tips of growing roots where invasion occurs, Tsai et al. (2019) found that J2 of the three RKN species (*M. incognita*, *Meloidogyne arenaria*, and *Meloidogyne enterolobii*) also displayed positive chemotaxis toward imbibing *Arabidopsis* seeds. Seed-coat mucilage, a gelatinous matrix of various cell-wall carbohydrates, is extruded from the seed coat upon imbibition. To determine whether mucilage is involved in RKN attraction to seeds, Tsai et al. (2019) analyzed the behavior of RKN in response to *Arabidopsis* mucilage mutant seeds. RKN attraction was reduced in a collection of mutant seeds that either cannot secrete mucilage into the apoplast (*mucilage-modified 1*, *mum1*; *glabra*, *gl2*; *apetala*, *ap2*; *mucilage-modified 4*, *mum4*; *transparent testa glabra*, *ttg1*) or do not extrude mucilage upon imbibition (*mucilage-modified 2*, *mum2*; *subtilisin-like serine protease*, *sbt1.7*). Furthermore, incubation of wild-type seeds with proteases or various polysaccharide-digesting enzymes decreased RKN attraction, implying that mucilage proteins and carbohydrates contribute to the response (Tsai et al., 2019). The

carbohydrate glucomannan was implicated in RKN attraction due to the substantial decrease in RKN attraction to seeds mutant for the glucomannan synthase (*cellulose synthase-like 2*, *csla2*), even though they extrude mucilage normally. However, the nature of glucomannan's role in RKN-host interactions remains unclear, as RKN attraction to different *endo- β -mannanase* (*Atman5*, *Atman6*, *Atman7*) mutants is for the most part unaltered. Notably, RKN attraction to dissected embryos or to mucilage separated from seeds was low. To reconcile these observations, Tsai et al. (2019) proposed a model in which an attractant is produced by an unknown enzyme upon imbibition, extruded in the seed-coat mucilage, and dispersed into the spermosphere, where it can be detected by nematodes.

While the nematodes are attracted to and accumulate around imbibing seeds, they do not infect the host at this stage. In addition, when continuously exposed to nematodes, roots of *csla2csla3csla9* triple-mutant seedlings become infected at a rate similar to that of wild-type, suggesting that attraction to root tips, where invasion occurs, is mediated by independent factors (Tsai et al., 2019). In that case, what are the attractants in root exudates? Another recent study provides some clues. Oota et al. (2019) stepped away from mutant analysis and instead employed chemical library screening and time-of-flight secondary ion mass spectrometry/scanning electron microscopy (cryo-TOF-SIMS/SEM) to reveal polyamines as potential RKN attractants. Among the screened synthetic polyamines, three to five carbon compounds with two terminal amino groups seem to specifically attract RKNs, showing 1,3-diaminopropane, putrescine, and cadaverine as the most promising candidates (Oota et al., 2019). Postulating cadaverine to be a plant stress signaling molecule, Oota et al. (2019) proposed a model in which cadaverine is synthesized in the root cortex and secreted into the rhizosphere to form a chemical gradient (visualized by cryo-TOF-SIMS/SEM) that RKNs detect as a marker of vulnerable stressed plants.

However, the mechanism by which these diamines attract RKN remains unclear. Oota et al. (2019) have suggested that their terminal amino groups alter the pH of the root rhizosphere as RKN accumulate in the pH range of 4.5–5.4 (Wang et al., 2009). However, terminal amino groups do not seem to be the definitive characteristic of attractants, given that 1,6-diaminohexane, 1,7-diaminoheptane, 1,8-diaminooctane, and 1,9-diaminononane do not attract RKN. It also seems that diamine content in root exudate is not the sole factor determining nematode attraction to hosts: despite having a lower diamine

