Mini Risk Assessment
Mediterranean cereal cyst nematode, *Heterodera latipons* Franklin
[Nematoda: Heteroderidae]

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Introduction

Heterodera latipons is a significant plant parasitic nematode pest of cereal crops, such as barley wheat, and to a lesser extent, oats and rye (Franklin 1969, Kort 1972, Sikora 1987b, Inserra et al. 2003). This nematode occurs primarily in the Mediterranean but has also been recorded in eastern and northern Europe, the Middle and Near East, North and South Africa, and Japan. It is also known as the cereal cyst nematode and the wheat cyst nematode (Handoo and Ellington 1998, Greco et al. 2002). The common name, 'cereal cyst nematode,' is somewhat unfortunate as it also refers to *Heterodera avenae*, a plant parasitic nematode occurs in Ontario [Canada] and Oregon (Norton et al. 1984).

Heterodera latipons is not known to occur in the US (USDA 1985). Greco et al. (2002) suggested that this nematode is likely to become established on temperate cereals in the US; damage may be severe but is difficult to predict based on historical records. A subsequent assessment concluded that *H. latipons* posed moderate risk relative to other exotic nematodes that might be introduced into the US, but the assessment encouraged further investigation (Inserra et al. 2003). The purpose of the current document is to further evaluate several factors that contribute to risks posed by *H. latipons* and apply this information for the refinement of sampling and detection programs.

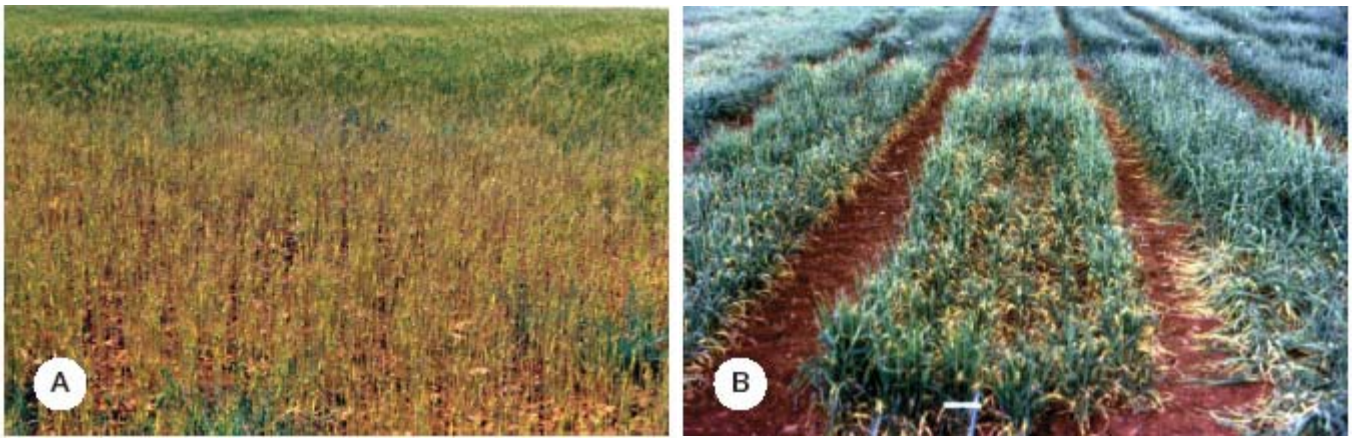

Figure 1. Symptoms of infection by *Heterodera latipons* in: A) Syrian durum and B) Cyprus barley. [Images from Greco et al. (2002).]

1. **Ecological Suitability. Rating: High.** *Heterodera latipons* is common in many cereal growing regions of Africa and Asia. Appendix A provides detailed records on the reported worldwide distribution of this nematode. In general, *H. latipons*

occurs in areas with a temperate to xeric (steppe or desert) climate. The currently reported distribution of *H. latipons* suggests that the pest may be most closely associated with biomes characterized as: temperate broadleaf and mixed forests; temperate coniferous forests; temperate grasslands, savannas and shrublands; Mediterranean scrub; montane grasslands; and desert and xeric shrublands. Montane grasslands do not occur in the US. Nevertheless, we estimate that >99% of the continental US could provide a suitable climate for *H. latipons* (Fig. 2). Only southern Florida, the very tip of southern Texas, and far south central Arizona are not likely to be suitable. See Appendix A for a more complete description of this analysis.

