CHAPTER 8

Foraging Nematodes—Hidden Enemies of Forage Production

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Nematodes are translucent, microscopic roundworms that inhabit all agricultural soils and typically measuring 0.25 to 1 mm (0.01 to 0.04 in) in length and only about 0.1 to 0.2 mm (0.004 to 0.008 in) in diameter (Fig. 1). Their abundance ranges from about 1000/l (1000/qt) soil in heavily tilled soil with low organic matter to about 50,000/l of fertile soil with a history or organic matter inputs. Most nematodes in soil are "free-living" species that feed on bacteria or fungi, and are known as bacterivores and fungivores, respectively. Although less abundant, there are also free-living nematodes that feed on a diversity of soil organisms (omnivores) and on other nematodes (predators). Most agricultural soils also harbour one to several species of nematodes that feed on plant roots.

All nematodes feeding on plant roots use piercing-sucking mouthparts called stylets to puncture the cell walls of fine roots and extract cellular contents (Fig. 1). Some species have particularly long stylets and feed only on root cells that can be reached from outside the root; these nematodes are known collectively as ectoparasites. Other species completely enter root

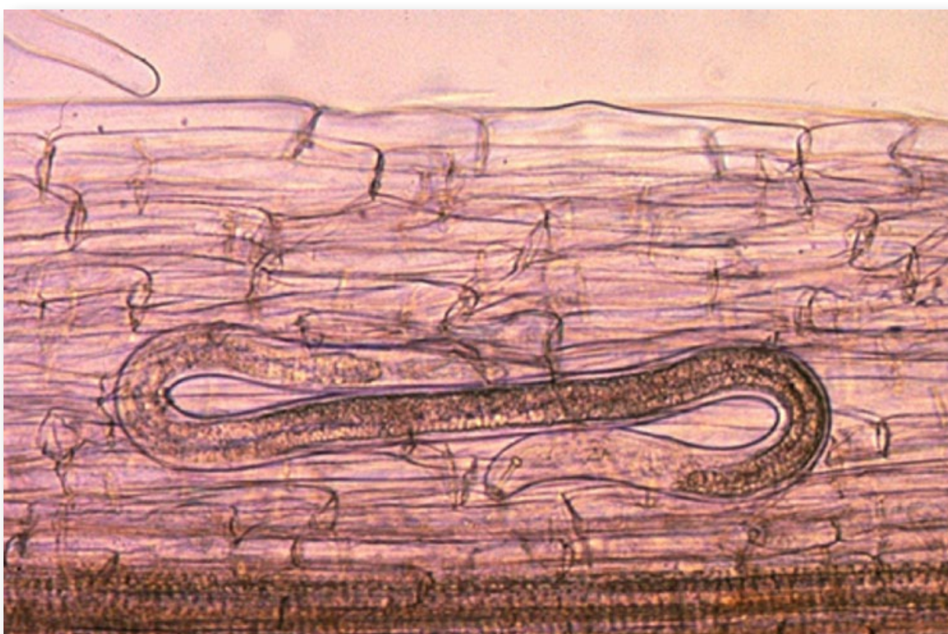

Figure 1. Adult female *Pratylenchus penetrans* in root tissue. Photo courtesy of Dan Wixted, University of Wisconsin.

tissue and move from cell to cell within the root as they feed; these nematodes are known as *migratory* endoparasites. The root-knot nematodes are *sedentary* endoparasites which establish permanent feeding sites within the root tissue, sometimes forming swellings known as galls. Some plant-feeding nematodes can be conceptualized as herbivores (e.g. ectoparasites and some migratory endoparasites with wide host ranges), while many others are conceptualized strictly as parasites. For the purpose of this article we will refer to them collectively as plant-feeding nematodes.

Many species of plant-feeding nematodes are well recognized as damaging pathogens of high value vegetable and fruit crops. Due to the lower value of forage crops, the impacts of nematodes on forage crops have not been studied as extensively and are not as well recognized. The research performed to date, however, indicates that plant-feeding nematodes can have important influences on forage production and deserve more research attention.

General nature of nematode damage

Much of the crop damage caused by plant-feeding nematodes is overlooked because aboveground symptoms are non-specific and difficult to distinguish from other stresses such as uneven water availability, salts, or even nutrient deficiencies. Even belowground, the symptoms of most nematodes are not obvious, and damage is typically manifest as reduced overall root growth. The primary exceptions are nematodes in the genus *Meloidogyne* (root-knot nematodes) which cause visible galls or "knots" on roots.

