Mini Risk Assessment
False Columbia Root-knot Nematode: *Meloidogyne fallax* Karssen
[Nematoda: Heteroderidae]

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Introduction

The plant parasitic nematode *Meloidogyne fallax* Karssen, also known as the false Columbia root-knot nematode, was named for its morphological similarity to *M. chitwoodi* (Karssen 1996). *M. fallax* is a significant pest primarily of potato in Europe (Brinkman et al. 1996, Karssen and van Hoenselaar 1998, EPPO 1999). This nematode occurs in the Netherlands, Belgium and Germany; outside of Europe, it has been reported from Australasia and South Africa (see Appendix A).

Meloidogyne fallax is not known to occur in the United States (Inserra et al. 2003). Because of its broad host range and geographic distribution, *M. fallax* has the potential to become established in the US if accidentally or intentionally introduced. This document evaluates several factors that influence the degree of risk posed by *M. fallax* and applies this information to the refinement of sampling and detection programs.

Figure 1. Carrot infected by *M. fallax*
(courtesy of Plant Protection Services, The Netherlands)

1. **Ecological Suitability. Rating: Medium.** *Meloidogyne fallax* has been reported from mild, temperate potato growing regions of Europe, Australasia and South Africa. Appendix A provides detailed records on the known worldwide distribution of this nematode. The currently reported distribution of *M. fallax*

suggests that the pest may be most closely associated with biomes characterized as: Mediterranean scrub; temperate broadleaf and mixed forests; and tropical and subtropical moist broadleaf forests. Consequently, we estimate that approximately 30% of the continental US could provide a suitable climate for *M. fallax* (Fig. 2). See Appendix A for a more complete description of this analysis.

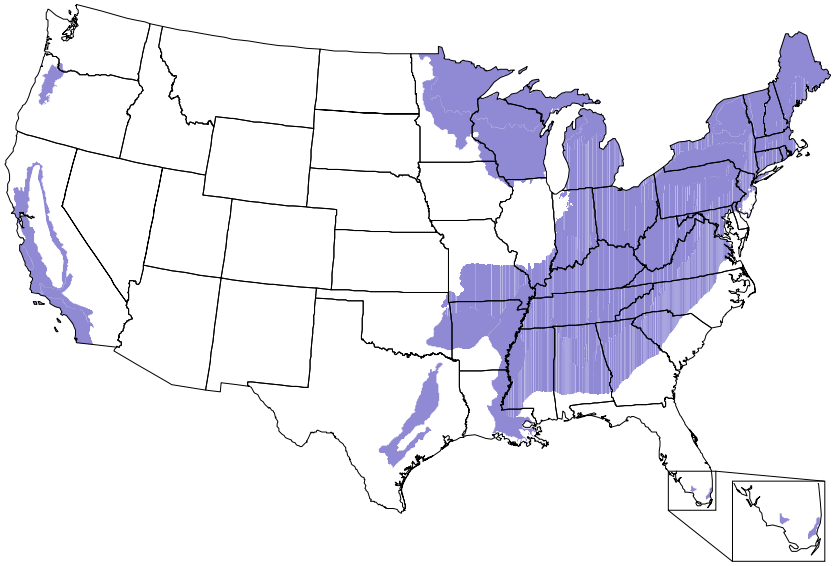

Figure 2. Predicted distribution (shaded purple) of *Meloidogyne fallax* in the continental US.

Figure 2 illustrates where *M. fallax* is most likely to encounter a suitable climate for establishment within the US. This prediction is based only on the known geographic distribution of the species. Because this prediction is based on coarse information, it would not be correct to conclude that *M. fallax* absolutely cannot establish in areas that are not highlighted. Rather, establishment in these areas is less likely. For initial surveys, efforts should be concentrated in the higher risk areas and gradually expanded as needed. Geographic areas that are not highlighted are not risk free.

2. **Host Specificity/Availability. Rating: High/High.** Table 1 lists host plants reported for *Meloidogyne fallax.* This nematode has been found in only a few field crops, primarily potato in Europe and New Zealand, carrot in Europe, and on peanut in South Africa. However, host rage tests have confirmed that *M. fallax*, like its close relative *M. chitwoodi*, can feed on several species of plants in multiple families (Table 1). Not all hosts are of equal quality. For example, corn (*Zea mays*) is, at best, a poor host for *M. fallax* and, at worst, a non-host (Brommer 1996, Karssen 1996, EPPO 1999, Inserra et al. 2003). Complications arise in determining nematode-host associations because populations of *M. fallax* may be mixed with, or occur adjacent to, populations of *M. chitwoodi. Meloidogyne fallax* and *M. chitwoodi* can be difficult to distinguish (see

'Taxonomic Recognition' below). It is reportedly rare for the two species to share the same host concomitantly (Karssen and van Hoenselaar 1998). However, Fourie et al. (2001) reported *M. fallax* on South African peanut in a mixed population with *M. chitwoodi* and *M. hapla*.

Table 1. Host plants of *Meloidogyne fallax*

Host(s)	**Reference(s)**
alfalfa (*Medicago sativa*)	(Inserra et al. 2003)
artichoke (*Cynara scolynus*)	(Inserra et al. 2003)
asparagus (*Asparagus officinalis*)	(EPPO 1999)
beet/sugarbeet[2](*Beta vulgaris*, *Beta vulgaris* subsp. *vulgaris*)	(Brommer 1996, Heijbroek et al. 1998, van der Beek et al. 1998a, van der Beek et al. 1998b, Korthals et al. 2000, Kok et al. 2001, Waeyenberge and Moens 2001, Inserra et al. 2003)
black salsify[2] /oyster plant[2] (*Scorzonera hispanica*)	(Brinkman et al. 1996, Brommer 1996, Karssen 1996, Heijbroek et al. 1998, Molendijk and Brommer 1998, EPPO 1999, Janssen et al. 1999, Korthals et al. 2000, Waeyenberge and Moens 2001, Inserra et al. 2003)
bleeding heart (*Lamprocapnos* =[*Dicentra*] *spectabilis*)	(Inserra et al. 2003)
buckwheat (*Eriogonum* sp.)	(Heijbroek et al. 1998)
carrot[1,2] (*Daucus carota* ssp. *sativus*)	(Brinkman et al. 1996, Brommer 1996, Heijbroek et al. 1998, Karssen and van Hoenselaar 1998, Molendijk and Brommer 1998, EPPO 1999, Korthals et al. 2000, Waeyenberge and Moens 2001, Inserra et al. 2003)
cereals (unspecified)	(Brommer 1996, Heijbroek et al. 1998)
corn (*Zea mays*)	(Brommer 1996, Waeyenberge and Moens 2001)
dahlia (*Dahlia* spp.)	(Brinkman et al. 1996)
daylily (*Hemerocallis* sp.)	(Inserra et al. 2003)
grasses (unspecified)	(Heijbroek et al. 1998, Waeyenberge and Moens 2001)
lacy phacelia (*Phacelia tenacetifolia*)	(Inserra et al. 2003)
lambsquarters (*Chenopodium album*)	(Heijbroek et al. 1998)
lettuce (*Lactuca sativa*)	(Inserra et al. 2003)
mustard, white (*Sinapis alba*)	(Heijbroek et al. 1998)
peanut/groundnut[1,2] (*Arachis hypogaea*)	(Fourie et al. 2001)
potato [1,2] (*Solanum tuberosum*)	(Brinkman et al. 1996, Brommer 1996, Karssen and van Hoenselaar 1998, Molendijk and Brommer 1998, Schmitz et al. 1998, van der Beek et al. 1998c, EPPO 1999, Janssen et al. 1999, Tastet et al. 1999, Korthals et al. 2000, Brommer and Molendijk 2001, Marshall et al. 2001, Nobbs et al. 2001, Inserra et al. 2003)
primrose (*Oenothera biennis, O. glazioviana* [=O. *erythrosepala*])	(Brinkman et al. 1996, van der Beek et al. 1998c, Inserra et al. 2003)
radish, fodder[2] (*Raphanus sativus*)	(Heijbroek et al. 1998)

Host(s)	Reference(s)
ryegrass/Italian ryegrass (*Lolium* sp., *Lolium perenne* ssp. *multiflorum*)	(Brommer 1996, Heijbroek et al. 1998, Korthals et al. 2000)
Strawberry (*Fragaria* sp.)	(Janssen et al. 1999)
tomato (*Solanum lycopersicum* =[*Lycopersicon esculentum*])	(Karssen 1996, Karssen and van Hoenselaar 1998, Schmitz et al. 1998, EPPO 1999, Inserra et al. 2003)
wheat (*Triticum* sp., *T. aestivum* and/or *T. durum*)	(Kok et al. 2001)

1. true (field) host
2. root crop and / or fruit develops in soil

See Appendix B for maps showing where various hosts are grown commercially in the continental US.