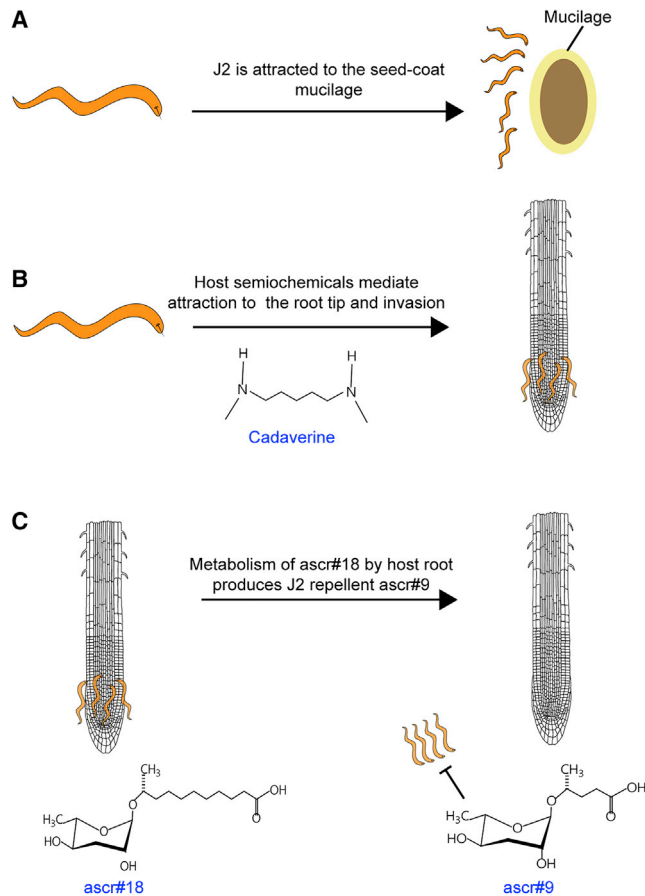


Figure 1. Recognition of and Response to Hosts by Nematodes Involves Multiple Stages during Their Interaction.

(A) Pre-infective juveniles (J2s) of root-knot nematodes are attracted to the seed-coat mucilage but they do not infect seeds.
(B) Naturally occurring polyamines including cadaverine from host roots mediate attraction and invasion of J2s to the root tip.
(C) Metabolic processing of nematode pheromones ascr#18 by host roots release ascr#9 into rhizosphere that is repellent to J2s.

content, soybean root exudate is a more effective attractant than tomato root exudate (Oota et al., 2019). This result ultimately implies that the ratio between RKN attractants and repellents is what determines the strength of nematode attraction to host plants.

ASCAROSIDES: NEW PLAYERS IN THE GAME

Recent findings suggest that plants can also modulate nematode infection by modifying nematode-produced pheromones (Manohar et al., 2020). Ascarosides, glycosides of the dideoxy sugar ascarylose with a fatty acid-derived side chain and in some cases other decorations, are widely utilized individually or as blends to mediate communication between nematodes.

It has been previously demonstrated that exposing plants to ascr#18, the most abundant ascaroside found in RKN and several other plant-parasitic nematode species, activates pattern-triggered immunity (PTI) in plants, similarly to pathogen-

associated molecular patterns derived from other pathogens (Manosalva et al., 2015). However, signaling pathways including the identity of the receptor involved in ascr#18 perception have not yet been identified. Using a metabolomics approach, Manohar et al. (2020) demonstrated that both monocot and dicot plants metabolize exogenously applied ascr#18 using the peroxisomal β -oxidation pathway. The shorter side-chained ascarosides, predominantly ascr#9, are then released from the roots. Assays with J2s showed that ascr#9, or the combination of ascr#9 and ascr#18, was repellent to the worms. Experiments further suggested that this repellency, rather than PTI, is the major cause of reduced infection by nematodes following treatment with ascr#18 (Manohar et al., 2020). While details remain to be determined, including the mechanism by which ascr#18 is internalized and delivered to the peroxisome, this work introduces another level of complexity in nematode recognition and infection of hosts.

The aforementioned recent studies have made it clear that a plethora of compounds from host root and seed exudates mediate nematode attraction to roots at different stages (Figure 1). Mucilage attraction positions the nematode near the root, where it is ready to invade upon germination. A second set of compounds may direct the nematode to invasion sites in the root. After arrival at the root, modification of nematode pheromones by the host may help to control the level of infection. Each step has the potential to explore for management by understanding this highly evolved interaction. This may be the tip of the iceberg as soil composition and other components of the rhizosphere interact with nematode attractants/repellents to mediate RKN response.

