Mini Risk Assessment
British root-knot nematode: *Meloidogyne artiellia* Franklin
[Nematoda: Meloidogynidae]

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Introduction

The plant-parasitic nematode *Meloidogyne artiellia* Franklin is a significant pest of several cereals, legumes, root and cruciferous crops and is adapted to survive cold and dry conditions (Sikora and Greco 1990, Riggs and Niblack 1993, Rivoal and Cook 1993). Despite what the common name may imply, the British root-knot nematode occurs in northern Europe, the Mediterranean, North Africa, the Middle East, Russia, and China.

Meloidogyne artiellia is not known to occur in the United States. An initial risk evaluation concluded that *M. artiellia* posed moderate risk relative to other exotic nematodes that might be introduced into the US (Inserra et al. 2003). The purpose of this document is to further evaluate several factors that contribute to risks posed by *M. artiellia* and apply this information to the refinement of sampling and detection programs.

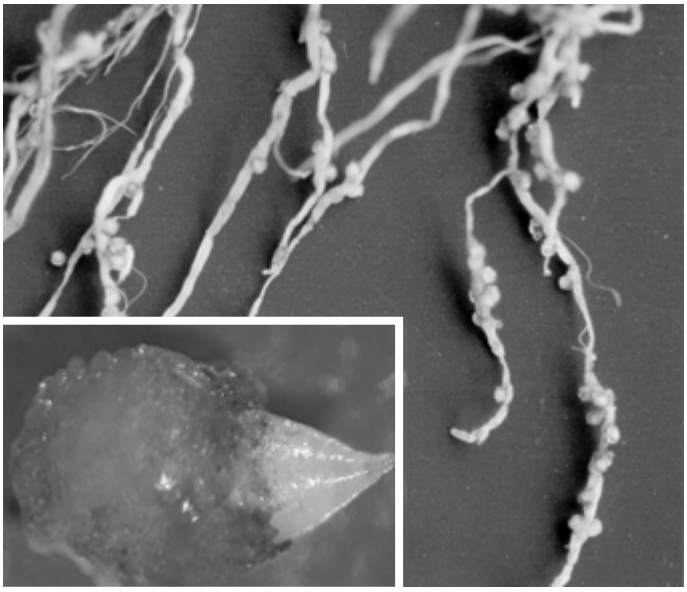

Figure 1. Galls on chickpea roots caused by *Meloidogyne artiellia*. Inset shows a swollen female.[Image from (Castillo et al. 2003).]

1. **Ecological Suitability. Rating: High.** *Meloidogyne artiellia* is present in parts of Europe, Africa, and Asia. Appendix A provides a detailed list of the reported worldwide distribution of this nematode. In general, *M. artiellia* occurs in dry to

temperate climates. The currently reported distribution of *M. artiellia* suggests that the pest may be most closely associated with biomes characterized as: Mediterranean scrub; temperate broadleaf and mixed forests; temperate coniferous forests; and tropical and subtropical moist broadleaf forests. Consequently, we estimate that approximately 49% of the continental US would have a suitable climate for *M. artiellia* (Fig. 2). See Appendix A for a more complete description of this analysis.

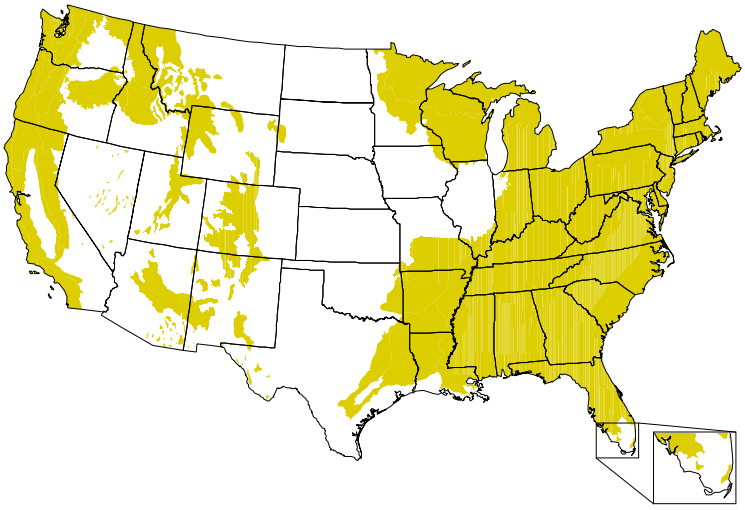

Figure 2. Predicted distribution of *Meloidogyne artiellia* in the continental US.

Figure 2 illustrates where *M. artiellia* is most likely to encounter a suitable climate for establishment within the continental US. This prediction is based only on the known geographic distribution of the species. Because this forecast is based on coarse information, areas that are not highlighted on the map may have some chance of supporting populations of this exotic species. However, establishment in these areas is less likely than in those areas that are highlighted. For initial surveys, survey efforts should be concentrated in the higher risk areas and gradually expanded as needed.

To our knowledge, *M. artiellia* has only been reported once within the United States (Lehman 2002). However according to Regulatory Nematologist Renato Inserra with the Florida Department of Agriculture and Consumer Services, this report was likely based on a misidentification of *M. mayaguensis*.

2. **Host Specificity/Availability. Rating: High/High.** *Meloidogyne artiellia* has more than 30 host plants. Table 1 summarizes literature reports of known host species.