3. **Survey Methodology. Rating: Low-Medium.** For consistency with other mini-risk assessments, a lower rating is given to this element because no trapping technologies (e.g., pheromone lures) are available to assist with surveys. Current techniques for nematode sampling should prove adequate to detect most infestations of new *Meloidogyne* spp. However, the success of the methods depends heavily on the amount of sampling that can be conducted. If only a modest sampling effort can be made, the likelihood of detecting infrequent, sparse infestations of nematode is low. In the remainder of this section, we outline considerations for sampling and make recommendations to improve the likelihood of detecting infestations.

   Goals. In this mini-PRA, we focus on the design of a survey to detect the presence of newly introduced *Meloidogyne* spp. rather than to determine the abundance or density of the species. Statistical approaches to the design of nematode surveys are relatively rare in the literature, whereas empirical approaches are far more common.

   Generalized approach. Vovlas and Inserra (1996) outline general considerations for conducting a survey for new *Meloidogyne* spp. In general, they recommend sampling root tissues to inspect for the presence of galled roots. They also note that soil samples may detect *Meloidogyne* spp., but these individuals may not be of particular concern. Many native or naturalized *Meloidogyne* spp. parasitize a number of weed hosts that may be found in orchards. Thus, careful examination of individuals will be necessary to confirm species identity.

   Alternatively, soil samples may be collected. General principles described by Greco et al. (2002) apply to *Meloidogyne* spp. Samples of soil or host roots must be collected with the purpose of obtaining males, juveniles, or nematodes within root tissues. Samples must then be processed to separate nematodes from soil and debris. Finally, nematodes must be prepared either for identification using morphological (e.g., perineal patterns) or molecular techniques. In the remainder of this section, we will focus on soil sampling. Soil sampling is typically based on the collection of cylindrical cores of soil. Frequently, a sample unit is

composed of several cores that are combined and mixed thoroughly. The number of sample units collected from a field is the sample size. Not all soil from each sample unit will necessarily be processed, rather nematodes will frequently be extracted from a soil subsample.

General procedures. Sampling may be conducted to detect the presence of new *Meloidogyne* spp. in an individual field or over a broader geographic area. For quarantine nematodes that are known to occur in the US (e.g., *Globodera rostochiensis*), it may be important to take sufficient samples to certify with a high degree of confidence that the probability of a nematode species being present in an individual field is very low. To achieve this goal, highly intensive sampling may be needed. Been and Schomaker (2000) proposed a sample unit of 50 cores (presumed to be 1 in diameter x 6 in deep) collected on a 5 m x 6 m (~16 ft x 20 ft) grid. This sampling procedure results in the collection of 2 kg soil per sample unit; a sample size of 6-7 units per hectare is recommended. Such a high level of sampling intensity provides a ≥90% probability of detecting nematode aggregations with ≥200 cysts/kg soil at their center. The sampling recommendations of Been and Schomaker (2000) are based on empirical observations of the size of nematode patches (or foci) when they occur in potato fields. Nevertheless, the same principles should apply to surveys for *Meloidogyne* spp., and the protocol should have a high probability of detecting members of the genus when they are present in a field.

In contrast, it may be more valuable (and perhaps even more cost effective) to use a smaller sample unit and/or sample size per field to maintain a high probability of finding an exotic nematode somewhere within a geographic area, even though the likelihood of finding a species in an individual field might be lower.

For regional surveys of nematodes, Prot and Ferris (1992) recommend a single composite sample of 10 cores per field. Cores should be collected approximately 55 m (180 ft) apart throughout the entire field. For most field and forage crops, soil samples should be collected at a depth of 15-40 cm (6 to 16 inches) within the root zone. Samples should be collected with an Oakfield- or Veihmeyer-sampling tube (~1 inch inner diameter). Soil samples should be collected from fields that include one or more hosts in the cropping rotation. The sampling recommendations from Prot and Ferris (1992) were based on observations from cotton and alfalfa. The sampling protocols have not been evaluated orchards, but the principles upon which the recommendations are based should still apply.

A 10-core, composite sample is particularly efficient at detecting nematodes when species are "frequent and abundant." Figure 3 illustrates this point. In the figure, "*k*" is from the negative binomial distribution and is a measure of the evenness of the nematode distribution within a field. Larger values of *k* indicate a more even distribution of nematodes across a field. During the early stages of an infestation, nematodes populations are likely to be tightly aggregated in discrete patches (with small values of *k*) within a field.

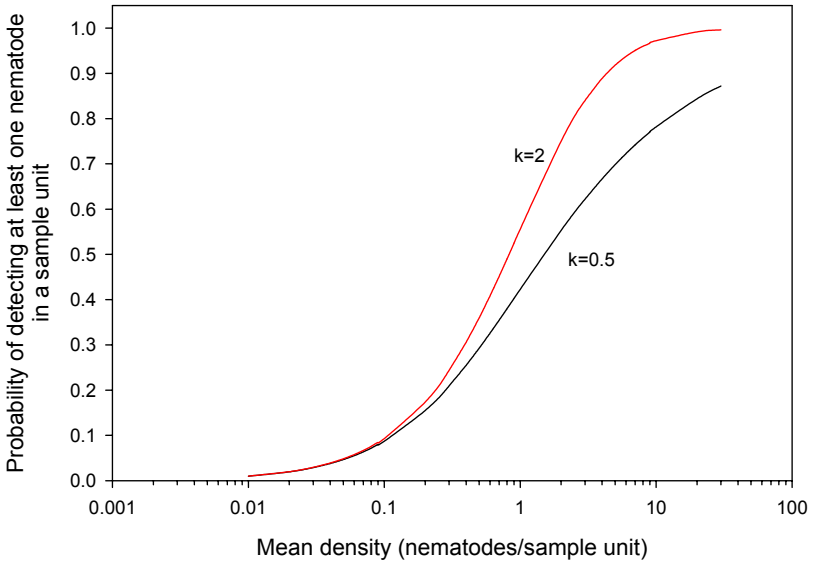

Figure 3. Influence of nematode density and spatial distribution on the likelihood of observing at least one nematode from a soil sample. Lines are based on the negative binomial distribution.

The number of fields that should be sampled to maintain a high probability of detection within a region depends on the chances that nematodes are found in an individual field. The chances that a nematode species will be detected when it is present within a field are influenced a number of factors. These include soil type, vertical distribution of nematodes within the soil profile, time of year, the number of soil samples that are collected, the unit size of those samples, the amount of soil that is processed (typically a subsample of the sample unit), and the method(s) of nematode extraction and identification. The vertical distribution of new *Meloidogyne* spp. is likely to be influenced by the distribution of roots. Figure 4 illustrates the influence of the anticipated frequency of infested fields and the probability of detecting a nematode species when it is present in a field on the number of fields that should be sampled to maintain a 95% confidence of finding the nematode when it is present. We assumed that it would be impractical for any group or agency to collect and process samples from more than 10,000 fields in a season. Generally, if 1 in 100 fields is infested (frequency = 10^{-2}), 600 to 6,000 fields must be sampled (depending on the likelihood of finding nematodes in an individual field) to have 95% confidence of finding an infestation within a broader geographical area.

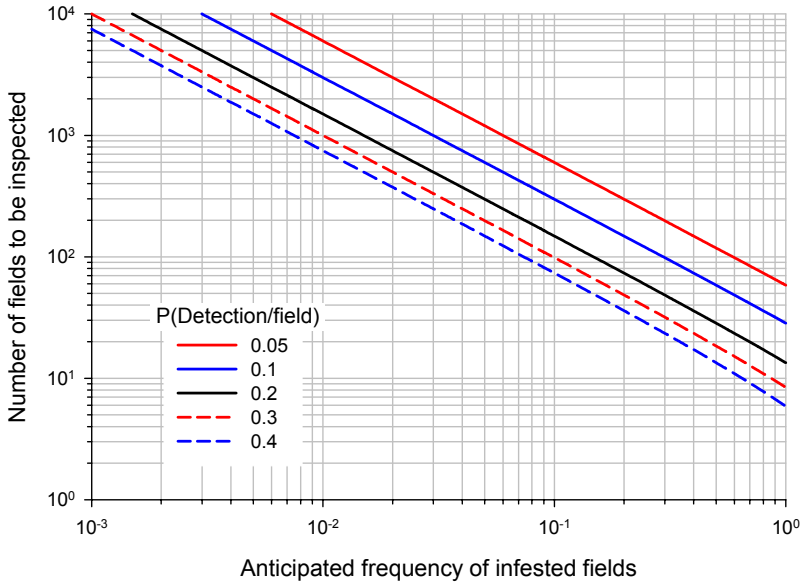

Figure 4. Influence of the frequency of infested fields and the likelihood of detecting an infestation in an individual field on the number of fields that should be inspected to have 95% confidence of detecting at least one exotic nematode within a region.

