Meloidogyne minor

Scientific Name
Meloidogyne minor (Karssen et al., 2004)

Type of Pest
Plant-pathogenic root-knot nematode

Taxonomic Position
Class: Secernentea **Order:** Tylenchida **Family:** Meloidogynidae

Reason for Inclusion in Manual
Solanaceous pest; Additional pest of concern; unconfirmed U.S. detection in Washington in 2012.

Pest Description
From Karssen et al., (2004):
Eggs: Eggs are 80 to 102.5 µm long, and 38.5 to 57.5 µm in diameter.

Males: The typical *M. minor* male measures 1040 µm long, 26.9 µm wide at the greatest diameter, 9.6 µm wide at the head, and 23.6 µm wide at the excretory pore. The testes are approximately 533 µm long.

The male body is vermiform and annulated, with a curved tail region. There are four incisures present in the raised lateral field, sometimes with one or two complete incisures in the middle. The outer bands are irregularly areolated. The head is not set off from the body and one post-labial annule is present often with one or two incomplete transverse incisures. The labial disc is rounded, elevated, and fused with anchor shaped submedial lips. The prestoma is hexagonal in shape, surrounded by six inner sensilla. There are four cephalic sensillae present on the submedial lips, close to the labial disc and marked by small slits. There are slit-like amphidial openings present between the labial disc and the prominent lateral lips. The cephalic framework is strongly sclerotized and the cestibule extension is distinct. The stylet is accompanied by a straight cone and cylindrical shaft. There are large transversely ovoid knobs slightly sloping backwards from the shaft. The dorsal gland orifice is close to the stylet knobs. The pharynx has a slender procorpus and oval-shaped metacorpus. The pharyngeal gland lobe ventrally overlaps the intestine, with two subventral gland nuclei present. The hemizonid is 3.5-4.5 µm long, anterior to the excretory pore. The testes are very long, monarchic, and have an outstretched germinal zone. The tail is usually curved ventrally. It is short and conical, with a bluntly rounded tip. The specula and gubernaculum is slender and curved ventrally. There are two small pores present on each speculum tip. The phasmids are small and located posterior to the cloaca.

Females: The average *M. minor* female measures 533 µm long by 330 µm wide at the greatest diameter, has a vulval slit length of 25.8 µm and a metacorpus length of 35 µm.

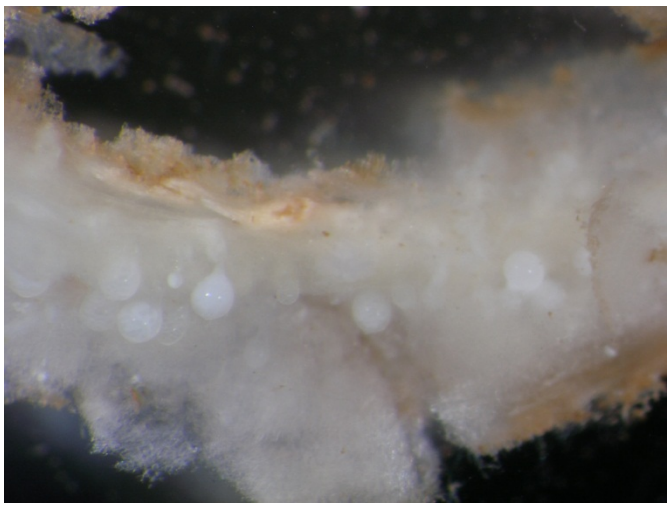

Figure 1. Pearly white *Meloidogyne minor* females infesting potato roots. Photo courtesy of Dr. Colin Fleming.