Table 1. Host plants of *Meloidogyne artiellia*

Host(s)	**Reference(s)**
alfalfa (*Medicago sativa*)	(DiVito et al. 1985, DiVito and Zacheo 1987, Greco and DiVito 1987, Greco and Sharma 1990, Greco et al. 1992b)
Artemesia sp.	(Shiabova 1981)
barley (*Hordeum vulgare*)	(Franklin 1961, Goodey et al. 1965, Kyrou 1969, Varo Alcala and Tobar Jimenez 1970, Tobar Jimenez 1973, Franklin 1978, Greco et al. 1984, Sharma 1985, Sikora 1987b, Sikora and Greco 1990, Potter and Olthof 1993, Rivoal and Cook 1993, Talavera and Tobar Jimenez 1997)
bean (*Phaseolus vulgaris*)	(Riggs and Niblack 1993)
Brassica spp.	(Jensen 1972)
broad bean (*Vicia faba*),	(Franklin 1961, Goodey et al. 1965, Jensen 1972, Tobar Jimenez 1973, Franklin 1978, Hooper 1983, Sharma 1985, Sikora and Greco 1990, Potter and Olthof 1993, Rivoal and Cook 1993, DiVito et al. 1994a, Talavera and Tobar Jimenez 1997)
brussels sprouts (*Brassica oleracea* var. *gemmifera*)	(Franklin 1961, Goodey et al. 1965, Franklin 1978, Sikora and Greco 1990, Potter and Olthof 1993)
cabbage/kale (*Brassica oleracea*),	(Franklin 1961, Goodey et al. 1965, Jensen 1972, Ritter 1972, Franklin 1978, Sikora and Greco 1990, Potter and Olthof 1993, Rivoal and Cook 1993, Karssen and van Hoenselaar 1998)
cauliflower (*Brassica oleracea* var. *botrytis*),	(Franklin 1978, Rivoal and Cook 1993)
celery (*Apium graveolens*)	(Zhang and Weng 1991)
cereals (unspecified)	(Ritter 1972, Shiabova 1981)
chickpea (*Cicer arietinum*)	(Varo Alcala and Tobar Jimenez 1970, Tobar Jimenez 1973, Franklin 1978, Mamluk et al. 1983, Greco 1984, Greco et al. 1984, Sharma 1985, Greco and DiVito 1987, Sikora 1987b, Sikora and Greco 1990, Greco et al. 1992a, Rivoal and Cook 1993, DiVito et al. 1994a, DiVito et al. 1994b, Talavera and Tobar Jimenez 1997, Karssen and van Hoenselaar 1998, Akem et al. 2000)
clover (*Trifolium pratense*)	(Franklin 1961, Goodey et al. 1965, Franklin 1978, Sikora and Greco 1990, Potter and Olthof 1993)
clover, crimson (*Trifolium incarnum*)	(Franklin 1978, Rivoal and Cook 1993)

Host(s)	Reference(s)
clover, white (*T. repens*)	(Franklin 1978, Rivoal and Cook 1993)
grasspea/lesser pea (*Lathyrus cicera*)	(Varo Alcala and Tobar Jimenez 1970, Tobar Jimenez 1973, Sharma 1985, Talavera and Tobar Jimenez 1997)
hard wheat (*Triticum durum*)	(Greco et al. 1984, Sikora 1987b, Sikora and Greco 1990)
legumes (unspecified),	(Ritter 1972)
lentil (*Lens* sp.)	(Greco et al. 1992a)
medic, annual (*Medicago rigidula*)	(Franklin 1978, Rivoal and Cook 1993)
medic, black (*Medicago lupulina*)	(Franklin 1961, Goodey et al. 1965, Franklin 1978, Sikora 1987a, Sikora and Greco 1990, Potter and Olthof 1993, Rivoal and Cook 1993)
medic/lucerne (*Medicago sativa*)	(Franklin 1961, Goodey et al. 1965, Franklin 1978, Sikora and Greco 1990, Potter and Olthof 1993)
medics, annual (*Medicago* spp.)	(Mamluk et al. 1983)
oats (*Avena sativa*)	(Franklin 1961, Goodey et al. 1965, Varo Alcala and Tobar Jimenez 1970, Franklin 1978, Sikora and Greco 1990, Potter and Olthof 1993, Karssen and van Hoenselaar 1998)
pea (*Pisum sativum*)	(Franklin 1961, Goodey et al. 1965, Varo Alcala and Tobar Jimenez 1970, Jensen 1972, Franklin 1978, Mamluk et al. 1983, Sikora 1987a, Sikora and Greco 1990, Abd El Moneim and Bellar 1993, Potter and Olthof 1993, Rivoal and Cook 1993)
radish (*Raphanus sativus*)	(Franklin 1978, Rivoal and Cook 1993)
rape/rutabaga (*Brassica napus*[=*B. napobrassica*])	(Franklin 1961, Goodey et al. 1965, Franklin 1978, Sikora and Greco 1990, Potter and Olthof 1993, Karssen and van Hoenselaar 1998)
rashad (*Nasturtium fontanum*)	(Franklin 1978, Rivoal and Cook 1993)
rough pea (*Lathyrus* sp.)	(Mamluk et al. 1983)
sorghum (*Sorghum vulgare*)	(Franklin 1978, Rivoal and Cook 1993)
sugarbeet (*Beta vulgaris* subsp. *vulgaris*)	(Ritter 1972)
sulla/French honeysuckle (*Hedysarum coronarium*)	(Franklin 1978, Rivoal and Cook 1993)
turnip (*Brassica rapa*)	(Franklin 1978, Rivoal and Cook 1993)

Host(s)	Reference(s)
vetch (*Vicia monanthos, V. narbonensis, V. sativa, V. villosa, Vicia* spp.)	(Varo Alcala and Tobar Jimenez 1970, Tobar Jimenez 1973, Franklin 1978, Mamluk et al. 1983, Sharma 1985, Sikora 1987a, Greco et al. 1992a, Abd El Moneim and Bellar 1993, Rivoal and Cook 1993, Talavera and Tobar Jimenez 1997, Karssen and van Hoenselaar 1998)
wheat (*Triticum aestivum* and/or *Triticum* spp.)	(Franklin 1961, Goodey et al. 1965, Kyrou 1969, Ritter 1972, Tobar Jimenez 1973, Franklin 1978, Sharma 1985, Oteifa 1987, Sikora 1987b, Sikora and Greco 1990, Potter and Olthof 1993, Talavera and Tobar Jimenez 1997)
wheat (*Triticum durum* and/or *T. vulgare*)	(Varo Alcala and Tobar Jimenez 1970, Franklin 1978, DiVito and Greco 1988b, Mor and Cohn 1989, Rivoal and Cook 1993, Karssen and van Hoenselaar 1998)

In host susceptibility studies, corn, cowpea, lupine and sainfoin (*Onobrychis viciifolia*) have been reported as "non-hosts"; oats have been reported as a "poor" host, while rye, turnip, vetch, wheat and other unspecified grasses have been reported as hosts with varying levels of resistance (DiVito et al. 1985, DiVito and Zacheo 1987, Greco and DiVito 1987, Greco and Sharma 1990, Greco et al. 1992b, Rivoal and Cook 1993). *Meloidogyne artiellia* has been observed on roots of chickpea and broad bean; however, symptoms were visible only on chickpea (DiVito et al. 1994a).

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.