Root knot nematodes are extracted from soil using a variety of techniques. Six methods (and subtle variations thereof) are particularly common: Baermann trays; Baermann trays with elutriation or sieving; centrifugal flotation; flotation-sieving; semiautomatic elutriation; and Cobb's decanting and sieving. These methods are described in detail by Barker (1985) and will not be repeated here. The efficiency of nematode extraction is influenced by the amount of soil that is processed at one time. Extraction efficiencies are greatest when 100 g (~70 cc) to 450 g (~300 cc) of soil are processed (Ingham and Santo 1994b). Extraction efficiencies for *Meloidogyne* spp. are frequently low and can vary between 13 and 45% (Barker 1985, Ingham and Santo 1994a).

Sub-sampling and extraction efficiency also affect the likelihood of detecting a nematode when it is present in a sample. Both factors reduce the likelihood that nematodes will be detected when they are present. Figure 5 illustrates the consequence of processing 300 cc of soil from every liter of soil that is collected from the field. The analysis behind Figure 5 assumes that at least one nematode is present in the sample. The likelihood of detection remains <90% until densities reach ~11-75 nematodes per liter of soil.

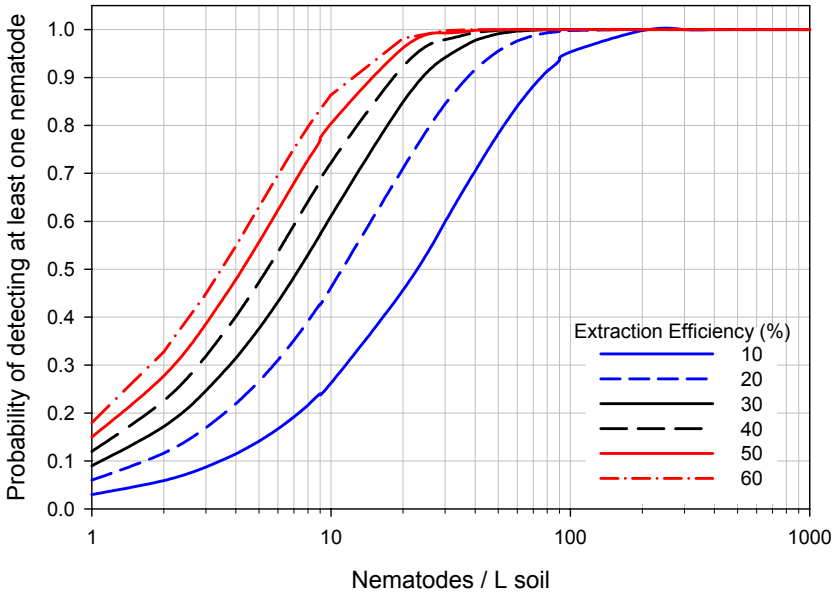

Figure 5. Influence of extraction efficiency and nematode density on the probability of detecting at least one nematode in 300 cc of a well-mixed, 1-liter soil sample.

4 **Taxonomic Recognition**. **Rating: Medium.** *Meloidogyne fallax* may occur in mixed or adjacent populations with *M. chitwoodi,* though it is reportedly rare for the two nematodes to share the same host (Karssen and van Hoenselaar 1998). Fourie et al. (2001) reported *M. fallax* on South African peanut in a mixed population with *M. chitwoodi* and *M. hapla*. Unless very closely examined, *Meloidogyne fallax*, *M. chitwoodi*, and *M. hapla* may be easily confused (Karssen 1996, CABI/EPPO 1997, Karssen and van Hoenselaar 1998, EPPO 1999, Castagnone-Sereno 2000, Fourie et al. 2001, Wishart et al. 2002). Before *M. fallax* was first recorded in New Zealand, it had been misidentified as *M. incognita* (Marshall et al. 2001). Advances in molecular techniques have improved diagnoses among morphologically similar nematodes. Common molecular techniques to identify *M. fallax* include isozyme electrophoresis, protein patterns and restriction fragment length polymorphism (PCR-RFLP) of ribosomal DNA (Esbenshade and Triantaphyllou 1987, Zijlstra et al. 1995, Karssen 1996, Petersen et al. 1997, van der Beek et al. 1997, Castagnone-Sereno et al. 1998, Schmitz et al. 1998, van der Beek et al. 1998a, van der Beek et al. 1998b, Castagnone-Sereno et al. 1999, Janssen et al. 1999, Tastet et al. 1999, Castagnone-Sereno 2000, Zijlstra 2000a, b, Tastet et al. 2001, Wishart et al. 2002).

For a detailed description of the taxonomy and morphology (including diagnostic characters) of *M. fallax*, see Appendix C.

5. **Entry Potential. Rating: Low.** Interceptions of "*Meloidogyne* sp." have been reported 212 times between 1985 and 2003. Annually, only about 12 (±3.8 standard error of the mean) interceptions have been reported nationally (USDA 2004). The majority of interceptions have been associated with airline passengers (44%). The remainders have been in permit cargo (31%), mail (20%), and general cargo (5%). The majority of interceptions were reported from Los Angeles (70%), with remaining interceptions coming from Miami (11%), and San Francisco (9%). These ports are the first points of entry for infested material coming into the US and do not necessarily represent the final destination of infested material. Movement of potentially infested material is more fully characterized in the next section.

   Meloidogyne fallax is most likely to be transported into the United States in infested plant material or infested soil. Approximately 5% of interceptions of "*Meloidogyne* sp." mention soil (USDA 2004). Infested soil may be associated with some commodities, but the greatest volumes are likely to be moved with international transport of equipment and machinery (Greco et al. 2002). Plant material is only likely to be infested if roots remain intact, as this nematode feeds strictly on root tissue. Thus, sugarbeets, black salsify, potatoes, and carrots [known hosts; see 'Host Specificity'] from infested countries have the potential to harbor this nematode. The relatively small size of this pest makes it difficult to detect during routine quarantine inspections at ports of entry. As a result, previous interception records of the pest may not accurately characterize the frequency at which this pest actually arrives in the US. As a result, we also examine PIN-309 records for interceptions of roots of potential host material. Between 1985 and 2004, beet/sugarbeet roots were intercepted 5 times (3 of these interceptions were from France where *M. fallax* has been detected in a greenhouse but has not been reported from the field). Black salsify roots are commonly eaten in Europe but have not been reported in interception records. Carrot (root) has been intercepted 9 times and peanuts have been intercepted only once. There are no interception records for potato tubers.

   Neither the nematode itself nor host plants from infested countries are intercepted frequently at US ports of entry. As a result, we assign a low rating to the potential for entry. However, potentially significant pathways (e.g., military equipment and soil contaminants of grain) have not been studied with any detail. Consequently, a great deal of uncertainty is associated with our rating.

6. **Destination of Infested Material. Rating: Medium.** When an actionable pest is intercepted, officers ask for the intended final destination of the conveyance. Materials infested with "*Meloidogyne* sp." were destined for 19 states (USDA 2004). The most commonly reported destination was California (77%), followed by Florida (7%), Texas (3%), New Jersey (3%), New York (1%), and Georgia (1%). We note that several of these states have a climate and hosts that would be suitable for establishment by *Meloidogyne fallax*.

7. **Potential Economic Impact. Rating: High.** The economic impact of *M. fallax* is difficult to measure because this species frequently occurs in mixed populations (Janssen 1997, Karssen and van Hoenselaar 1998, EPPO 1999, Fourie et al. 2001). As a result, it is possible to ascribe nematode damage within a field to *Meloidogyne* spp. in general but not *M. fallax* alone. *Meloidogyne* species are among the most economically important plant-parasitic nematodes found worldwide (DeGiorgi et al. 2002). Crop losses resulting from nematode damage to vegetables and grains are estimated at an average of 10-11% worldwide (Jensen 1972, Potter and Olthof 1993, Whitehead 1998, Tastet et al. 2001), but the economic impact from nematodes is thought to be grossly underestimated. If untreated, crop losses to potatoes grown in the Pacific Northwest region of the U.S. would amount to ca. $40 million (CABI/EPPO 1997, EPPO 1999, Bartlett 2000).

   Damage to host plants caused by root-knot nematodes involves impaired root growth (e.g., small gall formation, proliferation of lateral roots, or stimulation of giant cell growth at feeding sites in parenchyma and phloem) and impaired root function (contributing to chlorosis, stunted growth, nutrient deficiencies, and/or necrosis of above-ground plant parts). Symptoms of nematode damage may be similar to those caused by nutrient or water deficiency. Nematode infestation of plant roots limits water uptake. Infested plants may appear wilted under hot and sunny conditions, even with ample soil moisture (Hussey 1985). Symptoms may not be apparent until plants reach later stages of growth. Injured root tissue is susceptible to other disease-causing pathogens (Jensen 1972, Hesling 1978, Pitcher 1978, Sasser 1987, Eisenback and Hirschmann Triantaphyllou 1991, Tastet et al. 2001). Much of the visible damage to plant hosts is likely caused by a combination of biotic and abiotic factors (Jensen 1972, Hussey 1985, Swarup and Sosa-Moss 1990, Potter and Olthof 1993). Symptoms of infection of potatoes by *M. fallax* are described by den Nijs and Karssen (den Nijs and Karssen 2002):

   > "Above ground symptoms of heavily infested plants include stunting and yellowing, while below ground, galling is typical. The root galls produced by *M. chitwoodi* and *M. fallax* are comparable to those produced by several other root-knot species, i.e. relatively small galls in general without secondary roots emerging from them (as in *M. hapla*). On potato tubers *M. chitwoodi* and *M. fallax* have numerous small pimple-like raised areas on the surface (in *M. hapla* these swellings are not evident). However some potato cultivars, although heavily infected, are free of visible external symptoms. Internal potato tissue is necrotic and brownish, just below the peel."