The female body is relatively small, weakly annulated, pearly white, and is usually globose, sometimes elongated (Fig. 1). The neck region is distinct, and is often bent. The young females have a slight posterior protuberance. The head region is set off from the body, and the head cap region is distinct and highly variable in shape. The labial disk is elevated, and the lateral lips are prominent. The cephalic framework is weakly sclerotized. The stylet cone is slightly curved dorsally, with a cylindrical shaft. The knobs are transversely ovoid and slightly slope backwards from the shaft. The excretory pore is located near the stylet knob level. There are several small vesicles observed near the lumen lining of the metacorpus. The pharyngeal glands are variable in size and shape. The perineal pattern is small and rounded with fine striae, and the dorsal arch is low with coarse striae. The tail remnant area is distinct, without punctuations. In some patterns there are weak lateral lines present. The phasmids are small, usually not visible, and are located above the covered anus.

Second-stage Juveniles: The typical *M. minor* J2 measures 0.015 inches (348 µm) long, 13.3 µm around at the greatest diameter. The metacorpus is 3.3 µm long, and 2.9 µm wide.

The body is vermiform, relatively short and annulated. The anterior part tapers behind stylet-knob level, and the posterior part is slightly ventrally curved when heat relaxed. The lateral field has four incisures, and areolation is not visible. The head region is rounded and is not set off from the body. The cephalic framework is weakly sclerotized and the vestibule extension is distinct. The stylet is small with a straight cone and a cylindrical shaft. The knobs are transversely ovoid and slightly sloping backward. The metacorpus is relatively large and ovoid, and the triadiate lumen had clear sclerotized lining. The pharyngeal gland lobe is relatively long, with the ventral intestine overlap clearly visible along with three gland nuclei. The hemizonid is in the posterior region adjacent to the excretory pore and is 2-2.5 µm in length. The tail is straight, but can also be slightly curved ventrally sometimes. There is gradual tapering until the tail tip comes to a fine point. The rectum is usually weakly inflated. The hyaline tail terminus is distinct and relatively long and narrow. The anterior hypodermal part is rounded and relatively narrow. There are often one or two cuticular constrictions present on the tail terminus.

Biology and Ecology

Nematodes are unsegmented roundworms. Most plant parasitic types are very small and feed on roots by means of a stylet, a hollow needle-like structure used to pierce plant cells and withdraw nutrients. *Meloidogyne minor* has a life cycle very similar to most root-knot nematodes. This species favors sandy soils similar to those created on standard golf courses, hence the detection of this pest on golf greens in Washington state (McClure et al., 2012). *M. minor* reproduces via facultative meiotic parthenogenesis, with a haploid chromosome count of 17 (Karssen et al., 2004). Females lay eggs in gelatinous masses found on the surface or inside of gall tissue. The egg sac is initially sticky and hyaline in color, but becomes harder and darker brown with age. The eggs mature into first, then second-stage juveniles (J2s). Once the J2 molt is complete, the juveniles hatch and begin to search for another root location to bury into. Typically, the J2s begin to feed from the roots at the root tip and then move up the root to find a permanent spot. J2s feed on the protoxylem and protophloem cells in the root, which causes the cells to differentiate into specialized nurse (feeding) cells called giant cells. Once these cells develop, the J2s remain sedentary and enlarge. After feeding, the J2s differentiate into either male of females, and then continue to molt up through the fourth stage juvenile (J4) and eventually into adults (Perry et al., 2009).

Morris et al. (2011) found that the number of eggs per egg mass peaked in May, and again at a higher rate in September. The optimum hatching temperature ranges from 68 to 77 °F (20 to 25°C) and peaks at 73.4 °F (23°C). Less than 50% of the eggs hatch at 59°F (15°C) and less than 1% hatch at 50°F or lower (<10 °C). The highest rate of J2 activity occurred between 59 and 77 °F (15 to 25 °C) while any temperature below 50 °F resulted in drastically reduced activity. Experiments suggest that *M. minor* does not enter diapause, and it is important to note that although lower temperatures are not optimal, hatching is still possible (Morris et al., 2011). Hatching J2s have been detected in 7 to 12^{o}C (44.5 to 53.6^{o}F) soil in Ireland (Fleming, personal communication).