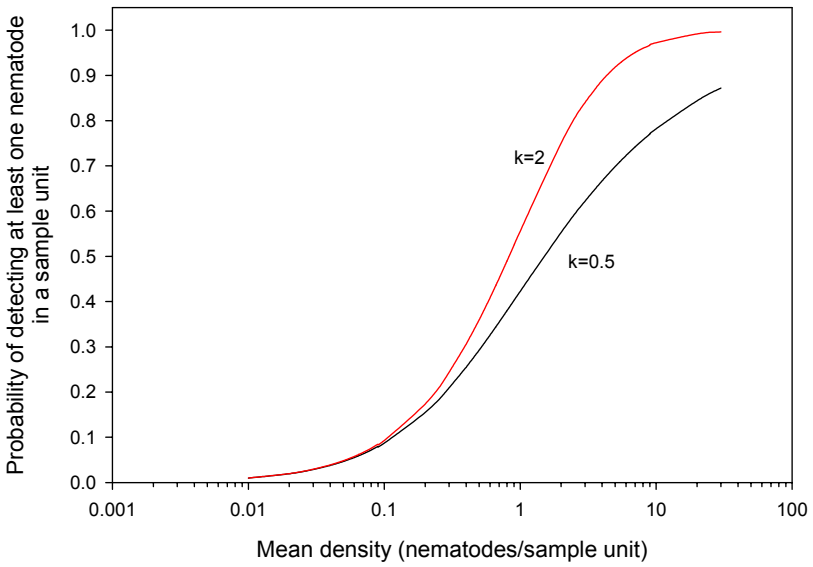

Figure 3. Influence of nematode density and spatial distribution on the likelihood of observing at least one nematode from a soil sample. Lines are based on the negative binomial distribution.

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.

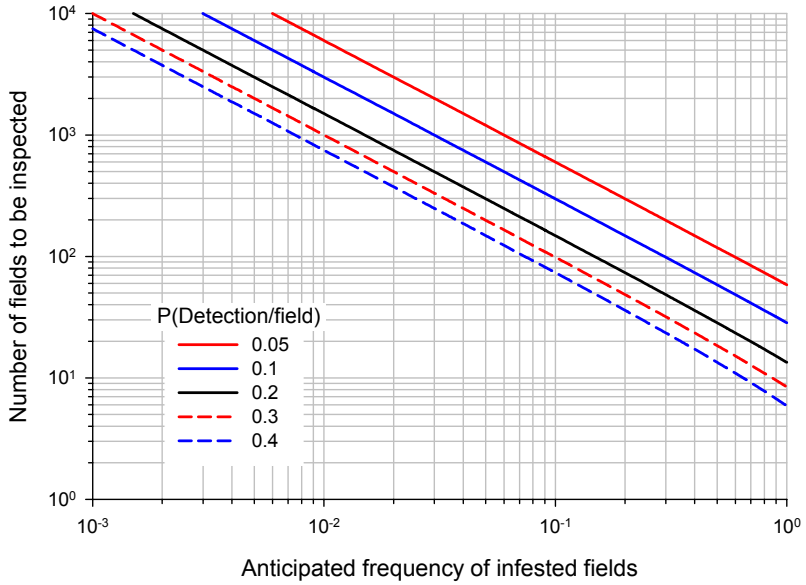

Figure 4. Influence of the frequency of infested fields and the likelihood of detecting an infestation in an individual field on the number of fields that should be inspected to have 95% confidence of detecting at least one exotic nematode within a region.

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.

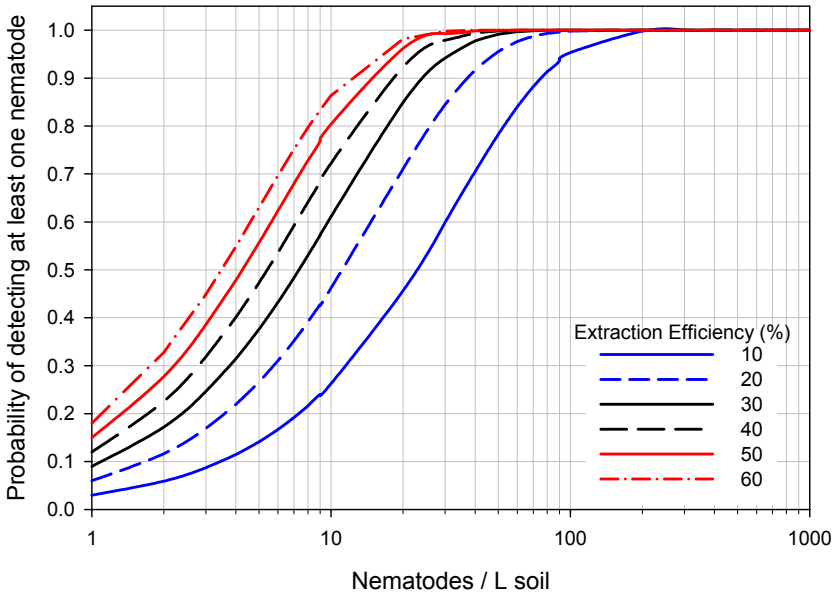

Figure 5. Influence of extraction efficiency and nematode density on the probability of detecting at least one nematode in 300 cc of a well-mixed, 1-liter soil sample.

4. **Taxonomic Recognition**. **Rating: Medium.** *Meloidogyne artiellia* may occur in mixed populations with closely related or other easily confused species. Swollen females and egg masses of *M. artiellia* are distinctly large but can easily be confused with other root-knot nematodes without close examination using magnification (Jensen 1972). Due to technological advances in identification techniques, differentiating among morphologically similar cyst nematodes can be accomplished most reliably by restriction fragment length polymorphism (PCR-RFLP) of ribosomal DNA (DeGiorgi et al. 1991, DeGiorgi et al. 1994, DeGiorgi et al. 2002).

   For a detailed description of the morphology and taxonomy of *M. artiellia*, 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 artiellia 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, radish, rutabaga, sugarbeet, and turnip [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. Thus, 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, radish (*Raphanus* spp.) roots were intercepted 9 times; sugarbeet/beet (*Beta* spp.) roots have been intercepted 6 times; and roots of rutabaga, turnip, or other *Brassica* spp. have been intercepted only once.

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 commodities) 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 some portion of each of these states has a climate and hosts that would be suitable for establishment by *Meloidogyne artiellia*.

7. **Potential Economic Impact. Rating: High.** The economic impact of *M. artiellia* is difficult to measure because this species occasionally occurs in mixed populations with other *Meloidogyne* spp. (Jensen 1972). *Meloidogyne* species are among some of the most economically important plant parasitic nematodes found worldwide (DeGiorgi et al. 2002). Crop loss resulting from nematode damage to vegetables and grains has been estimated at an average of 10-11% worldwide (Jensen 1972, Potter and Olthof 1993, Whitehead 1998, Nicol 2002), but the economic impact from nematodes is thought to be grossly underestimated.