Nematode populations usually have a patchy distribution in fields, and nematode damage is often observed as patches of poor, sometimes chlorotic growth within otherwise uniform fields. Patches of high nematode population densities may, however, correspond with other soil factors that also affect crop growth. For example, some nematodes reach higher population densities and cause more damage in coarse than in fine textured soils. As a result, poor crop growth observed on sandy soils can be the result of the interaction of low water or nutrient availability with nematode damage.

Given the subtle nature of nematode damage, how do we know that they affect forages?

Evidence for the impacts of nematodes typically comes from two different types of experiments. The most common approach involves growing the host plant species in soil to which a pure population of the nematode species of interest has been added, in comparison to plants grown in non-infested soil. This approach clearly isolates the effect of the nematode population of interest, but the plants are usually grown in pots in a greenhouse and do not experience environmental fluctuations. The effects of nematodes are mediated by environmental conditions and the effects may be overlooked when plants are grown under optimal conditions. The second approach involves comparing crop responses in nematicide-treated and non-treated crops in the field. This approach allows for observation of crop response under normally variable environmental conditions; however, nematicides can affect other deleterious organisms so positive crop responses can result from suppression of species such as herbivorous insects. Thus both techniques are needed to clarify the effects of nematodes.

Effects of nematodes on forage grasses

Several nematicide studies on mixed-species grazing land in the semi-arid western U.S. indicate that plant-feeding nematodes may suppress net primary production by 10–30% (Ingham and Detling 1990, 1984; reviewed in Stanton et al. 1981). In one study, the approximately 26% decrease in production attributed to plant-feeding nematodes was about 16X more than the amount of root tissue estimated to have been consumed by the nematodes (based on their biomass), indicating that impacts of the nematodes on plant physiology were beyond simple removal of root tissue (Ingham and Detling 1990). A series of greenhouse pot

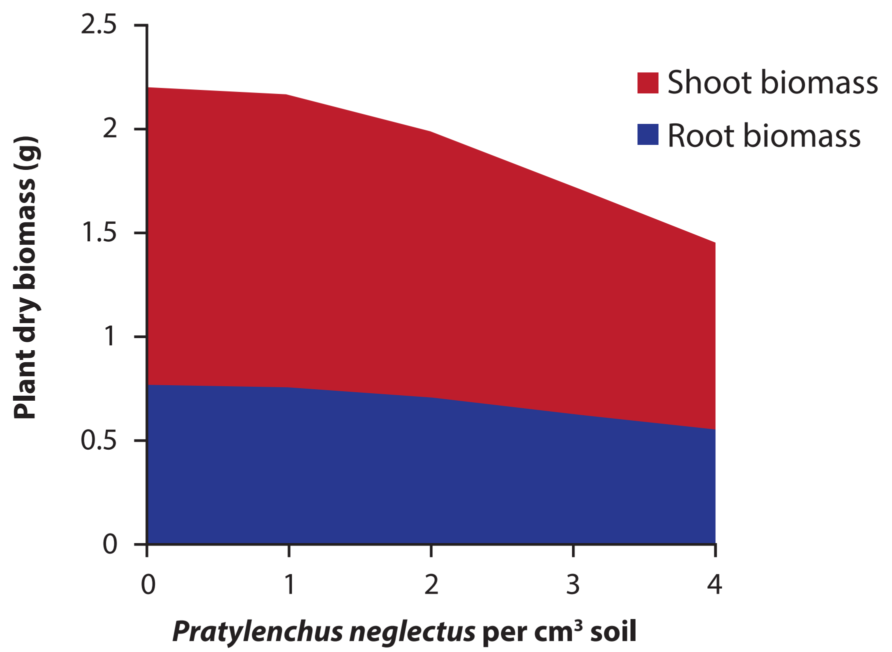

Figure 2. Effects of variable initial population densities of the root lesion nematode, *Pratylenchus neglectus*, on biomass of crested wheatgrass plants grown in a greenhouse for 170 days. Data are re-drawn from Griffin (1994) and averaged over five wheatgrass varieties.

studies reinforce the nematicide studies by demonstrating the potential for a common species of root lesion nematode, *Pratylenchus neglectus*, to reduce growth of several varieties of crested wheatgrass (*Agropyron cristatum, A. desertorum*), intermediate wheatgrass (*Thinopyrum intermedium*) and Snake River wheatgrass (*Elymus lanceolatus*) (Fig. 2; Griffin 1992, 1994). *P. neglectus* is distributed throughout western North America, and is well recognized as a significant pathogen of cereal crops in western North America and Australia (Smiley et al. 2005; Vanstone et al. 1998).