To cause aboveground disease symptoms, approximately 25 to 30 galls per 1000 cm^3 of soil must be present. There is a possibility for multiple generations per season since the time from one egg mass to another can be completed in 12 weeks. The majority of *M.* minor specimens were observed between 0 and 4 inches (0 to 10 cm) which could correlate to the higher root density of its turfgrass host at those depths (Morris et al., 2012).

Figure 2. Necrotic spots in potato tube infected with *Meloidogyne minor*. Photo courtesy of Dr. Colin Fleming.

Symptoms and Signs

On potatoes, *Meloidogyne minor* causes symptoms very similar to other root-knot nematodes. Root systems infected with *M. minor* can have pear-shaped galls up to 2 cm (0.787 inches) large. They are more commonly located at the beginning of the lateral roots leading to a thickened root base (Thoden et al., 2012). On tubers, *M. minor* causes numerous, small, raised areas that are apparent on the tuber surface. The females are present just below the peel and can cause small dots of brown necrotic tissue on the tuber cortex (Fig. 2) (Karssen et al., 2004).

Figure 3. Bentgrass turf affected by yellowpatch disease caused by *Meloidogyne minor*. Photo courtesy of Dr. Colin Fleming.

Recently, there have been reports of a yellow-patch disease on turf grass used for golf course greens. This disease has been traced to multiple nematode species, including *M. minor* (McClure et al., 2012). On turf grass, visible symptoms are typically seen during the warmer parts of the year when the grass is actively growing. These symptoms can include stunted growth, wilting, and discolored foliage. This causes the yellow patching on golf greens that gave the disease its name (Fig. 3). The location of the patches can vary and may remain visible from one season to the next, even while new patches are appearing in other areas. Galls on turfgrass can be prominent, or in some cases no obvious galls are present with females and egg masses inside slightly thickened roots. Regardless of whether the patch is visible or not, the actual nematodes remain in the soil throughout the year. Using only visible symptoms to diagnose a problem on turf grass, can be difficult since they are similar to symptoms caused by a variety of pathogens or nutritional deficiencies (Morris et al., 2012; Fleming, personal communication).

Pest Importance

Meloidogyne species are among the most important plant-parasitic pests in worldwide crop production. Crop losses from nematode damage alone are estimated at 10 to 11% worldwide, but this is thought to be an underestimate. If unregulated, crop losses to potatoes grown in the Northwest region of the United States could be as much as $40 million (Davis and Venette, 2004).

The presence of *M. minor* in Europe poses a threat to the potato trade there. In 2005, countries in the European Union produced 18.5% of the global share of potatoes. EU-25 trade in potato products (not including starch and sweet potatoes) was worth about €2.76 billion ($3.69 billion) in the period 2003/05 (EC, 2007). Total human consumption of potatoes amounted to roughly 38 million tons in the EU-25 during the period from 2001-2003.

In addition to the damage to consumable crops, *Meloidogyne minor* could have a detrimental effect on turfgrass in the United States, which has grown to become a multi-billion dollar industry. This root-knot nematode favors sand soils such as those at golf greens constructed according to the US golf association guidelines (McClure et al., 2012) as well as rye grass soccer fields. Turfgrass is an unusual crop in that its maintenance often leaves the plant in a stressed state. Common procedures on infected turf grass such as mowing, aeration, and actual play on the field can weaken the plant and exacerbate the damage caused by this root-knot nematode (Fleming et al., 2008). Turfgrass is the largest irrigated crop in the United States, and golf is a primary consumer and producer of turf. The impact of *Meloidogyne* sp. on the golf course industry, estimated to have an annual economic impact of $195 billion in the United States alone, is already significant and could become even greater if *M. minor* becomes established. McClure et al. (2012) surveyed 238 golf courses in the Western United States and found root-knot nematodes in 60% of the putting greens sampled.

M. minor is listed as a harmful organism in Peru and South Korea (USDA-PCIT, 2013). There may be trade implications with these countries if this nematode becomes established in the United States.