   Economic thresholds for *M. fallax* are not available. Thresholds for several other *Meloidogyne* spp on various hosts are summarized by Potter and Olthof (1993). Damage thresholds for other *Meloidogyne* spp. on potato range from 0.4-2.5 juveniles/ 100 cm^3 soil (Pinkerton et al. 1991, Korthals et al. 2000), or 20 eggs/100 cm^3 soil (Brodie et al. 1993, van der Beek et al. 1998c). For other vegetable crops, the threshold is approximately 0.5-2 juveniles/g of soil (Potter and Olthof 1993). In general, severity of damage caused by *Meloidogyne* spp. is

influenced by nematode species, host susceptibility, crop rotation, season, timing of planting and harvest, soil type and temperature (Potter and Olthof 1993). Economic losses from *Meloidogyne* spp. are most likely when soil temperatures are optimal for development and where crop rotations involve successive, susceptible hosts (Brodie et al. 1993). In addition to lowering the yield potential of crops, nematodes can adversely affect the marketability of produce. Nematodes can severely distort and blemish root crops. Acceptable levels of nematode infestation are very low (ranging from 0-5%) (Pinkerton et al. 1991, Brinkman et al. 1996, Brommer 1996, CABI/EPPO 1997, Molendijk and Brommer 1998, Bartlett 2000, Castagnone-Sereno 2000, Marshall et al. 2001); therefore, even produce used for processing may be rejected

8 Potential Environmental Impact. Rating: High. In general, newly established species may adversely affect the environment in a number of ways. Introduced species may reduce biodiversity, disrupt ecosystem function, jeopardize endangered or threatened plants, degrade critical habitat or stimulate use of chemical or biological controls. *Meloidogyne fallax* is likely to affect the environment in many of these ways.

Historically, the introduction of invasive agricultural pests has initiated control measures to avoid lost production (National Plant Board 1999). Consumer preferences for unblemished, high quality produce encourage the use of pesticides, while at the same time, negative public opinion regarding the use of pesticides on fruits and vegetables is a market concern (Bunn et al. 1990). Therefore, the establishment of any new pests of fruits and vegetables destined for fresh markets is likely to stimulate greater use of either chemical or biological controls to ensure market access.

Meloidogyne fallax has a wide host range including both monocots and dicots (see 'Host Specificity'). Appendix D summarizes state and federally listed threatened or endangered plant species (USDA NRCS 2004) found within plant genera known to be hosts (or potential hosts) for *M. fallax*. Plants listed in Appendix D might be suitable hosts for *M. fallax*, and thus, could be adversely affected by this nematode.

9 Establishment Potential. Rating: Low-Moderate. Our initial predictions suggest that approximately 1/3 of the US has a climate that could support populations of *M. fallax* (Fig. 2). Known host plants (especially potatoes) are only grown sporadically in areas judged to be climatically suitable for the nematode. Thus, establishment of this nematode is likely to depend heavily on the extent to which *M. fallax* can utilize "experimental" hosts (i.e., plants that will support nematode growth and reproduction in a greenhouse but have never been reported to sustain populations in the field). Experimental hosts (esp. corn and wheat) are more widely produced throughout the United States than are known hosts. It is important to note that the potential for the pest to arrive in the United States seems low based on available pest interception records. A low probability

of arrival lessens the chances for establishment by this pest. Finally, the propensity for the nematode to move after it is introduced seems limited as it has no stage for active, long-distance dispersal, though movement of other soil-borne nematodes by wind, water, and human activity has been noted (Potter and Olthof 1993).

See Appendix E for a more detailed description of the biology of *M. fallax*.

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Appendix A. Comparison of climate zones. To determine the potential distribution of a quarantine pest in the US, we first collected information about the worldwide geographic distribution of the species (Table A1). Using a geographic information system (e.g., ArcView 3.2), we then identified which biomes (i.e., habitat types), as defined by the World Wildlife Fund (Olson et al. 2001) occurred within each country or municipality reported An Excel spreadsheet summarizing the occurrence of biomes in each nation or municipality was prepared. The list was sorted based on the total number of biomes that occurred in each country/municipality. The list was then analyzed to determine the minimum number of biomes that could account for the reported worldwide distribution of the species. Countries/municipalities with only one biome were first selected. We then examined each country/municipality with multiple biomes to determine if at least one of its biomes had been selected. If not, an additional biome was selected that occurred in the greatest number of countries or municipalities that had not yet been accounted for. In the event of a tie, the biome that was reported more frequently from the entire species' distribution was selected. The process of selecting additional biomes continued until at least one biome was selected for each country. Finally, the set of selected biomes was compared to only those that occur in the US.

Table A1. Reported geographic distribution of *M. fallax*:

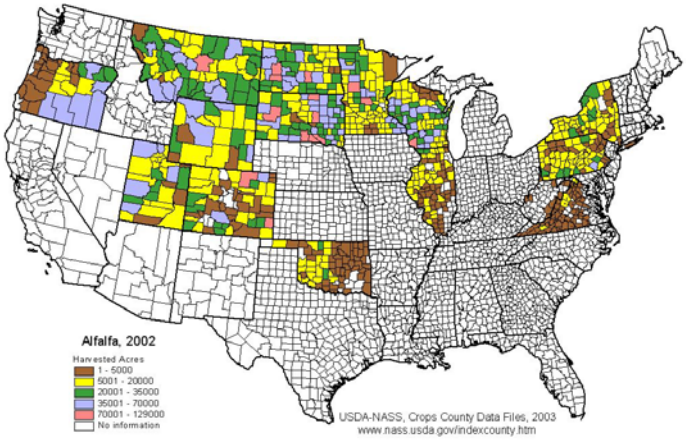
Locations	**Reference(s)**
Australia (SE: Adelaide Hills)	(Nobbs et al. 2001, Inserra et al. 2003)
Belgium (Antwerp and Limburg Provinces)	(Waeyenberge and Moens 2001, Inserra et al. 2003)
France (near *Paimpol in Province of Brittany)	(Daher et al. 1996, Tastet et al. 1999, Zijlstra 2000b, Wishart et al. 2002, Inserra et al. 2003, Mugniéry 2004)pers. comm.
Germany (Hamburg)	(Schmitz et al. 1998, Inserra et al. 2003)
Netherlands (SE: Limburg, Zuid-Holland Provinces, and throughout (unspecified)	(Brinkman et al. 1996, Brommer 1996, Karssen 1996, Heijbroek et al. 1998, Karssen and van Hoenselaar 1998, Molendijk and Brommer 1998, Schmitz et al. 1998, van der Beek et al. 1998a, van der Beek et al. 1998b, van der Beek et al. 1998c, EPPO 1999, Janssen et al. 1999, Tastet et al. 1999, Korthals et al. 2000, Brommer and Molendijk 2001, Kok et al. 2001, Wishart et al. 2002, Inserra et al. 2003)
New Zealand (Crosby Districts): North Island: Bay of Plenty, Northland, Taranaki, Wanganui, Wellington, and Waikato; South Island: Mid-Canterbury, Nelson, Southland including Centre and Dog Islands	(Crosby et al. 1976, Marshall et al. 2001)
South Africa (Northern Cape: Vaalharts)	(Fourie et al. 2001)

*=detected in a greenhouse, not obtained from a field sample

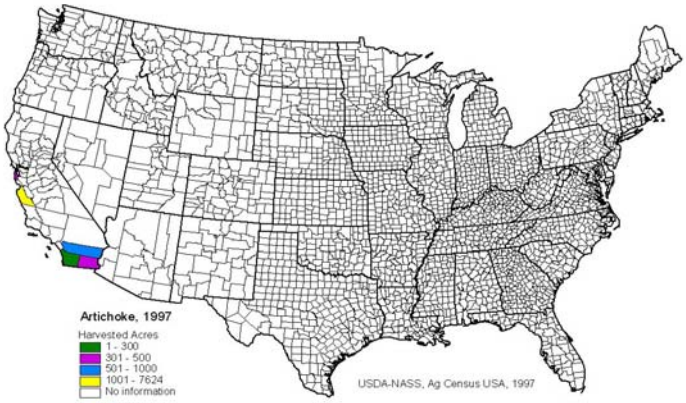
France was not included in our climatic analysis. Several reviews suggest that *M. fallax* occurs in France, however, its occurrence has been traced to a greenhouse detection. The source plant was thought to be a tomato plant imported from an area of Belgium where the nematode is well-established (Mugniéry 2004, pers. comm.).