FUNDING

The work in the Siddique lab is supported by the United States Department of Agriculture (project no. CA-D-ENM-2562-RR).

ACKNOWLEDGMENTS

We are thankful to Rangswamy Meganathan for drawing chemical structures. We also acknowledge Plant Illustrations (<https://doi.org/10.6084/m9.figshare.c.3701038.v12>) for root illustration. No conflict of interest declared.

Natalie Hamada^{1,3}, Henok Zemene Yimer^{1,3},
 Valerie M. Williamson² and Shahid Siddique^{1,*}

¹Department of Entomology & Nematology, University of California Davis, Davis, CA 95616, USA

²Department of Plant Pathology, University of California Davis, Davis, CA 95616, USA

³These authors contributed equally to this article.

*Correspondence: Shahid Siddique (ssiddique@ucdavis.edu)
<https://doi.org/10.1016/j.molp.2020.03.005>

REFERENCES

- Kihika, R., Murungi, L.K., Coyne, D., Ng'ang'a, M., Hassanali, A., Teal, P.E.A., and Torto, B. (2017). Parasitic nematode *Meloidogyne incognita* interactions with different *Capsicum annum* cultivars reveal the chemical constituents modulating root herbivory. *Sci. Rep.* 7:2903.
- Kirwa, H.K., Murungi, L.K., Beck, J.J., and Torto, B. (2018). Elicitation of differential responses in the root-knot nematode *Meloidogyne incognita* to tomato root exudate cytokinin, flavonoids, and alkaloids. *J. Agric. Food Chem.* 66:11291–11300.

Spotlight

- Lindford, M.B.** (1939). Attractiveness of roots and excised shoot tissues to certain nematodes. *Proc. Helminthol. Soc. Wash.* **6**:11–18.
- Manohar, M., Tenjo-Castano, F., Chen, S., Zhang, Y.K., Kumari, A., Williamson, V.M., Wang, X., Klessig, D.F., and Schroeder, F.C.** (2020). Plant metabolism of nematode pheromones mediates plant-nematode interactions. *Nat. Commun.* **11**:208.
- Manosalva, P., Manohar, M., von Reuss, S.H., Chen, S.Y., Koch, A., Kaplan, F., Choe, A., Micikas, R.J., Wang, X.H., Kogel, K.H., et al.** (2015). Conserved nematode signaling molecules elicit plant defenses and pathogen resistance. *Nat. Commun.* **6**:7795.
- Murungi, L.K., Kirwa, H., Coyne, D., Teal, P.E.A., Beck, J.J., and Torto, B.** (2018). Identification of key root volatiles signaling preference of tomato over spinach by the root knot nematode *Meloidogyne incognita*. *J. Agric. Food Chem.* **66**:7328–7336.

Molecular Plant

- Oota, M., Tsai, A.Y.-L., Aoki, D., Favery, B., Ishikawa, H., and Sawa, S.** (2019). Identification of naturally occurring polyamines as nematode *Meloidogyne incognita* attractants. *Mol. Plant* <https://doi.org/10.1016/j.molp.2019.12.010>.
- Savary, S., Willocquet, L., Pethybridge, S.J., Esker, P., McRoberts, N., and Nelson, A.** (2019). The global burden of pathogens and pests on major food crops. *Nat. Ecol. Evol.* **3**:430–439.
- Sikder, M.M., and Vestergård, M.** (2020). Impacts of root metabolites on soil nematodes. *Front. Plant Sci.* **10**:1792.
- Tsai, A.Y.L., Higaki, T., Nguyen, C.N., Perfus-Barbeoch, L., Favery, B., and Sawa, S.** (2019). Regulation of root-knot nematode behavior by seed-coat mucilage-derived attractants. *Mol. Plant* **12**:99–112.
- Wang, C.L., Bruening, G., and Williamson, V.M.** (2009). Determination of preferred pH for root-knot nematode aggregation using Pluronic F-127 gel. *J. Chem. Ecol.* **35**:1242–1251.