   Worldwide, more than 13% yield loss in chickpeas may be attributed to damage from plant parasitic nematodes (Sasser 1987, Riggs and Niblack 1993). *Meloidogyne artiellia* damages chickpea crops in Italy, Spain and Syria,

especially when crop rotations involve more than one suitable host. Damage thresholds for *Meloidogyne* spp. occurring on chickpea range from 0.01-1.0 juveniles/ g soil (Riggs and Niblack 1993). *Meloidogyne artiellia* also reportedly lowers the yield potential of wheat in Greece and Israel (Kyrou 1969, Rivoal and Cook 1993), and barley in Greece (Sikora 1987b, Nicol 2002). Studies on durum wheat in Italy show 90% yield loss when population densities reach 32 eggs and juveniles/ml soil (DiVito and Greco 1988b, Rivoal and Cook 1993).

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).

Severity of damage caused by *Meloidogyne* can be species specific and also may vary by host, crop rotation, season and soil type (Greco et al. 1992b, Potter and Olthof 1993). Similarly, economic thresholds may vary primarily depending on these same factors. Some thresholds have been developed for vegetable crops where the average is approximately 0.5-2 juveniles/g of soil. Thresholds have been established for several *Meloidogyne* species on various hosts and are summarized by Potter and Olthof (1993) Yield loss with reference to a threshold or nematode population density has been reported for only a few crops (Potter and Olthof 1993). Because root crops can be severely misshapen by nematodes, damage has a direct impact on marketability, and therefore acceptable thresholds are very low (low to zero).

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 artiellia* 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 artiellia has a moderate host range, feeding primarily on cereals hosts, legumes, root and cruciferous crops [see #2-Host Specificity]. Appendix D summarizes federally listed threatened or endangered plant species (USDA NRCS 2004) found within plant genera known to be hosts (or potential hosts) for *M. artiellia*. Plants listed in Appendix D might be suitable hosts for *M. artiellia*, and thus, could be adversely affected by this nematode.

9. **Establishment Potential. Rating: High.** Our initial predictions suggest that approximately one half of the US has a climate that could support populations of *M. artiellia* (Fig. 2). Known host plants (esp. wheat, oats, and green-/dry-edible beans) are common in these climatically suitable areas. Thus, upon arrival into the United States, the chances for establishment are relatively high. However, we note that the likelihood for introduction seems low based on current interception records.

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

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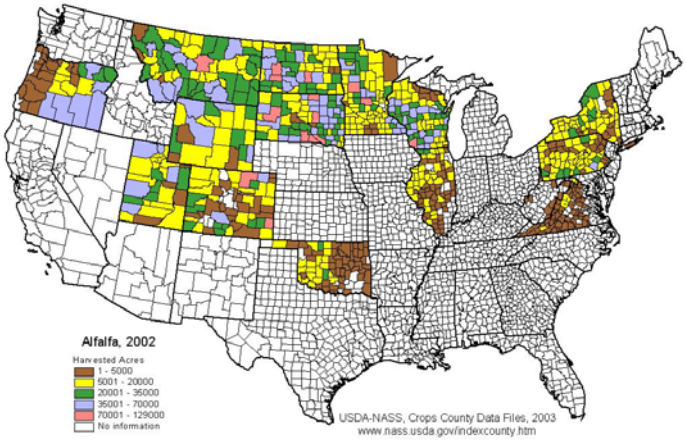
Appendix A. Comparison of climate zones. To determine the potential distribution of a quarantine pest in the US, we first collected information about the worldwide geographic distribution of the species (Table A1). Using a geographic information system (e.g., ArcView 3.2), we then identified which biomes (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. artiellia*:

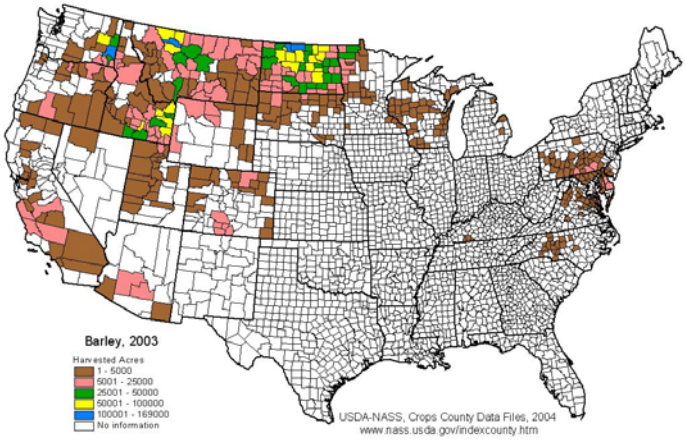
Locations	**Reference(s)**
Algeria (Northwest: Hattab and Tiared)	(DiVito et al. 1994a)
China (Fujian Province: Sanming)	(Zhang and Weng 1991)
England (Eastern)	(Franklin 1961, Jensen 1972, Sikora and Greco 1990, Karssen and van Hoenselaar 1998)
France (Laons, Champagne)	(Ritter 1972, Franklin 1978, Karssen and van Hoenselaar 1998)
Greece	(Kyrou 1969, Franklin 1978, Sikora 1987b, Karssen and van Hoenselaar 1998)
Israel (Southern)	(Mor and Cohn 1989)
Italy	(Greco 1984, Greco and DiVito 1987, DiVito and Greco 1988b, DiVito et al. 1994a, Karssen and van Hoenselaar 1998)
Morocco (Fes, Settat, Sept Maarif, Safi)	(DiVito et al. 1994a)
Russia (Siberia: Altai Territory)	(Shiabova 1981)
Spain	(Varo Alcala and Tobar Jimenez 1970, Tobar Jimenez 1973, Franklin 1978, Sharma 1985, Greco and DiVito 1987, Sikora and Greco 1990, DiVito et al. 1994a, Talavera and Tobar Jimenez 1997, Karssen and van Hoenselaar 1998)
Syria	(Mamluk et al. 1983, Greco et al. 1984, Sharma 1985, Al-Ahmad 1987, Greco and DiVito 1987, Sikora 1987b, Sikora and Greco 1990, Greco et al. 1992a, Abd El Moneim and Bellar 1993, DiVito et al. 1994a)
Tunisia (Oued Meliz)	(DiVito et al. 1994a)
Turkey (Usak)	(DiVito et al. 1994b)

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

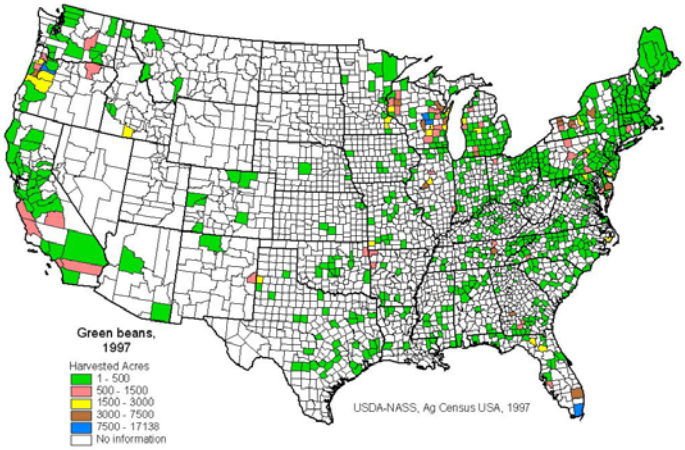
Map 1. Alfalfa (*Medicago sativa*)