Known Hosts

Major hosts: *Agrostis stolonifera* var. *stolonifera* (creeping bentgrass), *Lolium perenne* (perennial ryegrass), and *Solanum tuberosum* (potato) (Turner and Fleming, 2005; Fleming, personal communication).

Minor hosts: *Anagallis arvensis* (scarlet pimpernel), *Festuca* sp. (fescue), *Medicago lupulina* (black medick), *Poa* sp. (bluegrass), *Phleum pratense* (timothy), *Trifolium pratense* (red clover), *Trifolium repens* (white clover), *Trifolium* sp. (clover) (Turner and Fleming, 2005; Lammers et al., 2007).

Experimental hosts: *Avena sativa* (oat), *Dacus carota* (carrot), *Hordeum vulgare* (barley), *Lactuca sativa* (lettuce), *Lolium multiflorum* (Italian ryegrass), *Lolium perenne* (perennial ryegrass), *Lolium* sp. (ryegrass), *Medicago sativa* (alfalfa), *Phacelia tanacetifolia* (phacelia), *Solanum esculetum* (tomato), *Triticum sativum* (wheat), *Vicia sativa* (vetch) (Karssen et al, 2004; Turner and Fleming, 2005; Lammers et al., 2007).

Known Vectors or Associated Organisms

As a soil-borne nematode, this pest is not vectored by another organism. It is commonly found in mixed infections with *M. naasi*. There are no data detailing how this interaction affects symptomology, but there is speculation that *M. naasi* could be one

parent of *M. minor* due to similarities in morphology and esterase activity patterns (Karssen et al., 2004).

Known Distribution

Europe: Belgium, Ireland, Netherlands, Portugal, United Kingdom (Morris et al., 2011; Viaene et al., 2007; Lammers et al., 2007; McClure et al., 2012).

North America: United States (McClure et al., 2012).
Meloidogyne minor was recently detected in Washington state(King and Snohomish counties) in samples taken from a golf course greens exhibiting symptoms of yellow patch disease (McClure et al., 2012).

Note: There has been no official APHIS confirmation of the detection of *M. minor* in the United States. *Meloidogyne minor* was recently detected from Washington state (McClure et al., 2012), and we are awaiting confirmation from an official sample (NPAG, 2013).

South America: Chile (McClure et al., 2012).

Pathway

The most important possible pathways for entry are commercial potato trade and the transfer of soil and infected plant material on golfing equipment. There are some hygienic regulations in the UK, but it is by no means universal. There have only been a few positive confirmations of *M. minor* in potato fields compared to the identification of *M. minor* on turfgrass, so the movement of infected golf equipment could be of greater concern (Lammers et al., 2007). Infected construction sand is likely a source of the spread of this nematode in new sports facilities in Europe (Fleming, personal communication).

There are no regulations on the import of plant material of *Trifolium pratense* (red clover) and *T. repens* (white clover), both natural hosts of *M. minor*. While seed material is subject to FSA-A (subject to sampling as an agricultural seed), there are no other restrictions on seed import. There have been 63 shipments of *Trifolium sp*. propagative material from known *M. minor* host countries since 2003, with at least one coming from each host country (AQAS, 2013). Some of the shipments indicated import of plant material (up to 977 plant units). There were also 29 interceptions of *Trifolium* sp. propagative material intended for propagation or consumption since 2003.

There are no regulations on the import of *Daucus carota* plant material into the United States (USDA, 2013). *D. carota* is an experimental host of *M. minor*. This could be an open pathway if *D. carota* is confirmed as a natural host.

Since 2003, there were 24 shipments of *Agrostis* sp. propagative material from known host countries and no recorded interceptions (AQAS, 2013). The import of *Agrostis* sp. propagules other than seeds is prohibited from all countries except Canada (USDA, 2013).