Appendix B. Commercial production of hosts of *Meloidogyne fallax* in the continental US.

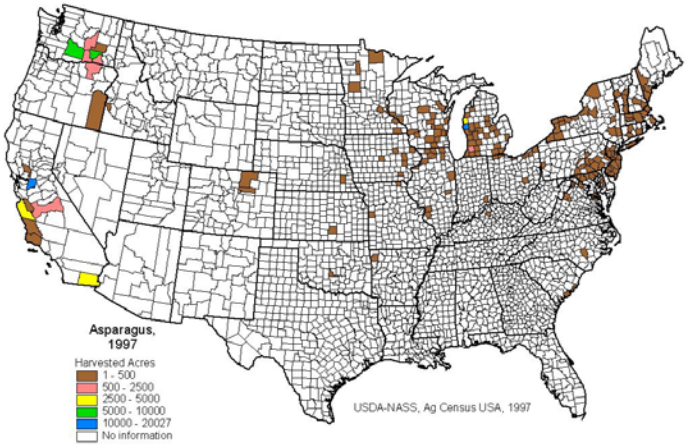
Map 1. Alfalfa (*Medicago sativa*)



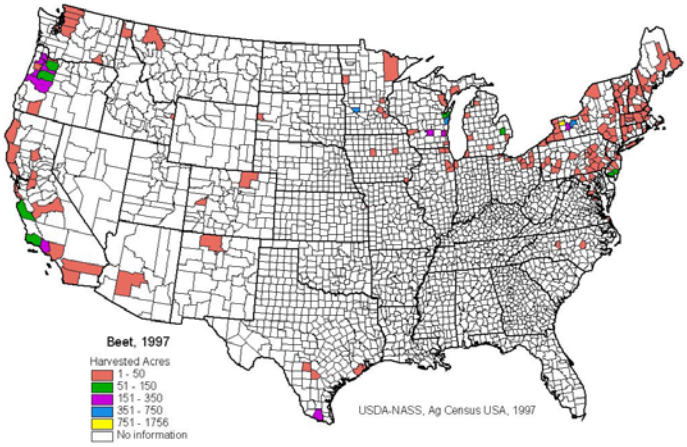
Map 2. Artichoke (*Cynara scolynus*)



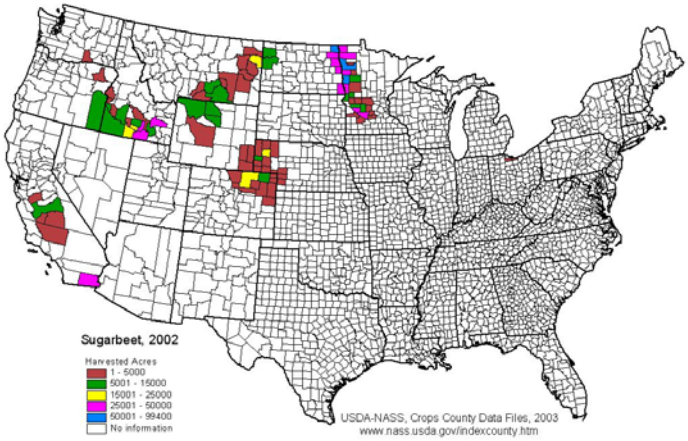
Map 3. Asparagus (*Asparagus officinalis*)



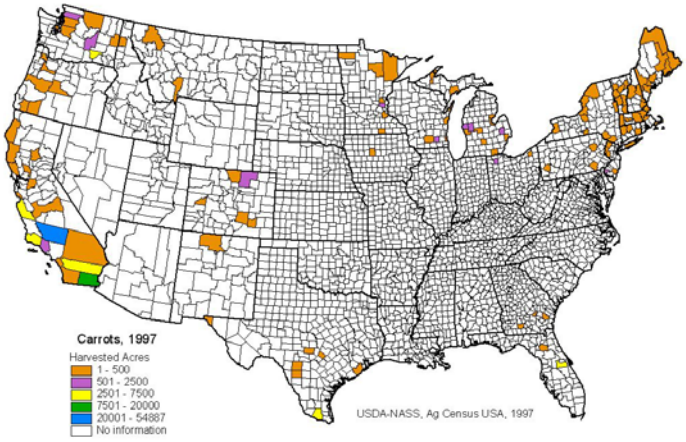
Map 4. Beet (*Beta vulgaris*)



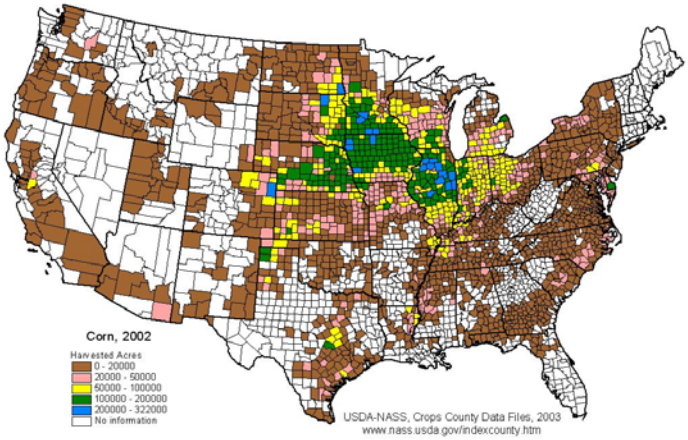
Map 5. Sugarbeet (*Beta vulgaris* subsp. *vulgaris*)



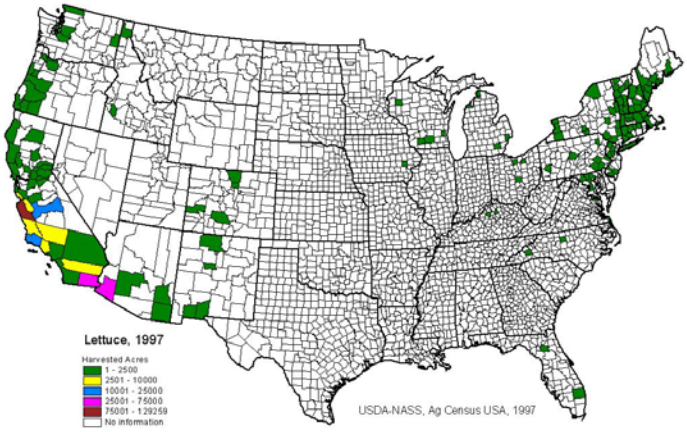
Map 6. Carrot (*Daucus carota*)



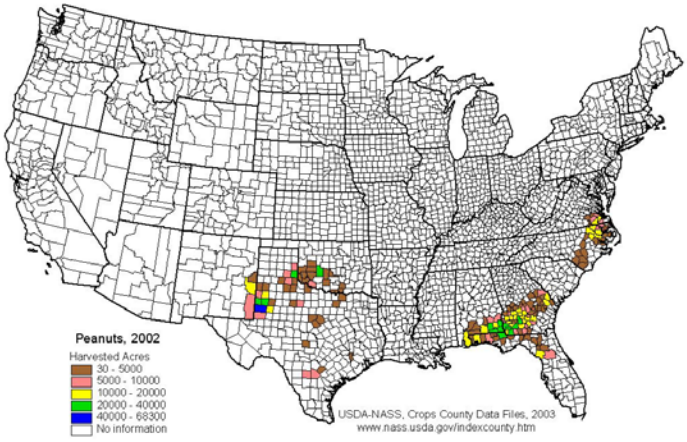
Map 7. Corn (*Zea mays*)



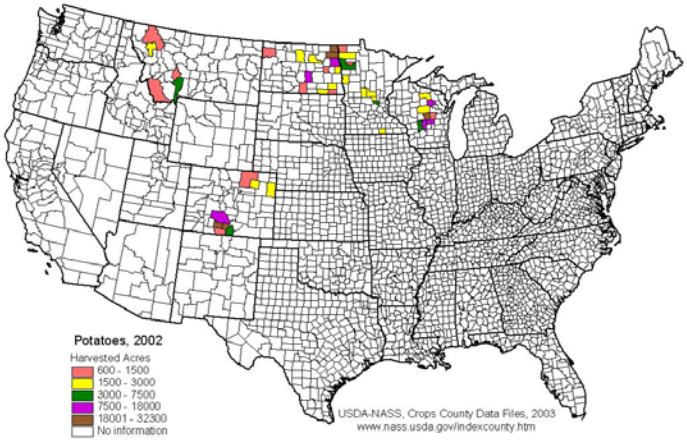
Map 8. Lettuce (*Lactuca sativa*)