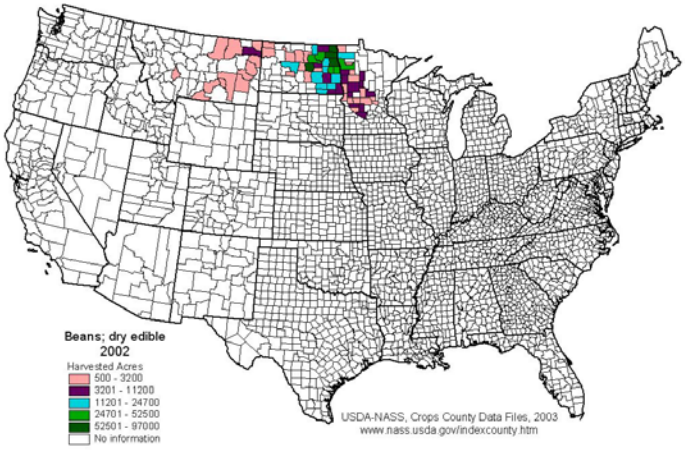
Map 2. Barley (*Hordeum vulgare*)



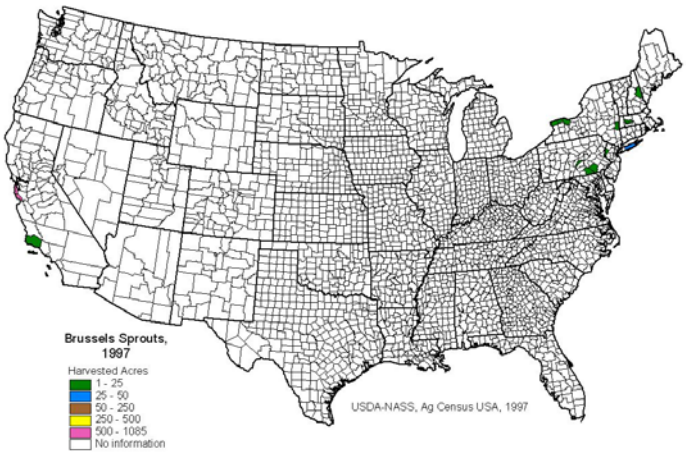
Map 3. Beans, green (*Phaseolus vulgaris*)



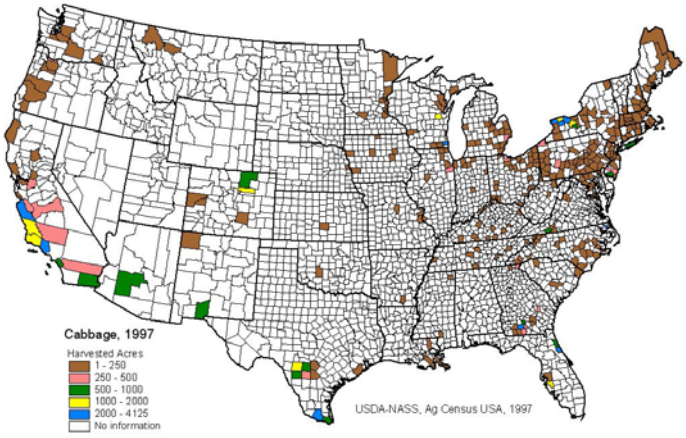
Map 4. Beans, dry/edible (*Phaseolus vulgaris*)



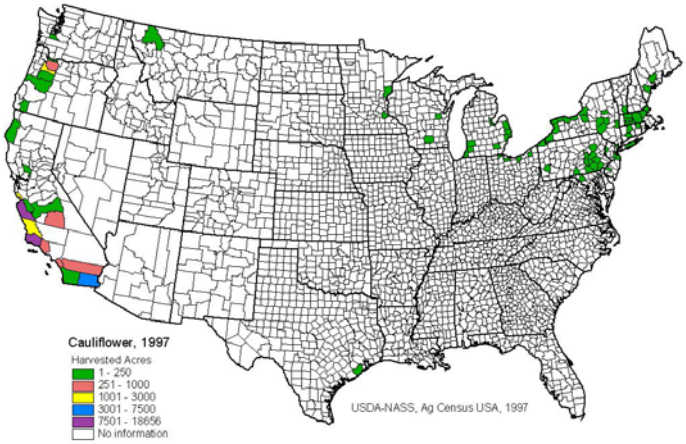
Map 5. Brussels sprouts (*Brassica oleracea* var. *gemmifera*)



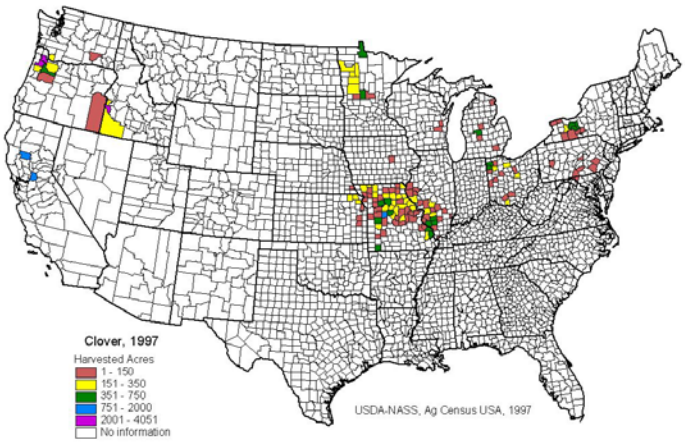
Map 6. Cabbage (*Brassica oleracea*)



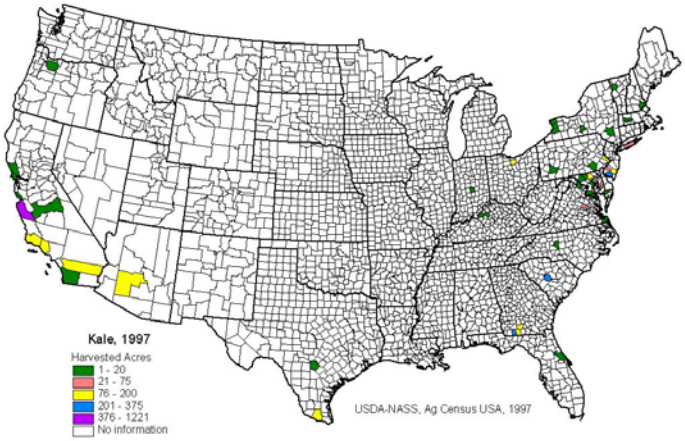
Map 7. Cauliflower (*Brassica oleracea* var. *botrytis*)