Nematicide experiments in the southeastern U.S. have similarly shown that mixed communities of nematodes can have significant effects on production and persistence of phalaris (*Phalaris aquatica*) and tall fescue (*Festuca arundinacea*) (Hoveland et al. 1975). Nematode species in those trials included *Trichodorus christei* (stubby root nematode), *Tylenchorhynchus claytoni* (stunt nematode) and *Hoplolaimus galeatus* (lance nematode). In greenhouse studies, mixed populations of plant-feeding nematodes reduced forage yields of small- and large-rooted genotypes of tall fescue by 9 and 50%, respectively (Elkins et al. 1979). Nematicide and greenhouse experimental approaches were both used to show that another species of root lesion nematode, *Pratylenchus penetrans,* is able to reproduce on a wide variety of forage grasses grown in the upper midwest and eastern parts of North America and the nematode was found to significantly reduce growth of reed canarygrass, perennial ryegrass and tall fescue (Thies et al. 1995). Over all grass species, there was a significant negative correlation between nematodes per gram of root and root weights.

Fungal endophytes

Fungal endophytes in the genus *Neotyphodium* commonly colonize tall fescue, perennial ryegrass and other *Lolium* species. These endophytes can produce ergot alkaloids that are toxic to cattle, but they have also been associated with increased grass tolerance to water stress and resistance to insect herbivores. Research has shown that the presence of these endophytes can also confer resistance to plant-feeding nematodes in the genera *Pratylenchus* (Kimmons et al. 1990; West et al. 1988), *Meloidogyne* (Kimmons et al. 1990) and *Tylenchorhynchus* (West et al. 1988). *Helicotylenchus pseudorobustus*, an ectoparasite in the group known as spiral nematodes, is commonly associated with grasses but is the only plant-feeding nematode species tested to date that is not inhibited by endophyte (Kimmons et al. 1990; Davis et al. 2004).

Endophyte-infected and endophyte-free tall fescue was grown in nematode infested soil under a range of soil moisture regimes (West et al. 1988). In addition to reducing nematode populations in the rhizosphere, presence of the endophyte increased grass production and reduced canopy temperature relative to air temperature under water stress conditions, but not under well-irrigated conditions. These results suggest that the drought tolerance so commonly associated with endophyte may be at least partially the result of resistance to plant-feeding nematodes.

Recently, strains of *N. coenophialum* have been identified that do not produce the ergot alkaloids that are responsible for toxicity to livestock. Growth of two root lesion nematode species, *P. scribneri* and *P. zeae,* was compared on tall fescue with ergot-producing and non-ergot strains of the endophyte (Timper et al. 2005). One non-ergot endophyte did not confer nematode resistance in either tall fescue cv Georgia 5 or cv Jesup, while another non-ergot endophyte conferred some nematode resistance in tall fescue cv Georgia 5 but not cv Jesup. This suggests that alkaloid synthesis due to the endophyte was not the direct cause of nematode resistance, and that plant genotype may also be involved. Another study compared wild-type endophyte with 'gene-knockout' mutants lacking alkaloid production, and also concluded that nematode resistance is not determined by production of alkaloids alone (Panaccione et al. 2009).

Discovery of the role of endophytes has presented exciting opportunities for managing plant-feeding nematodes in forage crops, and for using grass cover crops to manage nematode populations in horticultural crop rotations. It also raises questions about earlier research on nematode-grass interactions, which includes contradictory results regarding whether tall fescue and perennial ryegrass are hosts for root lesion nematodes in particular. Unknown variation in endophyte status of plants could have led to some confusion in earlier studies, and future research on nematode-grass relationships must take into account the endophyte status of the host plant species.

Effects of nematodes on forage legumes

The impact of plant-feeding nematodes has been studied much more extensively on productivity of forage legumes than of grasses, and excellent reviews have been written for alfalfa (Griffin 1998), clovers and other legumes (Pederson and Quesenberry 1998; Mercer and Watson 1996).

Alfalfa

The alfalfa stem nematode, *Ditylenchus dipsaci*, is the most important nematode pest of alfalfa. This nematode differs from the majority of parasitic nematodes in that it is primarily an endoparasite of crowns, stems and occasionally leaves. As it feeds on plant cells and exudes enzymes within stem tissues, it causes stems to become shortened, swollen and discoloured; under conditions of high humidity and temperature, stems will become necrotic and die. The stem nematode is a serious pest in areas of high rainfall or in irrigated fields within semi-arid regions. It can survive for long periods in an anhydrobiotic (dry) state in dried infested plant material or dry soil. The stem nematode also can invade alfalfa seed, and it is spread from field to field by movement of infested plant material or seed.