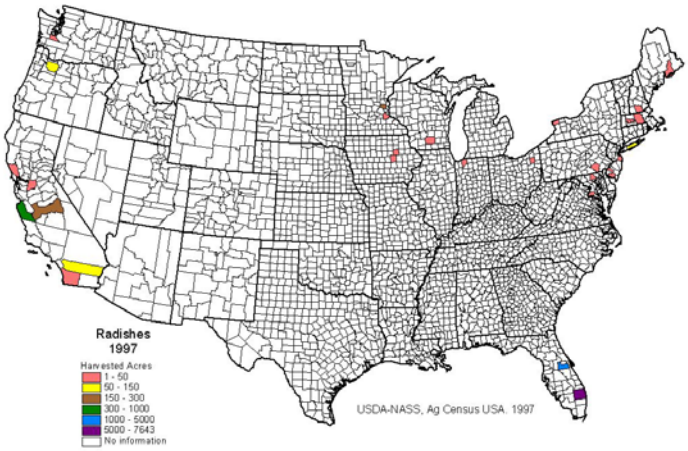
Map 9. Peanut (*Arachis hypogaea*)



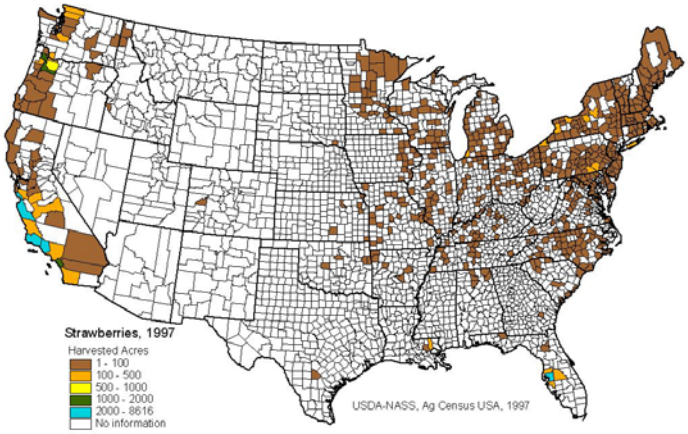
Map 10. Potato (*Solanum tuberosum*)



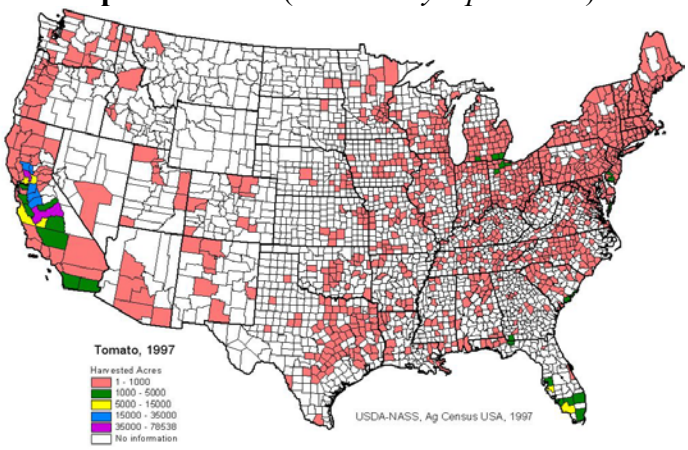
Map 11. Radish (*Raphanus sativus*)



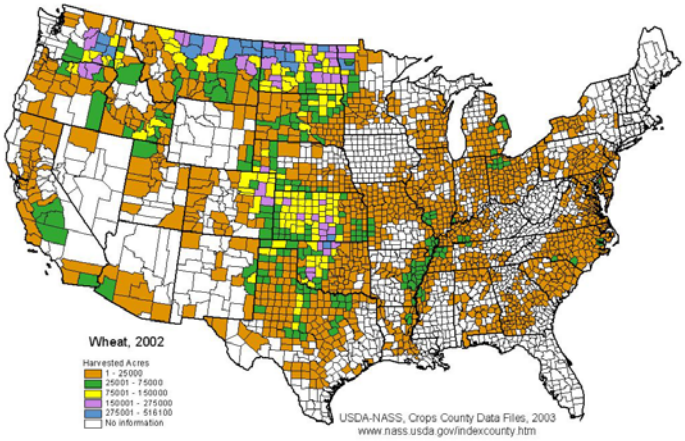
Map 12. Strawberry (*Fragaria* sp.)



Map 13. Tomato (S*olanum lycopersicum*)



Map 14. Wheat (*Triticum* spp.)



Appendix C. Taxonomy and Morphology of *Meloidogyne fallax*

Important diagnostic characters of several *Meloidogyne* spp. including *M. chitwoodi* have been reviewed by Eisenback and Hirschmann Triantaphyllou (1991) but these will not be presented here. The European Plant Protection Organization provides a brief morphological comparison of *M. fallax* to similar *Meloidogyne* spp. (EPPO 1999). The following descriptions of *M. fallax* females, males, second-stage juveniles, eggs, and diagnostic features are quoted from Karssen (Akem et al. 2000).

Meloidogyne fallax Karssen, 1996

Synonyms

Meloidogyne chitwoodi B-type Karssen (1995)

Female (See Figs. C1 (A-K))

(n=30)
length 404.1-720.3 µm;
greatest body diameter 256.2-464.1 µm;
stylet 13.9-15.2 µm;
stylet knobs 3.8-4.4 µm width;
2.0-2.5 µm height
vulval slit length 20.2-28.4 µm;
vulval-anus distance 12.6-19.0 µm;
metacorpus valve length 10.1-13.9 µm
metacorpus valve width 89.7-103.6 µm
excretory pore-anterior end 12.6-32.9 µm

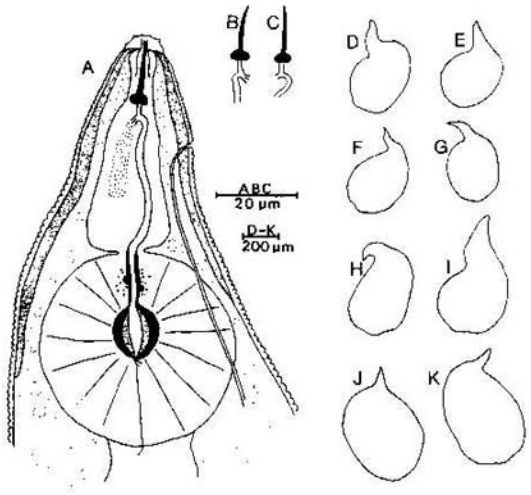


Figure C1. *Meloidogyne fallax* n. sp. females. **A:** Pharyngeal region (lateral view); **B, C:** Stylets (lateral view); **D-K:** Female body shapes [Quoted and reproduced from (Karssen 1996)].

Body annulated, pearly white, globular to pear shaped with slight posterior protuberance and distinct neck region projecting from the body axis at an angle of up to 90º to one side (Fig. C1(D-K)). Head region set off from body, marked with one or two annules. Head cap distinct but variable in shape; labial disc slightly elevated. Cepahlic framework weakly sclerotized; vestibule extension distinct. Stylet cone dorsally curved and shaft cylindrical; knobs large, rounded to transversely ovoid, slightly sloping posteriorly from the shaft. Excretory pore located between head end and metacarpus levels. One or two large vesicles and several smaller ones located along the lumen lining. Pharyngeal glands variable in size and shape (Fig. C1(A)). Perineal pattern ovoid to oval shaped, sometimes rectangular; dorsal arch ranging from low to moderately high, with coarse striae. Tail terminus indistinct without punctuations. Phasmids small and difficult to observe. Perivulval area devoid of striae.

Male (See Figs. C2(A-G))
(n=30)
length 736.2-1520.1 µm
greatest body diameter 27.2-43.8 µm
a=21.2-53.5 µm
c=82.7-201.7
[DeMan's indices (modified) from Jones (1965): a=length/greatest diameter; c=length/length of tail (anus to tip)]
stylet 18.9-20.9 µm
stylet knobs 3.8-5.1 µm width;
2.5-3.2 µm height
spicules 22.1-29.7µm

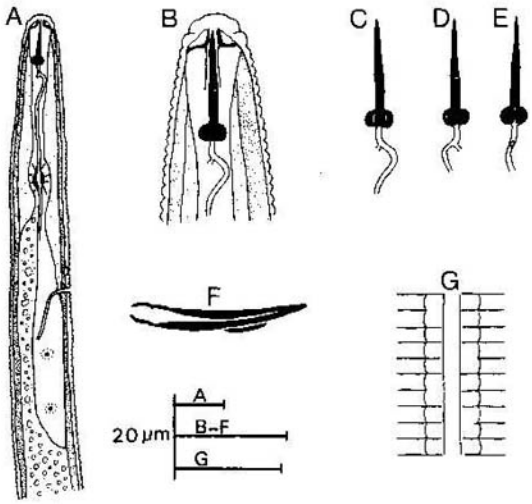


Figure C2. *Meloidogyne fallax* n. sp. males. **A:** Pharyngeal region (lateral view); **B:** Head end (lateral view); **C-E:** Stylets (lateral, lateral, ventral view respectively); **F:** Spicule and gubernaculum (lateral view); **G:** Lateral field at mid-body [Quoted and reproduced from (Karssen 1996)].