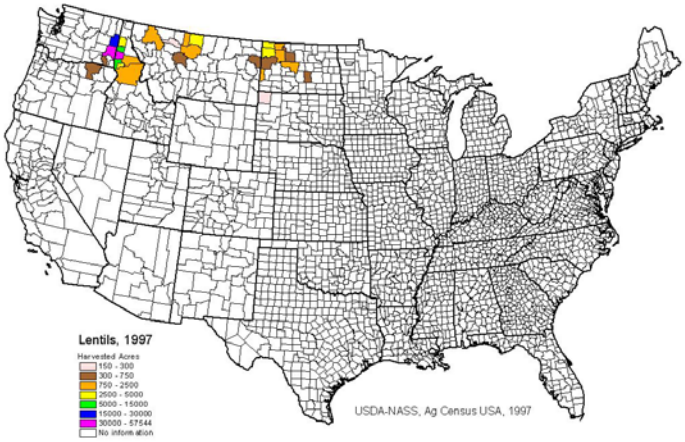
Map 8. Clover (*Trifolium* spp.)



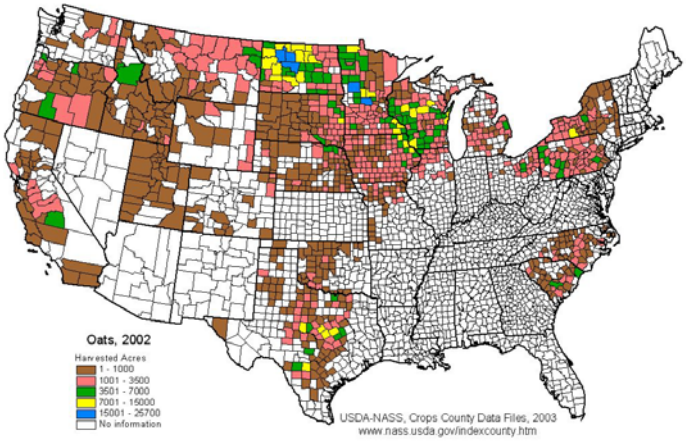
Map 9. Kale (*Brassica oleracea*)



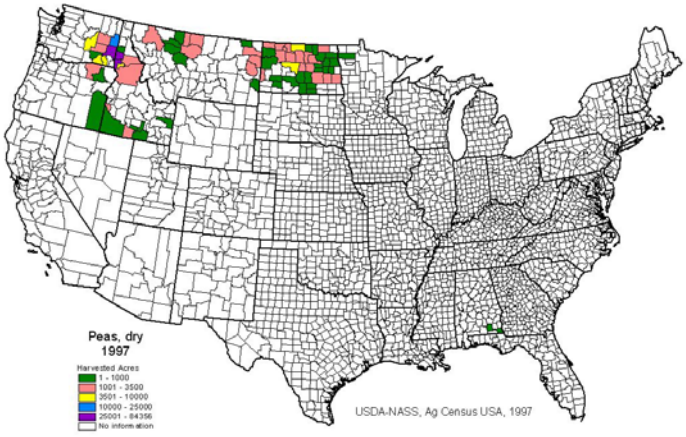
Map 10. Lentils (*Lens* sp.)



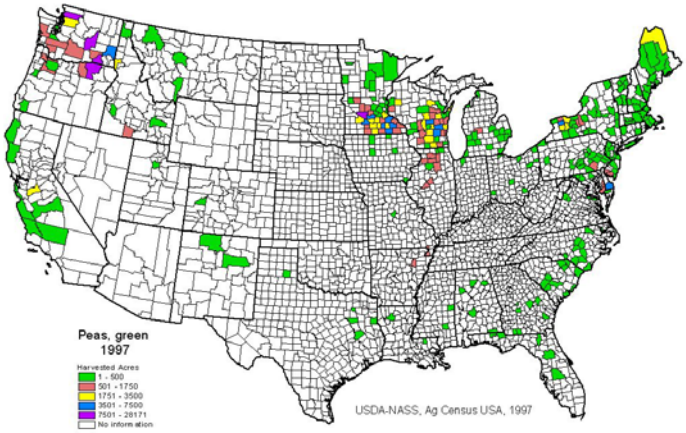
Map 11. Oats (*Avena sativa*)



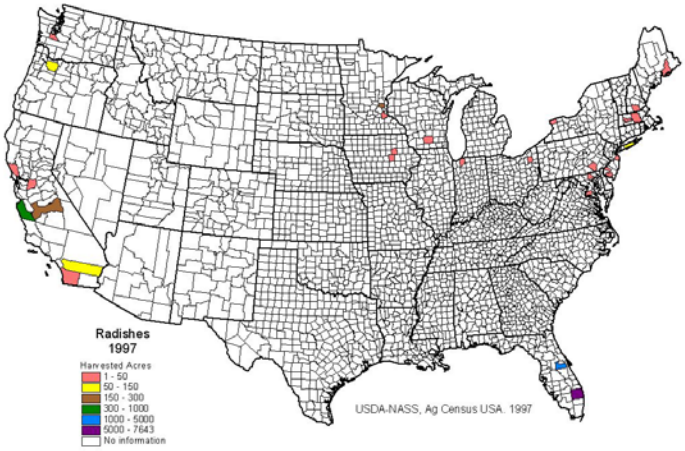
Map 12. Pea, dry (*Pisum* sp.)



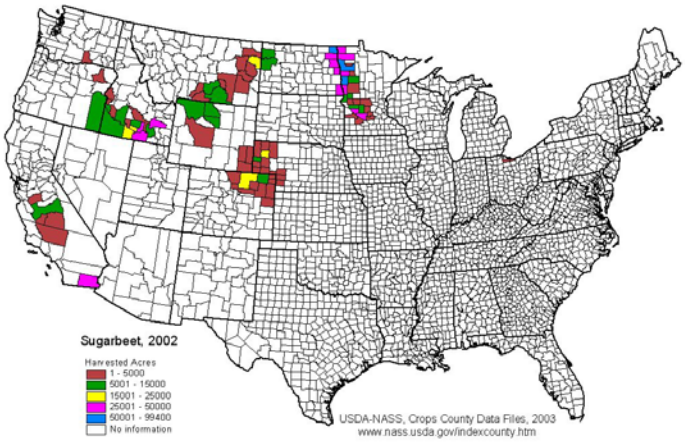
Map 13. Pea, green (*Pisum* sp.)