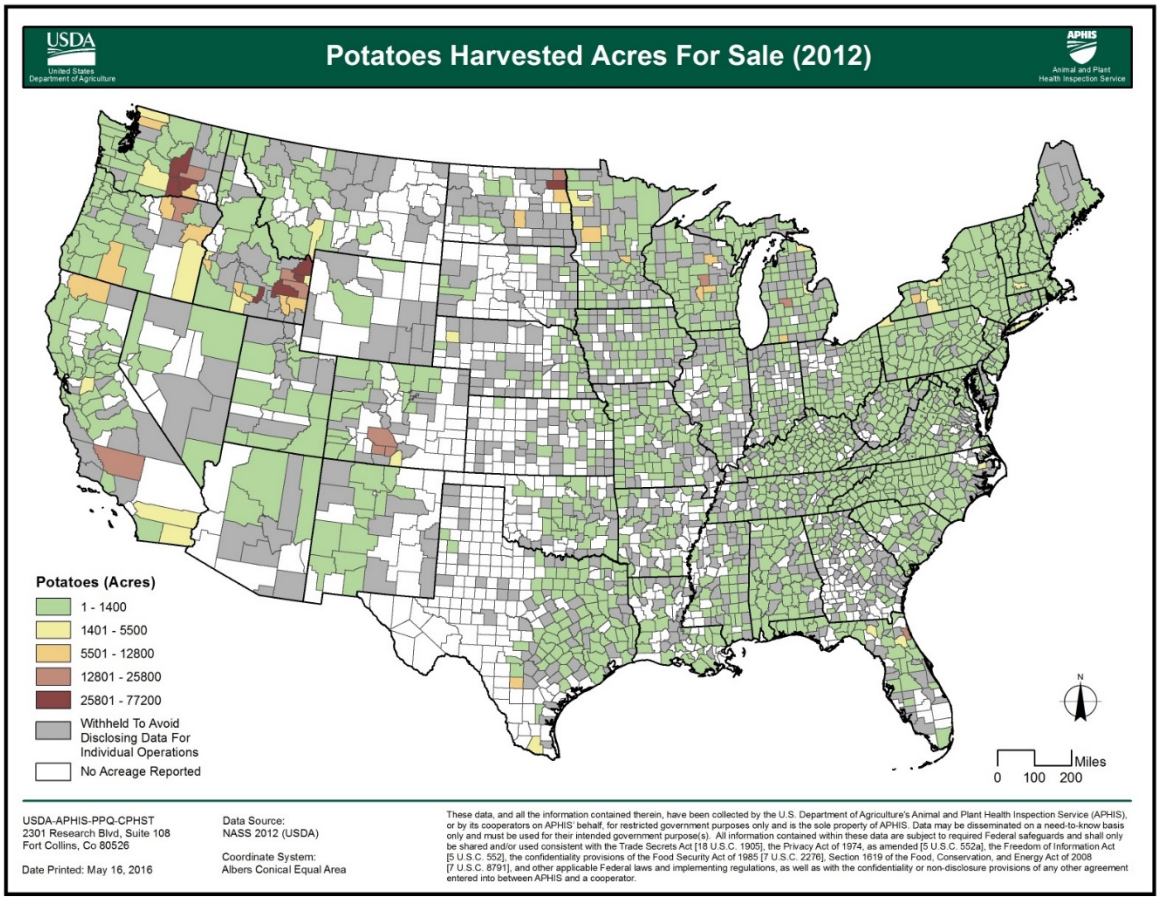

Figure 4. Potato commodity acreage map. Map courtesy of USDA-APHIS-PPQ-CPHST.

Since 2003, there were 78 shipments of *Solanum sp*. propagative material from known host countries. While the majority of these shipments appear to be seed, several of them were measured in plant units and may have been plant material. There have also been 113 interceptions of *Solanum* sp. propagative material since 2003, including 102 from the Netherlands alone (AQAS, 2013). Effective May 20, 2013, under federal regulation 7 CFR 319.37-2a, import of all *Solanum* sp. propagules except seeds from countries other than Canada is prohibited pending a pest risk analysis of *Tuta absoluta*, the tomato leafminer (USDA, 2013).