Alfalfa is also often parasitized by root knot nematodes (*genus Meloidogyne*). Alfalfa can be attacked by the northern root-knot nematode (*Meloidogyne hapla*), the southern root knot nematode (*Meloidogyne incognita*), and two species limited to subtropical regions (*M. javanica* and *M. arenaria*). A fifth species of interest, *M. chitwoodi*, is not generally considered a pest of alfalfa, but one race of the species is capable of infecting alfalfa (Santo and Pinkerton 1985). The northern root knot nematode is the most widely distributed species in North America; unlike *M. incognita,* it can survive where soil freezes. *M. hapla* can build up to high population densities in alfalfa fields (e.g. > 1 juvenile nematodes/cm^3 or >16/ in^3 soil), which can cause seedling mortality and significantly reduce yields. High populations of *M. hapla* also increase susceptibility of alfalfa to Phytophthora root rot, and can destroy *Rhizobium* nodules and hence reduce nitrogen fixation (Gray et al. 1990).

Root lesion nematodes (*Pratylenchus* species) are often overlooked as pests of alfalfa due to the more easily recognized symptoms of stem nematodes and root knot nematodes. Nonetheless, alfalfa is well known to be susceptible to both *P. penetrans* (Fig. 3) and *P. neglectus* (e.g. Griffin 1994, 1993a, 1994b; Griffin and Gray 1990). Root lesion nematodes reduce alfalfa cold hardiness (Suzuki and Willis 1974) and increase susceptibility to Fusarium (Mauza and Webster 1982).

Clovers and other forage legumes

The complex of nematode species affecting clovers and other forage legumes is similar to alfalfa. However, clover crops are also attacked by the clover cyst nematode, *Heterodera trifolii*. This nematode, which has been found in most clover-growing regions of the world, has been shown to reduce yield of white clover by 14 - 46% and of red clover by 27%, with corresponding reductions in protein content (Pederson and Quesenberry 1998).

Due to their widespread occurrence and potential to cause damage, root knot nematodes appear to be the primary concern for clover crops in North America. As with alfalfa, root-knot nematodes can significantly reduce N-fixation root growth in clover. Indeed, identification and incorporation of resistance to root knot nematodes and the clover cyst nematode into new clover varieties has been a goal of breeding programs in the U.S. (Pederson and Quesenberry 1998) and New Zealand (Mercer and Watson 1996). *Meloidogyne incognita* and *M. hapla* are the species most commonly associated with clover in North America, but *M. trifoliophila* has been identified as the dominant species affecting clover in New Zealand. *M. trifoliophila* has also been identified in Tennessee (Bernard and Eisenback 1997), but the extent of its distribution in North America is not known.

Root lesion nematodes can also cause measurable reductions in yields of clover (Pederson and Quesenberry 1998), and attempts have been made to identify resistance to root lesion nematodes in red clover genotypes (Kimpinski et al. 1992).

Influences of belowground herbivory on soil ecology

Beyond their effects on forage production, plant-feeding nematodes can also affect fluxes of C and N through the soil ecosystem and influence interactions between plant species. Experiments on clover and grasses (Bardgett et al. 1999; Denton et al. 1999) using ^{14}C labelling showed that plant-feeding nematodes increase allocation of photoassimilate from leaves to roots and this leads to increased root exudation and rhizosphere microbial activity. Another study showed that transfer of ^{15}N from a legume (red clover) to a grass (perennial ryegrass) was correlated with the number of nematodes feeding on clover roots (Dromph et al. 2006). The prevailing hypothesis is that nematode feeding in clover causes greater turnover and subsequent mineralization of