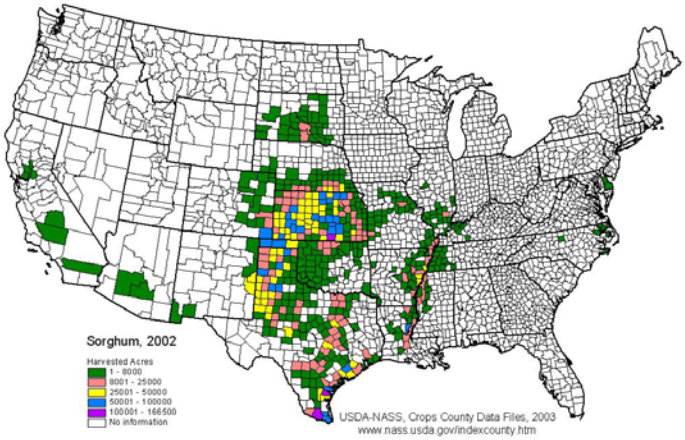
Map 14. Radish (*Raphanus sativus*)



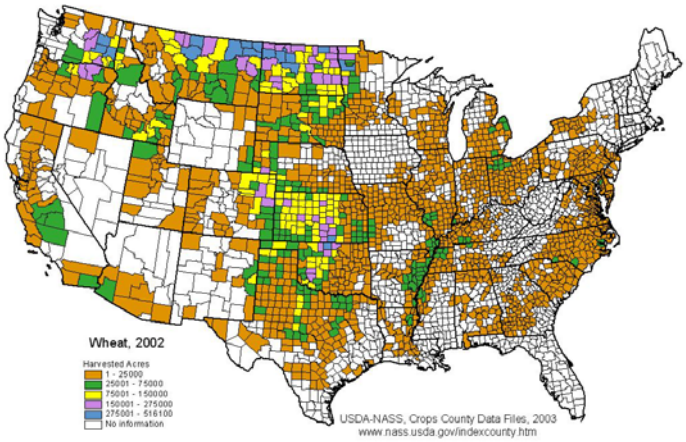
Map 15. Sugarbeet (*Beta vulgaris* subsp. *vulgaris*)



Map 16. Sorghum (*Sorghum vulgare*)



Map 17. Wheat (*Triticum* spp.)



Appendix C. Morphology of *Meloidogyne artiellia.*

Greco et al., (1992a), Eisenback and Hirschmann Triantaphyllou (1991), and Karssen and Van Hoenselaar (1998) provide detailed morphological comparisons between *M. artiellia* and several closely related species, including (but not limited to) *M. acrita*, *M. arenaria*, *M. incognita*, and *M. javanica*. Franklin (1961) provides a detailed key and description of *M. artiellia* for identification purposes. The following description of *M. artiellia* is quoted from excerpts of Franklin (1961, 1978).

Female (Figs. C1 (A, D-F))
(n= 8-10 specimens)
length 650-760 µm;
width 340-460 µm;
stylet 12-16 µm;
vulva 15-22 µm.

Body swollen, pear- or flask-shaped, tapering gradually anteriorly to a small head; smooth, rounded posteriorly, with terminal vulva. Annules visible in neck region and around tail....

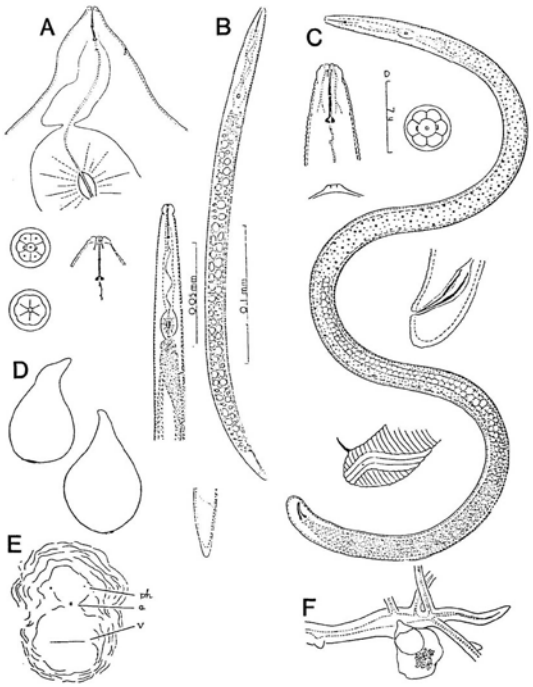


Figure C1. *Meloidogyne artiellia*. **A.** Female: anterior, face and skeleton, lip region and stylet. **B.** Juvenile: full body, anterior and tail. **C.** Male: full body, anterior, transverse section of lateral field, face and posteriors (spicules and lateral field). **D.** Female body shapes. **E.** Perineal pattern. **F.** Root with female [Quoted from (Franklin 1961) and reproduced from (Taylor 1987)].

The broad "neck" narrows abruptly at the head which is 4-5µ across. In face view there appear to be six almost equal lips, and a small labial cap around the mouth aperture. The amphids open as short slits on the inner edge of the lateral lips. Each of the four sub-lateral lips has a small papilla, but nonewas visible on the lateral lips. Optical sections show a delicate, six-radiate skeletal structure around the anterior end of the stylet, but it disappears below the level of the lips. Dorsal views of the head show a constriction on the lateral lips about one-third behind the anterior edge. These lips could therefore be described as consisting of two unequal annules. The excretory pore lies ventrally one or two stylet lengths behind the head....

The cuticular pattern round the vulva and anus is characteristic. It is formed of striae and ridges of the cuticle, the latter being more pronounced nearer the vulva an anus. In general outline the pattern is roughly that of a figure eight, the upper, smaller area enclosing the phasmids which are usually quite distinct, the anus situated at the centre and the vulva occupying the diameter of the lower, larger part of the pattern. At the top of the arch, which is morphologically the dorsal part of the tail, the pattern is usually angular. Cuticular folds curve towards the anus from each side, but leave a smooth unpatterned area around the vulva. The vulva is further from the anus in relation to the

tail length than in most other species of the genus. The distance from the anus to vulva is about three times that from the anus to a line joining the phasmids. The exact position of the tail tip is difficult to determine because the lateral lines are marked only by the position of the phasmids and by slight irregularities in the striae....

Egg (n=20)
length 75-111 µ;
breadth 34-43 µ.