Potential Distribution within the United States

A commodity acreage map is available for potato, a primary host of *M. minor* (Fig. 4). The top potato producing states in 2015 were: Idaho, Washington, Oregon, North Dakota, Colorado, and Wisconsin (USDA-NASS, 2016).

In addition, turfgrass is grown throughout the United States (McClure et al., 2012), and much of the United States has a similar climate to the countries in Europe where this nematode is present (see distribution section).

Survey

CAPS-Approved Method*:

Soil sampling: Send sample to nematology diagnostic lab where nematodes will be extracted and identified.

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

From Morris et al. (2011): "Use a soil corer to a depth of 10 cm. Rinse roots gently with tap water to remove soil. Place egg masses in hatching chambers of plastic cylinders 15mm high x 10 mm diameter (0.59 x 0.393 inches) with 20 µm mesh at the bottom where J2s can migrate freely. Suspend each cylinder in a 24 well flat bottom tissue culture plate containing 500µl (0.017 oz.) of tap water into which the J2s will migrate".

From Thoden et al. (2012): "Take soil cores samples 13 mm (0.512 inches) in diameter, 25 cm (9.84 inches) in depth. Take a subsample of 100 ml (6.10 in^3) of soil from each core. Sieve sample with mesh 180 µm in size with water. Extract nematodes using an Oostenbrink elutriator".

Note: It is recommended to incubate *M. minor* samples for at least 4 weeks. Occasionally, in soil samples with low numbers of *M. minor*, only *M. naasi* is detected after 2 weeks of incubation, while after 4 weeks *M. minor* appears from the same material (Karssen, personal communication).

Key Diagnostics/Identification

CAPS-Approved Method*:

Morphological: Characteristics of the males, females, and juveniles (Karssen et al., 2004).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods:

Zijlstra (2000) developed a SCAR-PCR to identify *M. hapla*, *M. chitwoodi*, and *M. fallax*. This PCR was designed before the description of *M. minor*, and subsequent research by Nischwitz et al. (2013) showed that *M. minor* amplification also occurs and cannot be distinguished from *M. fallax* amplification. While this SCAR-PCR could be used to detect *M. minor*, it is not advised to use this protocol if a mixed presence of both *M. fallax* and *M. minor* is possible in the same sample.

Karssen et al. (2004) isolated DNA from J2s using the High Pure PCR Template Preparation Kit from Roche with the instructions for the mammalian tissue protocol. After eluting the DNA, they used primers they had developed amplify a specific segment of the internal transcribed spacer region.

McClure et al. (2012) lysed J2 specimens and used a Taq Core kit from Qiagen using primers designed to amplify the D2-D3 region of the 28S gene and the internal transcribed spacer region.

There is a commercially available Real-Time PCR identification kit from Clear®Detections that is specific for *Meloidogyne minor* (Clear®Detections, 2012). Note: This test has not been validated for regulatory samples.

De Weerdt et al (2011) developed a rapid real-time PCR assay to identify *M. minor* whose amplicon has a product of 64 base pairs.

Easily Confused Pests

Meloidogyne minor may be confused with numerous other species of root-knot nematodes. These include *M. hapla*, *M. incognita,* and *M. fallax.* Morphologically, *M. minor* is also very similar to *M. chitwoodi* and *M. microtyla*. However it differs from them in knob shape, perineal pattern shape, male head shape, and most juvenile characteristics. There are also differences in host range, isozyme patterns and molecular sequence between the species. The differences in the morphology of *M. minor* also exclude it from the '*graminis*-group' of nematodes that include other typical golf-grass nematodes such as *M. graminis* and *M. Maryland* (Karssen et al., 2004). Research by McClure et al. (2012) showed that many infested turf samples contained a mixture of *Meloidogyne* spp., and molecular analysis was required to distinguish the species in the samples.

It is possible that there has been hybridization between two existing *Meloidogyne* species, producing a new species in *M. minor*. Molecular studies in the Netherlands have identified similarities between *M. minor* and *M. hapla* (Karssen et al., 2004).

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Draft log

July, 2016: Updated maps, Potential Distribution sections