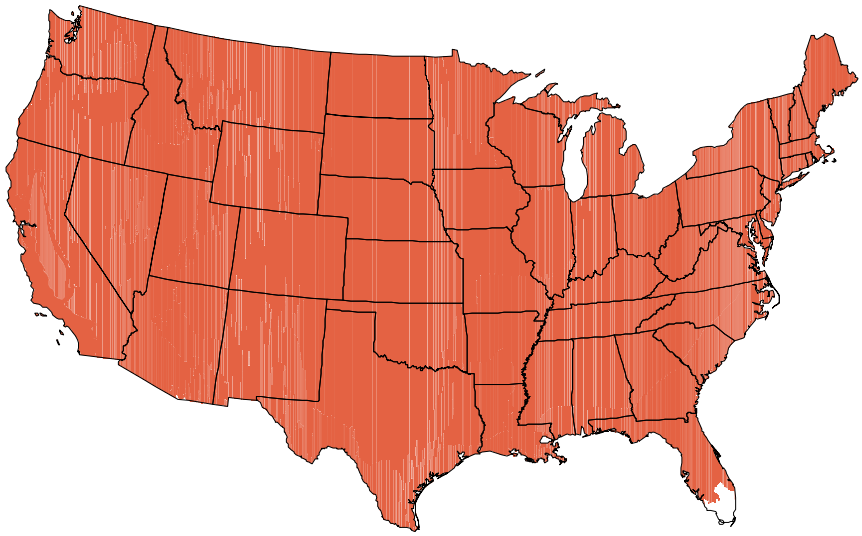

Figure 2. Predicted distribution (shaded red) of *Heterodera latipons* in the continental US.

Figure 2 illustrates where *H. latipons* is most likely to occur within the US, 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 *H. latipons* absolutely could not establish in areas that are not highlighted on either map (e.g., southern Florida). Rather, establishment in these areas is less likely. For initial surveys, efforts should be concentrated in the higher risk areas and gradually expand as needed. Of course, geographic areas that are not highlighted are not risk free.

Our analysis also predicts that *H. latipons* could find a suitable climate in much of Canada (map not shown). Interestingly, *H. latipons* has been reported from an unknown host on Prince Edward Island, Canada (Mulvey and Golden 1983, Norton et al. 1984, Ebsary 1986). Although cysts of *H. trifolii* are more commonly recovered from samples collected in PEI (Kimpinski 2004), Dr. Bruce Hopper (2004) confirms the presence of *H. latipons* on the island.

2. **Host Specificity/Availability. Rating: Low/High.** Table 1 lists host plants reported for *Heterodera latipons.* The quality of these hosts may vary. For example, bread wheat (*Triticum durum*) has been noted as a poor host (Greco et al. 2002). Carrot may also be a poor host as *H. latipons* was reported at low population densities and without causing notable damage (Tacconi 1976). No other authors have reported carrot as a host.

   Some host associations may be difficult to establish reliably. For example, Mackintosh (1970) reported marram grass as a host of *H. latipons*, however this was probably a misidentification of *H. hordecalis* (Cook 2004, Hockland 2004). Although *H. latipons* may be found on grasses that occur in association with marram grass, the nematode is not known to reproduce on marram itself (Cook 2004). Furthermore, depending on soil type and crop rotation, *H. latipons* can occur in mixed or adjacent populations with *Heterodera avenae* (Franklin 1969, Kort 1972, Stoyanov 1982, Oteifa 1987, Rivoal and Cook 1993, Fourie et al. 2001). *Heterodera latipons* and *H. avenae* can be difficult to distinguish (see 'Taxonomic Recognition' below).

