

Plant Infection by Root-Knot Nematode

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Abstract Plant-parasitic nematodes, particularly the sedentary endoparasitic forms, are cosmopolitan pests, collectively causing over \$100 billion in annual crop loss worldwide. In the past decade, significant progress has been made in identifying genes and their products that define key aspects of the host–parasite interface, including enzymes and proteins with direct roles in virulence and resistance. However, little remains known about how a host is identified or how the development of the nematode is coupled to establishment of the parasitic interaction. Here, we consider the role of signaling molecules and their interplay with nematode development from hatch through primary interaction with the plant.

1 A Brief Introduction to Root-Knot Nematode

Although plant-parasitic nematodes are found in three of the five major clades of the phylum Nematoda (Blaxter et al. 1998), much of the damage to crops is caused by the approximately 60-member tylenchid genus, *Meloidogyne* (Sasser and Freckman 1986; Koenning et al. 1999). Reflecting the gross symptoms exhibited by roots infected with these nematodes (Fig. 1), the common name for *Meloidogyne* spp. is “root-knot nematode(s).” More than 2,000 plant species have been designated as hosts to root-knot nematodes, and most cultivated crops are attacked by at least one root-knot nematode species (Sasser 1980). Since its description as a genus (Chitwood 1949), root-knot nematodes have been particularly favored for research by plant nematologists in large measure because of their importance as agricultural parasites. Beyond this, however, the motivation to study root-knot nematode has sprung from scientific curiosity regarding the many intriguing features of their

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Fig. 1 Symptoms of root-knot nematode infection. Root systems of *Medicago truncatula* plants inoculated with *Meloidogyne incognita*. The plant on the *left* carries a gene conferring resistance to *M. incognita*, whereas the plant on the *right* is susceptible. Characteristic root knots (galls) are evident on the roots of the infected plant

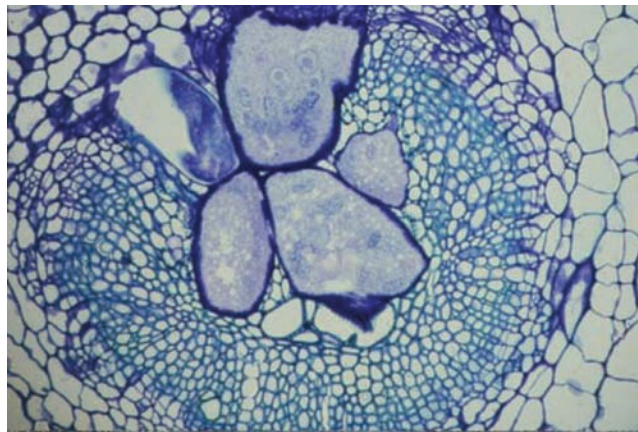


Fig. 2 Transverse section through a mature root gall induced by *Meloidogyne incognita* in tomato, stained with toluidine blue. Four giant cells are evident in the center of the vascular cylinder, surrounded by numerous small cells. The head of the nematode has contracted during fixation, leaving a partially hollow space adjacent to the giant cells

parasitic lifestyle, the most striking of which is the induction of so-called “giant cells” in the host root vasculature (Fig. 2). Induction of giant cells uniquely defines the *Meloidogyne*–host interaction and is central to it because these cells apparently serve as the sole food source for the developing worm.