Male (Fig. C1(C))
(n= 7-15)
length 0.82-1.37 µm;
width 23-36 µm;
stylet 17-27 µm;
a=31-40;
*b=10-15;
c=60-100
[DeMan's indices (modified) from Jones (1965):
a=length/greater diameter; b=length/distance from head end to end of oesophagus; c=length/length of tail (anus to tip)]
*Measurements for b were made from the anterior end to the posterior edge of the oesophageal bulb, as the end of the glandular region overlaps the intestine and is difficult to define.

Body annulated, annules about 1.5 µ wide. Lateral fields with four incisures at the tail, but along the greater part of their length a fifth incisure is present in the centre of each field. The lateral fields continue round the tail which is twisted through about 90º. Phasmids small, approximately adanal.

Head with labial cap and six nearly equal lips. Face views shows the slit-like amphid openings on the lateral lips; papillae not seen, nor was the stellate skeletal structure, such as that in the female. In dorso-ventral view a constriction is seen on the lateral lips about one-third from the front. A tubular guide surroundsthe anterior end of the stylet which has well-developed, rounded, basal knobs....

Pro-corpus narrow, two to three body-widths long, followed by a spindle-shaped muscular corpus about twice as long as wide. The oesophageal glands stretch for about three body-widths ventro-laterally along the intestine. Nerve ring one bulb-length behind muscular bulb. Two body-widths behind the oesophageal bulb is a conspicuous hemizonid and immediately behind it is the excretory pore with its duct running back for a short distance.

Spicules typical for the genus, curved with anterior thicker part and tapering posteriorly to a point. A small gubernaculum, about one-third the length of the spicules, lies dorsally in the cloaca wall. Tail very slightly longer than the anal body diameter.

Larva (Fig. C1(B))
(n=10-20)
body length 301-370 µm;
body breadth 10-16 µm;
tail length 18-26 µm;
stylet length 14-16 µm.

...The most striking feature of the larvae is the short tail with rounded tip. It is about 24.5 µ long, and two and one half times as long as the body diameter at the anus.

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

Meloidogyne artiellia has the potential to adversely affect threatened and endangered plant species. However, because *M. artiellia* is not known to be established in the US and threatened and endangered plant species do not occur outside the US, it is not possible to confirm the host status of these rare plants from the scientific literature. From available host records, *M. artiellia* is known to feed primarily on species within the families Brassicaceae, Fabaceae and Poaceae. From these host records, we infer that threatened or endangered plant species which are closely related to known host plants might also be suitable hosts (Table D1). For our purposes closely related plant species belong to the same genus.

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

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
Hedysarum coronarium	*H. alpinum*	alpine sweetvetch		MI (E)
Lathyrus cicera, L. sativus, Lathyrus sp.	*L. holochlorus*	thinleaf pea		WA (T)
	L. japonicus	beach pea		OH (T) PA(T)
	L. japonicus var. *maritimus*	beach pea		IL(E) IN(E) VT(T)
	L. ochroleucus	cream pea		IL(T) IN(E) NJ(E) OH(T) PA(T)
	L. palustris	marsh pea		KY(T) MD(E) PA(E) VT(T)
	L. torreyi	Torrey's pea		WA(T)
	L. venosus	veiny pea		IN(T) NJ(E) NY(E) OH(E)
Trifolium incarnum, T.	*T. amoenum*	showy Indian clover	E	

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

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
pratense, T. repens	*T. calcaricum*	running glade		TN(E)
	T. owyheense	Owyhee clover		OR(E)
	T. reflexum	buffalo clover		IL(E) IN(E) KY(E) MD(E) OH(E) TN(E)
	T. stoloniferum	running buffalo clover	E	IN(E) KY(T) MO(E) OH(E)
	T. thompsonii	Thompson's clover		WA(T)
	T. trichocalyx	Monterey clover	E	CA (E)
	T. virginicum	Kates Mountain clover		MD (T) PA(E)
Vicia monanthos, V. narbonensis, *V. sativa V. villosa, Vicia* spp.	*V. americana*	American vetch		MD(E)
	V. caroliniana	Carolina vetch		NJ(E)
	V. ocalensis	Ocala vetch		FL(E)

1. E= Endangered; T=Threatened

Appendix E. Biology of *Meloidogyne artiellia*.

Population phenology

DiVito and Greco (1988a) have investigated the biology of *M. artiellia* on chickpea under Mediterranean climate conditions using growth chamber and microplot studies. Unless noted otherwise, much of the following information about development stages is summarized from their work (DiVito and Greco 1988a, Greco et al. 1992b). Like several nematodes, *M. artiellia* is adapted to cool and dry conditions and has the ability to enter into an inactive, quiescent state to survive environmental stresses (Jensen 1972). In climates with cool, wet winters and warm, dry summers, *M. artiellia* is active during spring and winter months and inactive from late spring through summer. Typically one generation is completed in the Mediterranean under non-irrigated conditions.

Stage specific biology

Development time depends on temperature. Temperatures of 10ºC and 30ºC have been reported as unfavorable for root penetration, development and egg production, while temperatures in the 15-25ºC range are considered optimal. *M. artiellia* has a reported threshold temperature of 10ºC.

Adult

This species reproduces asexually, but has the ability to produce sexually when conditions are appropriate (DeGiorgi et al. 2002). Adults have reportedly occurred 14-18 days following root penetration at optimal temperatures between 15-25ºC. Females develop after 230-240 degree days over 10ºC. Females swell, producing large gelatinous egg masses or sacs, containing between 500-1000 eggs. The egg sac is deposited on either galled root surfaces or inside root galls (Hussey 1985).

Egg

Egg hatch may or may not involve stimulation from the host root (Hussey 1985). Hatching can occur for an extended period at temperatures between 5-10ºC. Hatch is most rapid at temperatures between 15-25ºC. Eggs will not hatch under extended dry periods but may persist in soil or dry roots awaiting more favorable moist soil conditions.

Larva

Emergence occurs under moist soil conditions; juveniles may become inactive under dry conditions. *Meloidogyne* larvae and eggs can be easily distributed by irrigation ditches, and in areas of saturated soil, larvae may survive under water for up to three weeks (Milne 1972). *M. artiellia* can also reportedly survive in "fallow" fields for 1-2 years (Jensen 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). The second is the only stage when juveniles are mobile and are thought to be attracted to host plant roots (Hussey 1985). They may feed singly or in a group. If a larva cannot find a suitable feeding site on a host, it will continue searching until its energy is depleted. When a suitable site is selected the 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, 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 complete (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 within 1-2 days following root penetration (Hussey 1985). A female larva will swell as it feeds until development is completed. Total development time varies from approximately 20 days at 25ºC to 55 days at 10ºC. Following chickpea root penetration, third and fourth stage juveniles have been observed 3-5 and 10-12 days, respectively, at 15-25ºC (DiVito and Greco 1988a).