Table 1. Host plants of *Heterodera latipons.*

Host(s)	**Reference(s)**
barley (*Hordeum vulgare*)	(Franklin 1969, Kort 1972, USDA 1985, Oteifa 1987, Sikora 1987b, Philis 1988a, b, Sabova et al. 1988, Swarup and Sosa-Moss 1990, Mor et al. 1992, Rivoal and Cook 1993, Philis 1995, 1997, 1999, Ismail et al. 2000, Greco et al. 2002, Mokabli et al. 2002, Nicol 2002, Inserra et al. 2003)
canary grass (*Phalaris* sp., *P. minor, P. paradoxa*)	(Mor et al. 1992, Mor and Sturhan 2000, Greco et al. 2002)
carrot (*Daucus carota*)	(Tacconi 1976)
cereals (unspecified)	(Rivoal and Cook 1993, Rumpenhorst et al. 1996, Nicol 2002, Maafi et al. 2003)
marram grass (*Ammophila arenaria*)	(Mackintosh 1970, USDA 1985, Cook 2004, Hockland 2004)
oats (*Avena sativa*)	(Franklin 1969, Kort 1972, Cohn and Ausher 1973, Romero 1980, USDA 1985, Mor et al. 1992, Greco et al. 2002, Mokabli et al. 2002, Inserra et al. 2003)
peanut (*Arachis hypogaea*)	(Fourie et al. 2001)
rye (*Secale cereale*)	(Franklin 1969, Kort 1972, USDA 1985, Greco et al. 2002, Inserra et al. 2003)
sugarbeet (*Beta vulgaris* subsp. *vulgaris*)	(Talatschian and Achyani 1976)
wheat (*Triticum* spp., *T. aestivum* and/or *T. durum*)	(Franklin 1969, Kort 1972, Mulvey 1972, Stoyanov 1982, Mulvey and Golden 1983, USDA 1985, Oteifa 1987, Sikora 1987b, Sabova et al. 1988, Swarup and Sosa-Moss 1990, Mor et al. 1992, Rumpenhorst et al. 1996, Rivoal et al. 2000, Greco et al. 2002, Nicol 2002, Inserra et al. 2003)

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 infestations of *H. latipons*. 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 *H. latipons* 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. Greco et al. (2002) outline general considerations for conducting a survey for *H. latipons*. Samples of soil or host roots must be collected with the purpose of obtaining cysts. Samples must then be processed to separate cysts from soil and debris. Finally, cysts 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 *H. latipons* 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 cm 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.
   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 (Mor et al. 1992). 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.

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.

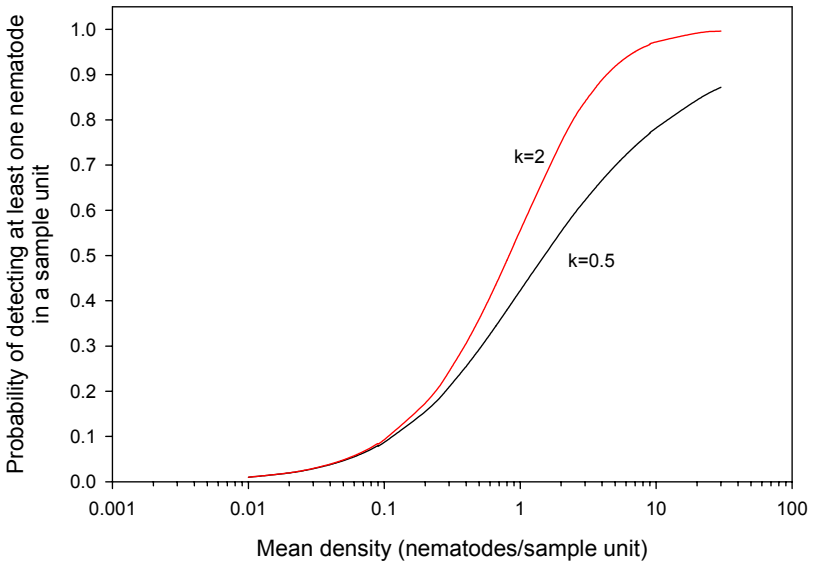

Figure 3. Influence of nematode density and spatial distribution on the likelihood of observing at least one nematode from a soil sample. Lines are based on the negative binomial distribution.

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 *H. latipons* 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. Generally, if 1 in 100 fields is infested (frequency = 10^{-2}), 300 to 1,500 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.