Very briefly, root-knot nematodes hatch in the soil as motile, vermiform larvae (Fig. 3) able to locate, penetrate and migrate within plant roots (Fig. 4), ultimately



Fig. 3 Newly hatched *Meloidogyne* J2. Arrows point to some of the numerous lipid storage vesicles throughout the nematode's body, and the bar (S) indicates the retracted stylet

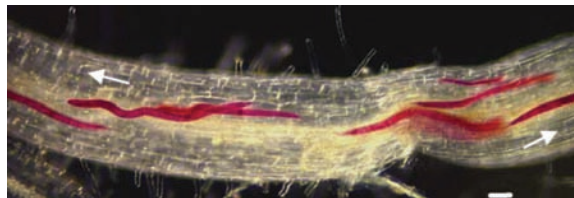


Fig. 4 Root-knot nematode J2 migrating through cleared *Lotus japonicus* roots. The worms were stained with acid fuchsin

reaching the developing vascular cylinder where the giant cells are established. Giant cell formation, coupled with expansion and proliferation of nearby pericycle and cortical cells, results in the characteristic root-knot gall. Like many other root-colonizing organisms, root-knot nematodes reside in the apoplast once inside the plant, obtaining nutrition from the symplast through an as yet poorly understood process. Mature females lay eggs out into the soil to complete the lifecycle.

This chapter focuses on the biological events that lead the root-knot nematode to its selection of a host and the irrevocable commitment by the parasite to a sedentary lifestyle. In other words, we consider the events that occur between hatch and the first meal, ending our discussion prior to giant cell ontogeny and operation (Gheysen and Mitchum 2008; Berg et al. 2008). Our focus is on the nematode rather than the host, although in reality both must play equally in the host–parasite interaction.

Our intent is twofold. First, we will discuss events that take place prior to root penetration, arguing that nematode behavior reflects responses to multiple environmental signals. Because little is known yet about the nature of such signals, this will be a short section by necessity. One considerable impediment to progress stems from the fact that the biology prior to host penetration occurs within the complex four-dimensional milieu that is the rhizosphere and surrounding soil. Although some studies attempted to make direct observations of nematodes in the soil (e.g., Pitcher 1967), most of our current understanding comes from analysis of in vitro systems. In the soil, the host for the root-knot nematode is very literally a “moving

target” that is not well modeled in vitro. Similarly, once the nematode has penetrated its host, direct experimental manipulation becomes extremely difficult. Not surprisingly, most of what is known of the biological events associated with root-penetration and subsequent migration comes either from destructive analysis (e.g., following fixation and staining) or from inference based on in vitro experiments.

For at least 40 years (e.g., Bird 1964), a particular focus has been on the proteins secreted by the root-knot nematode second-stage juvenile (J2) during and after migration through the root, and a picture is emerging of the myriad roles played by these proteins (e.g., Baum et al. 2007). We will make no further mention of these proteins in this chapter, not to diminish their importance, but because they are discussed in detail elsewhere in this volume (Davis et al. 2008). Our second goal, therefore, is not to describe the “machinery” deployed by the J2, but rather to frame the events that lead to host selection as a behavioral response by the nematode that culminates in an irrevocable developmental commitment. Despite only a limited data set in this area, a picture is emerging of complex communication between host and parasite that likely influences the behavior of both. Additional signaling between other environmental components, including other rhizosphere organisms, contributes to the complexity. Deciphering these networks may be an important step towards truly understanding plant infection by root-knot nematode.

2 The Root-Knot Nematode Larva at Hatch

Like all nematodes, root-knot nematode embryogenesis/morphogenesis occurs within an environmentally resilient egg, whose shell is principally composed of protein (50%), chitin (30%), and lipid (Bird and McClure 1976). The egg is the most robust life stage of the nematode and precludes passage of even small molecules (such as the fungal toxin α -amanitin) that readily penetrate the cuticle of hatched stages (Rogalski and Riddle 1988). Rendering the egg sensitive to α -amanitin requires the drastic treatment of chitinase digestion followed by mechanical stripping of the vitelline membrane (Edgar et al. 1994). Thus, for root-knot nematodes there is no evidence of the developing larvae perceiving external clues, but it is not inconceivable that such events may take place. Indeed, other tylenchid nematodes, particularly *Globodera* species, almost completely depend on perception of a host-derived signal to induce substantial hatch. On the basis of purification from potato root diffusate, one component of the hatch signal has been proposed to be *trans*-2-(2,13-dihydroxy-9-methoxy-7,7,16-trimethyl-5,10,20-trioxo-19-oxahexacyclo[9.7.0.1³.6.0³.8.1¹².15.0¹².16]-eicosa-1(11),8-dien-15-yl) cyclopropanecarboxylic acid (Mulder et al. 1996).