Body vermiform, slightly tapering anteriorly, bluntly rounded posteriorly. Cuticle with distinct transverse striae. Lateral field with four incisures; outer bands irregularly areolated; a fifth broken longitudinal incisure is rarely present near mid-body. Head slightly set off, with a single post-labial annule (sometimes called head region) usually partly subdivided by a transverse incisure (Fig. C2(B)). Labial disc rounded, elevated and fused with medial lips. Prestoma hexagonal in shape with six inner cephalic sensilla adjacent to the rim. Medial lips crescent shaped with raised edges at lateral sides. Four cephalic sensilla small and marked by cuticular depressions on the medial lips. Amphidial openings appear as elongated slits between labial disc and medium sized lateral lips. Cephalic framework moderately sclerotized, vestibule extension distinct. Stylet cone straight; shaft cylindrical; knobs large and rounded, set off from the shaft. Pharynx with slender procorpus, meta corpus oval shaped with pronounced valve. Ventrally overlapping pharyngeal gland lobe variable in length (Fig. C2A)). Hemizonid, 2-3 µm in length, two to four annules anterior to excretory pore. Testis usually long, monorchic, with reflexed or outstretched germinal zone. Tail short and twisted. Spicules slender, curved ventrally; gubernaculum slightly crescent shaped (Fig. C2(F)). Phasmids located anterior to cloaca.

Second-stage juvenile (See Figs. C3(A-F))
(n=30)
length 381.4-435.2 µm;
greatest body diameter 13.3-16.4 µ;
tail 46.1-55.6 µm;
tail terminus length 12.2-15.8 µm;
stylet 10.1-11.4 µm.

Body moderately long, vermiform, tapering at both ends but posteriorly more than anteriorly. Body annules small but distinct. Lateral field with four incisures, not areolated. Head region truncate, slightly set off from body. Head cap low and narrower than head region (Fig. C3(B)). Cephalic framework weakly sclerotized, vestibule extension distinct. Stylet slender and moderately long, cone straight; shaft cylindrical; knobs distinct, rounded and set off from the shaft. Pharynx with faintly outlined procorpus and oval shaped metacorpus with distinct valve. Oesophageal gland lobe variable in length, overlapping intestine ventrally. Hemizonid distinct at the level of the excretory pore. Moderately sized tail, gradually tapering until hyaline tail terminus, with inflated proctodeum (Fig. C3(C-F)). Phasmids difficult to observe, small, slightly posterior to anus. A rounded hypodermis marks the anterior position of the smooth hyaline tail terminus ending in a broadly rounded tip. Terminus generally marked by faint cuticular constrictions.

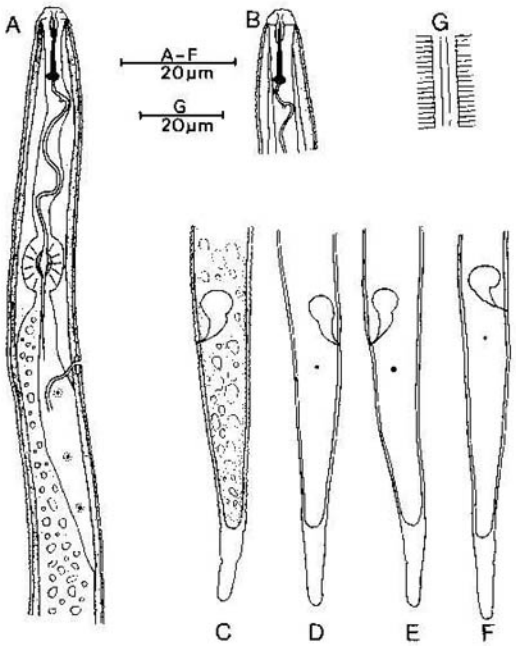


Figure C3. *Meloidogyne fallax* n. sp. second-stage juveniles. **A:** Pharyngeal region (lateral view); **B:** Head end (lateral view); **C-F:** Tail shape variation (lateral view); **G:** Lateral field at mid-body [Quoted and reproduced from (Karssen 1996)].

Egg (n=30)
length 89.7-103.6 µm
width 34.1-44.2 µm

Diagnosis and Relationship

M. fallax n. sp. is characterized by a dorsally curved female stylet 14.5 µm (13.9-15.2) long with rounded set off stylet knobs. Oval shaped perineal pattern with coarse striae and moderately high dorsal arch. Male stylet length 19.6 µm (18.9-20.9) with prominent set off rounded knobs. The labial disc is elevated, crescent shaped medial lips at lateral side and distinct lateral lips. The J2's hemizonid is at the same level with the excretory pore. Tail and hyaline tail length 49.3 µm (46.1-55.6) and 13.5 µm (12.1-15.8), respectively. *M. fallax* n. sp. reproduces by facultative meiotic parthenogenesis, the haploid chromosome number is n=18 (H.v.d. Beek, pers. comm)(van der Beek and Karssen 1997). *M. fallax* n. sp. is characterized by an unique malate dehydrogenase (MDH) pattern, not described by Esbenshade and Trianaphyllou (1987), and the lack of any major esterase (EST) band. In combination these patterns are useful to differentiate *M. fallax* from other known Meloidogyne species. Beside the mentioned difference in isozyme patterns between *M. chitwoodi* and *M. fallax*. n. sp., biochemical differentiation was also confirmed by restriction analysis of ribosomal (ITS) DNA (Zijlstra et al. 1995). *Meloidogyne fallax* n. sp. differs from the morphologically close related *M. chitwoodi* Golden et al., 1980 by greater female and male stylet length, absence of small, irregular outlined male and female stylet knobs (Eisenback and Hirschmann Triantaphyllou 1991), male labial disk elevated, longer juvenile body-, tail-, and hyaline tail length, different hyaline tail shape, hemizonid position, esterase and malate dehydrogenase patterns; from *M. hapla* Chitwood, 1949 by the absence

of fine, smooth striae, rounded and flattened dorsal arch and tail area punctuations in the female perineal pattern, broader J2 tail and tail terminus with distinct hyaline part, shorter female and male stylet length, and the absence of small rounded stylet knobs; from *M. artiellia* Franklin, 1961 and *M. ardenensis* Santos, 1968 by much greater J2 body-, tail-, and hyaline tail length and by hemizonid position relative to excretory pore; from *M. naasi* Franklin, 1965 by smaller J2 body-, tail-, and hyaline tail length and the absence of vesicles in the juvenile metacorpus. The presence of vesicles or vesicle-like structures in the metacorpus of the female *Meloidogyne* species was reported as "unique" with the description of *M. chitwoodi* Golden et al., 1980, although first reported in *M. kikuyensis* De Grisse, 1961 and *M. oryzae* Maas et al., 1978. These structures are also described in *M. hispanica* Hirschnmann, 1986, *M. maritima* Jepson, 1987, *M. konaensis* Eisenback et al., 1995, *M. fallax* n. sp. and some *M. hapla* populations from The Netherlands (Karssen, unpubl.). Therefore these vesicles are not useful as a discriminating character for *M. chitwoodi* identification.

Appendix D. Threatened or endangered plants potentially affected by *Meloidogyne fallax*.

Meloidogyne fallax has the potential to adversely affect threatened and endangered plant species. However, because *M. fallax* only occurs outside the US and threatened and endangered plant species under consideration only occur within the US, it is not possible to confirm the host status of these rare plants from the scientific literature. From available host records, *M. fallax* is known to feed primarily on species within the families Apiaceae, Asteraceae, Fabaceae, and Solanaceae. From literature documenting natural and potential (experimental) hosts, we infer that threatened or endangered plant species which are closely related to either known or potential host plants might also be suitable hosts (Table D1). For our purposes closely related plant species belong to the same genus. Note that, as discussed under 'Host Specificity/Availability,' though *M fallax* may successfully feed and complete its life cycle on several plants, it has been found in agriculture on peanut, potato, and carrot, and is thought to be economically important to black salsify carrot, potato and tomato production in Europe (Brinkman et al. 1996, Karssen and van Hoenselaar 1998, Molendijk and Brommer 1998, EPPO 1999, Fourie et al. 2001, Marshall et al. 2001, Inserra et al. 2003).

Table D1: Threatened and endangered plants in the conterminous U.S. that are potential hosts for *Meloidogyne fallax*.