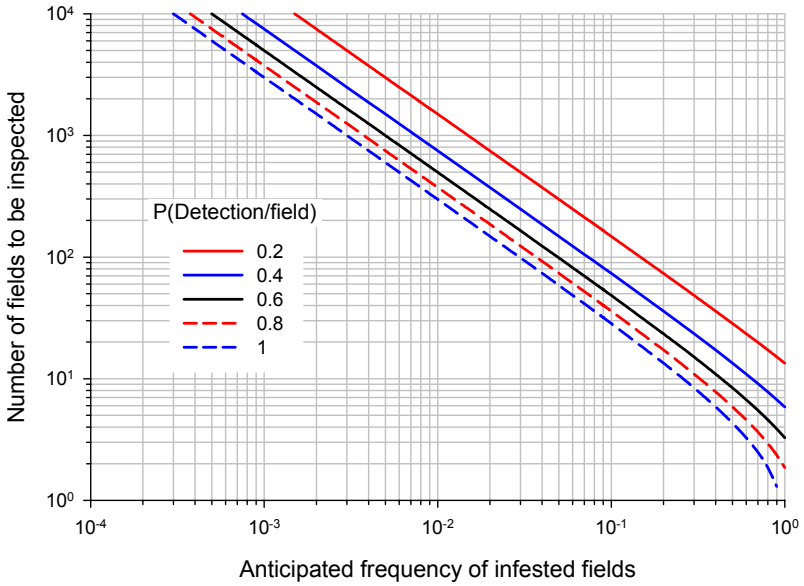

Figure 4. Influence of the frequency of infested fields and the likelihood of detecting an infestation in an individual field on the number of fields that should be inspected to have 95% confidence of detecting at least one exotic nematode within a region.

Cyst nematodes are often extracted from soil using some form of elutriation or flotation. The Fenwick flotation can (Fig. 5), or a modification thereof, is frequently used for this purpose. Cysts are collected on 60 or 80 mesh sieves (reviewed in Eisenback and Zunke 1998). Vermiform nematodes (particularly second stage juveniles) will be caught more effectively on 400 mesh sieves. 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 1994, Turner 1998). Extraction efficiencies for cyst nematodes using these amounts of soil with flotation can vary between 50% (Eisenback and Zunke 1998) and 80% (Ingham and Santo 1994).

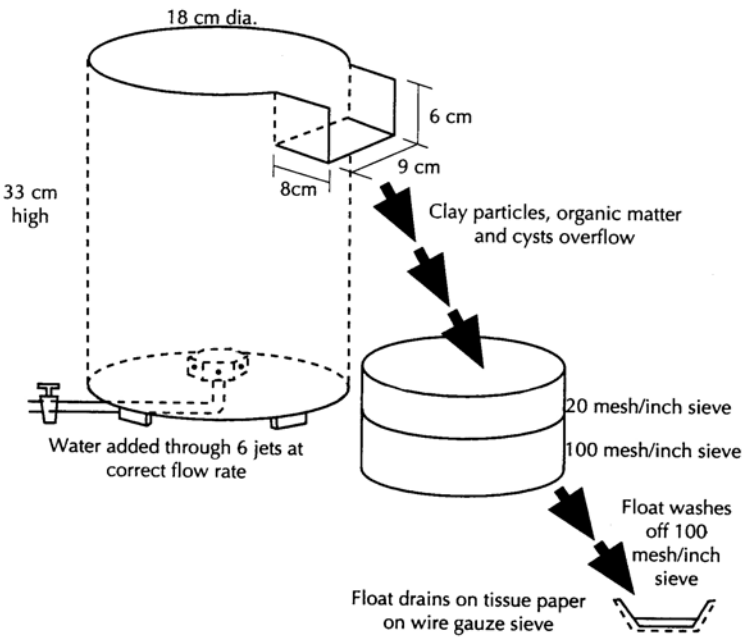

Figure 5. Schematic of a Fenwick flotation can used to extract nematode cysts from soil samples. [Reproduced from Ingham and Santo (1994).]

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 6 illustrates the consequence of processing 300 cc of soil from every liter of soil that is collected from the field. The analysis behind Figure 6 assumes that at least one nematode is present in the sample. The likelihood of detection remains <90% until densities reach ~9-11 nematodes per liter of soil.