Immediately prior to hatch, the root-knot nematode eggshell undergoes structural transformation, rendering it permeable to a number of reagents, such as the electron microscopy fixative/stain osmium tetroxide, to which younger eggs are resistant (e.g., Fig. 5 in Bird and Bird 1991). Unlike most nematodes, root-knot nematodes undergo the first of their larval four molts within the egg, thus hatching

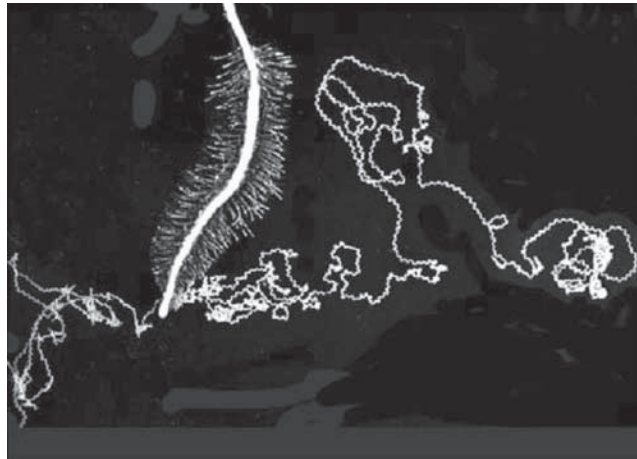


Fig. 5 Root-knot nematode attraction to root. Darkfield image of the track left in an agar surface by two root-knot nematode individuals as they migrated to the root tip

as a J2. Very little is known about the relative importance of eggs and J2s in dormant periods of root-knot nematode such as over wintering or between hosts; however, it is likely that the J2 is the predominant dormant stage because hatch does not require an external cue. Indeed, numerous lines of evidence point to the root-knot nematode (and other tylenchid nematodes) J2 as being analogous to the dauer (“enduring”) stage of *Caenorhabditis elegans* (Riddle and Georgi 1990; Bird and Opperman 1998; Opperman and Bird 1998). The dauer was first described as an adaptation by animal-parasitic nematodes (Fuchs 1915), but subsequently appreciated as a phylum-wide phenomenon, extending to plant-associated genera as well (e.g., Fuchs 1937; Bird and Buttrose 1974). Dauers share the properties of arrested development, motility, non-feeding, non-ageing and hence longevity (Cassada and Russell 1975; Klass and Hirsh 1976; Riddle and Albert 1997), attributes that accurately describe root-knot nematode J2s. *C. elegans* dauers also exhibit characteristic morphological features, such as sparse (compared to L3) luminal microvilli, numerous lipid storage vesicles, and a denser cuticle that results in elevated detergent resistance; these features all have been found in tylenchid J2s (Endo 1988; Opperman and Bird 1998) (Fig. 3). A consequence of the suspension of ageing by the root-knot nematode dauer is that the time spent as a J2 largely determines the egg-to-egg time for any given individual.

Dauers have been most extensively studied in *C. elegans*, where they function as facultative, alternative stage-three larvae (L3) and serve as a binary switch to broadly couple larval development to sexual maturity with “boom” (L3) or “bust” (dauer) conditions. On the basis of an elegant amalgam of genetic, biochemical and developmental experiments (reviewed by Riddle and Albert 1997), it was shown that the stage-one larvae (L1) integrates the environmental cues of “dauer-pheromone” and “food signal” (and, to a lesser extent, temperature) to instruct the stage-two larvae (L2) development and the product of the L2 molt (Golden and Riddle 1982).



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