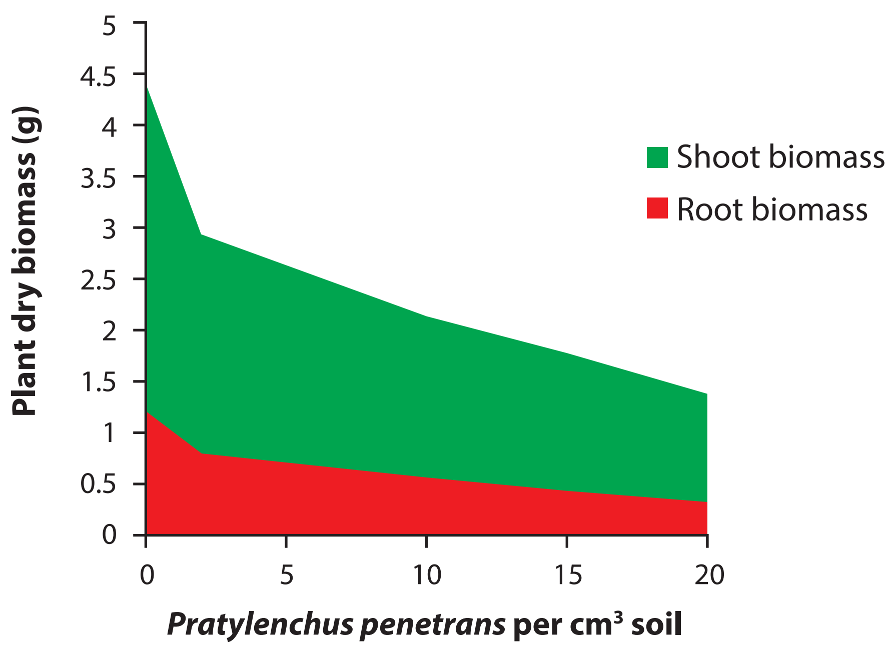

Figure 3. Effects of variable initial population densities of the root lesion nematode, *Pratylenchus penetrans,* on biomass of 'Lahontan' alfalfa plants grown in a greenhouse for 150 days. Data are re-drawn from Griffin (1993) and averaged over four different nematode populations.

N in roots. Plant-feeding nematodes in a grass pasture have been estimated to consume 5 - 8% of total standing root biomass on average, but within field, spatial variation ranged from 1 - 40% (Verschoor 2002), indicating that the patchiness of nematode populations may contribute to spatially dynamic fluxes in C and N and interactions among plant species and the spatial variability characteristic of pastures.

Plant-feeding nematodes can influence competitive interactions between plant species and influence plant succession in mixed stands. By studying relative suppression of growth of different grassland plant species in soil with fauna from different stages of succession, researchers have shown that soil fauna (primarily plant-feeding nematodes) can selectively suppress the dominant plant species in early stages of succession, helping to facilitate succession (De Deyn et al. 2003). Using similar experimental approaches, it was found that negative effects of nematodes exceed the positive effects of the mutualistic fungi, AMF (mycorrhizae) in *Festuca rubra* and *Carex arenaria* (Olff et al. 2000). Interestingly, the suppression in growth of both plant species was greater when inoculated with nematodes from areas with declining rather than increasing nematode populations, illustrating the role of belowground herbivory on the shifting mosaics of plant community composition. While our understanding of the influences of plant-feeding nematodes and belowground herbivory on vegetation dynamics is still very limited, it is clear that these microscopic, soil-dwelling organisms must no longer be overlooked in the quest for complex forage communities.

Influences of management practices on nematodes

Intensive and relatively costly practices (e.g. preplant fumigation, specific nematicides, green manure/biofumigant cover crops) developed for nematode management in high value horticultural crops, are not suitable for extensive forage production. Instead, resistant plant genotypes and beneficial endophytes will be needed. There may also be opportunities to minimize impacts of nematodes through better understanding of crop management on their ecological relationships and population dynamics.

Populations of plant-parasitic nematodes generally increase with crop N status. For example, long-term use of both manure and N-fertilizer increased population densities of *Pratylenchus penetrans* in tall fescue (Forge et al. 2004). Similarly, *Pratylenchus* species increased in fertilized clover-ryegrass pastures in New Zealand (Sarathchandra et al. 2001) and a species of *Helicotylenchus* increased in fertilized tall grass prairie in Kansas (Todd et al. 1996). The incorporation of manures and composts into soil has been associated with nematode suppression in some horticultural crops, but this suppression has not been observed in forage crops. Indeed, one study observed an increase in *Pratylenchus* population densities in manure-amended plots of tall fescue relative to non-amended but fertilized plots (Forge et al. 2005).

While the benefits of fertilization to forage production would likely offset any negative effect of increased nematode populations, the buildup of nematode populations with high inputs could possibly diminish the *efficiency* of N uptake by forage crops, reducing the efficiency of N use by the crop and render crops more vulnerable to environmental stresses, and perhaps hasten the decline of stands (Bittman et al. 2007); however, none of these outcomes have been demonstrated to date. The interactions between long-term nutrient applications, plant-parasitic nematodes, and forage production and persistence deserve additional research.

References available online at www.farmwest.com

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