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
Chenopodium album	*C. album* var. *missouriense*	Missouri lambsquarters		NY (E)
	C. berlandieri var. *boscianum* [=*Chenopodium boscianum*]	pitseed goosefoot		NH (E)
	C. berlandieri var. *macrocalycium*	pitseed goosefoot		NY (E)
	C. foggii	Fogg's goosefoot		PA (E)
	C. humile	marshland goosefoot		ME (T)
	C. rubrum	red goosefoot		ME (T) NH (T) NJ (E) NY (T)
	Chenopodium simplex [=*Chenopodium gigantospermum*]	mapleleaf goosefoot		MD (E)
	C. standleyanum	Standley's goosefoot		MD (E)

Table D1: Threatened and endangered plants in the conterminous U.S. that are potential hosts for *Meloidogyne fallax*.

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
Lamprocapnos =[*Dicentra*] *spectabilis*	*Dicentra canadensis*	squirrel corn		CT (T) ME (T) NH (T) NJ (E)
	D. eximia	turkey corn		MD (T) NJ (E) PA (E)
Eriogonum sp.	*E. alpinum*	Trinity buckwheat		CA (E)
	E. apricum var. *apricum*	Ione buckwheat		CA (E)
	E. chrysops	bitterroot buckwheat		OR (T)
	E. clavellatum [=*Eriogonum pelinophilum*]	Comb Wash buckwheat	E	
	E. codium	basalt desert buckwheat		WA (E)
	E. crosbyae	Crosby's buckwheat		OR (T)
	E. ericifolium var. *thornei*	Thorne's buckwheat		CA (E)
	E. grande var. *timorum*	San Nicolas Island buckwheat		CA (E)
	E. gypsophilum	Seven River Hills buckwheat	T	NM (E)
	E. kelloggii	Red Mountain buckwheat		CA (E)
	E. kennedyi var. *austromontanum*	southern mountain buckwheat	T	
	E. longifolium var. *gnaphalifolium*	longleaf buckwheat	T	FL (E)
	E. longifolium var. *harperi*	Harper's buckwheat		TN (E)
	E. ovalifolium var. *vineum*	Cushenbury buckwheat	E	
	E. ovalifolium var. *williamsiae*	Williams' buckwheat	E	
Lactuca sativa	*L. floridana*	woodland lettuce		MI (T) NY (E)

Table D1: Threatened and endangered plants in the conterminous U.S. that are potential hosts for *Meloidogyne fallax*.

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
	L. hirsuta	hairy lettuce		IL (T) MD (E) NY (E) OH (E) VT (T)
	L. tatarica var. *pulchella* [=*Lactuca pulchella*]	blue lettuce		MI (T)
Oenothera biennis, *O. glazioviana* [=*O. erythrosepala*]	*Oenothera argillicola*	shalebarren evening-primrose		PA (T)
	O. californica ssp. *eurekensis* [=*Oenothera avita* ssp. *eurekensis*]	Eurela Dunes evening-primrose	E	
	O. clelandii	Cleland's evening-primrose		OH (E)
	O. deltoides ssp. *howellii*	Antioch Dunes evening-primrose	E	CA (E)
	O. humifusa	seabeach evening-primrose		NJ (E)
	O. laciniata	cutleaf evening-primrose		NY (E)
	O. linifolia	threadleaf evening-primrose		KY (E)
	O. macrocarpa Nutt. ssp. *macrocarpa*	bigfruit evening-primrose		TN (T)
	O. oakesiana [=*Oenothera parviflora* var. *oakesiana*]	Oakes' evening-primrose		NY (T) OH (T)
	O. parviflora	northern evening-primrose		OH (T)
	O. perennis	little evening-primrose		IA (T) IL (T) IN (T) KY (E)
	O. pilosella ssp. *sessilis*	meadow evening-primrose		AR (T)

Table D1: Threatened and endangered plants in the conterminous U.S. that are potential hosts for *Meloidogyne fallax*.

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
	O. triloba	stemless evening-primrose		KY (T)
	O. wolfii	Wolf's evening-primrose		OR (T)
Phacelia tenacetifolia	*P. argentea*	sanddune phacelia		OR (T)
	P. argillacea	Attwood's phacelia	E	
	P. covillei	Coville's phacelia		MD (E)
	P. formosula	Northpark phacelia	E	
	P. franklinii	Franklin's phacelia		MI (T)
	P. gilioides	Brand's phacelia		IL (E)
	P. insularis var. *insularis*	northern Channel Islands phacelia	E	
	P. lenta	sticky phacelia		WA (T)
	P. ranunculacea	oceanblue phacelia		IN (E) OH (E)

E= Endangered; T=Threatened

Appendix E. Biology of *Meloidogyne fallax*

Population dynamics

Meloidogyne fallax is closely related to *M. chitwoodi*, with the former possibly being a new race of the latter (CABI/EPPO 1997). The two nematodes are very similar in many aspects of their basic biology, particularly with respect to host penetration, general development and reproduction (CABI/EPPO 1997, EPPO 1999). Less information has been published for *M. fallax* than for *M. chitwoodi*. As a result, we provide relevant information about the biology of *M. chitwoodi* to provide insights on the way *M. fallax* may respond to environmental conditions. We present information for *M. fallax* specifically when it is available.

Meloidogyne chitwoodi is biologically active at lower temperatures than many other *Meloidogyne* species (Brodie et al. 1993). Therefore, this species can initiate infection earlier in the season than other root-knot nematodes and can complete more than one generation per year (Brodie et al. 1993). *Meloidogyne chitwoodi* is active at temperatures as low as 6°C (Brodie et al. 1993). *Meloidogyne chitwoodi* completes a generation in 3-4 weeks under optimal conditions; development occurs above a threshold temperature of 5ºC within 600-800 and 500-600 degree days, respectively for the first and second generations (CABI/EPPO 1997). Van der Beek et al. (1998c) suggested that *M. fallax* has a shorter life cycle than *M. chitwoodi*. In a study on population dynamics of *M. chitwoodi* and *M. fallax* on potato in the Netherlands, at least 2 generations occurred, with the second generation beginning in the third week of June (Brommer and Molendijk 2001). In the Netherlands it is plausible that *M. fallax* could achieve 2-3 generations annually (Brommer and Molendijk 2001). Similar to *M. chitwoodi*, *M. fallax* can overwinter in the egg or juvenile stage (CABI/EPPO 1997), though overwintering in the egg stage is more likely (Brodie et al. 1993).

Stage specific biology

Adult

Although males can be found in populations of *Meloidogyne* spp., their contribution to the reproductive success of females is unclear. Females can reproduce asexually, and this mode of reproduction maintains reproductive isolation in mixed populations of *M. chitwoodi* and *M. fallax* (van der Beek et al. 1998a, van der Beek et al. 1998b). Females produce large gelatinous egg masses or sacs containing "hundreds of eggs" (Janssen 1997). The egg mass is deposited on either galled root surfaces or inside root galls (Hussey 1985, Inserra et al. 2003). In potatoes, females with egg masses were found 10 mm inside the surface of a tuber (Marshall et al. 2001).

Egg

Egg hatch may or may not involve stimulation from the host root (Hussey 1985). Hatching can occur quickly and for an extended period depending on temperature (Janssen 1997). Eggs will not hatch in dry soils and may persist in soil or dry roots for extended periods awaiting more favorable moisture conditions.

Larva

Emergence occurs under moist soil conditions; juveniles may become inactive under dry conditions. *Meloidogyne* larvae can be easily distributed by irrigation water (as can eggs), and in areas of saturated soil larvae may survive under water for up to three weeks (Milne 1972). There are four juvenile stages. The first stage occurs inside the egg. Following a molt and emergence, second stage juveniles move out of the egg and invade the host plant roots (Hussey 1985). Females are only mobile during the second juvenile stage and are attracted to host plant roots (Hussey 1985, Eisenback and Hirschmann Triantaphyllou 1991). Juveniles may feed singly or in a group. After egg hatch, larvae will continue to search for suitable feeding sites until their energy reserves are depleted if a host cannot be found. When a suitable site is located, a larva will penetrate the root, usually near or behind the root cap, at lateral root initials or in galled root tissue near an embedded adult female. The site where one juvenile enters the root may attract others (Hussey 1985). The juvenile moves through the root to the region of cell differentiation or to regions of lateral root emergence, settles and becomes inactive while feeding. Feeding induces cells in the primary phloem or parenchyma to swell and form "giant" or "nurse" cells on which juveniles feed until development is completed (Hussey 1985). If the plant does not form giant cells as the nematode attempts to establish a feeding site, the larva may not complete its development and leave in search of another root, or die of starvation in the process(Jensen 1972, Hussey 1985). When giant cell formation occurs, tissues surrounding the feeding nematode begin transforming at approximately the same time, producing a gall or "knot" within 1-2 days following root penetration (Hussey 1985). A female larva will swell as it feeds, also swelling apical roots, until development is completed (Inserra et al. 2003). Total development time varies from 2-3 months (Janssen 1997).