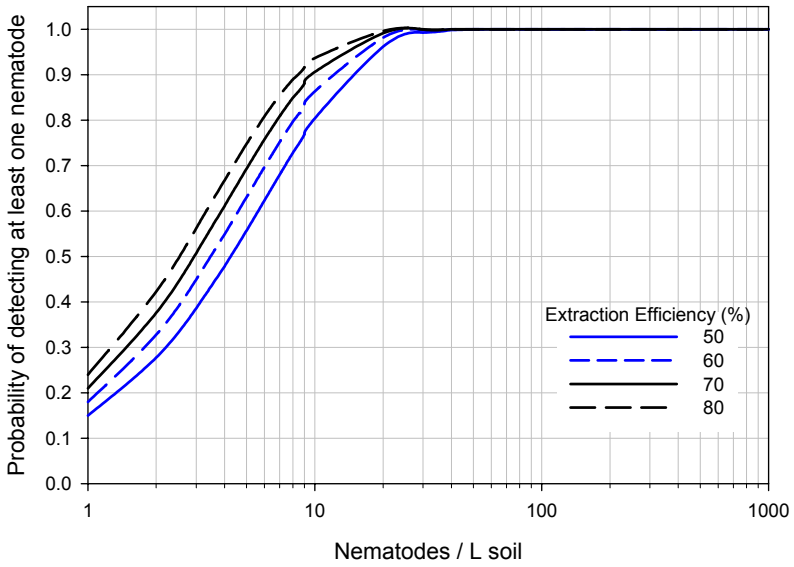

Figure 6. Influence of extraction efficiency and nematode density on the probability of detecting at least one nematode in 300 cc of a well-mixed, 1-liter soil sample.

4. **Taxonomic Recognition**. **Rating: Medium.** *Heterodera latipons* may occur by itself or in mixed populations that include closely related *H. avenae* or *H. trifolii* (Stoyanov 1982, Mor et al. 1992, Kimpinski 2004). *H. latipons* has been confused with several other cyst nematode species that parasitize cereals, including (but not limited to) *H. avenae, H. bifenestra, H. filipjevi, H. hordecalis, H. mani, H. pakistanensis, H. zeae*, and a more taxonomically distant species, *Punctodera punctata* (Kort 1972, Nicol 2002, Cook 2004). Franklin (1969), who first described *H. latipons*, compared morphological characters of *H. avenae, H. tucomanica* and *H. latipons* (see Appendix C). Due to technological advances in molecular diagnostics, differentiating among morphologically similar cyst nematodes can be done most reliably by restriction fragment length polymorphism (PCR-RFLP) of ribosomal DNA (Subbotin et al. 1999, Subbotin et al. 2001, Handoo 2002, Nicol 2002, Maafi et al. 2003, Rivoal et al. 2003).

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

5. **Entry Potential. Rating: Low.** Interceptions of *Heterodera latipons* or "*Heterodera* sp." have been reported 40 times between 1985 and 2003; *H. latipons* was reported only once (USDA 2004). Annually, only about 1 (± 0.3 standard error of the mean) interception has been reported nationally (USDA 2004). Although the single interception of *H. latipons* was associated with the root of a sugarbeet in a ship's stores, the majority of interceptions have been associated with airline passengers (40%). The remainders have been in ships' stores (25%), permit cargo (20%), general cargo (7.5%), mail (2.5%), and

miscellaneous other places (5%). The majority of interceptions were reported from JFK International Airport (27%) and Miami (23%) with remaining interceptions coming from New Orleans, Elizabeth (NJ), Jacksonville, Atlanta, Los Angeles, Chicago, Presidio (TX), San Francisco, Seattle, Albany, and Hoboken in approximately equal numbers. 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.

Heterodera latipons is most likely to be transported to the United States in infested plant material or infested soil. Approximately 50% of interceptions of *Heterodera latipons* or "*Heterodera* sp." mention soil (USDA 2004). Infested soil may be associated with some commodities, but the greatest volumes are most likely to be moved with international transport of equipment and machinery. Greco (2002) suggested that the return of military equipment from Iraq after the first Gulf War in the early 1990's had the potential to introduce nematode cysts. This possibility presents itself again. Occasionally soil peds may be found in grain shipments. To our knowledge, soil contaminants in grain shipments have never been examined for plant parasitic nematodes. As this nematode feeds strictly on roots, plant material is only likely to be infested if roots remain intact. Thus, carrot, peanut, and sugarbeet [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.

Beet roots have only been intercepted 3 times and peanuts 7 times between 1985 and 2004 from countries that have reported infestations of *H. latipons* (USDA 2004). Carrot roots have been intercepted approximately 18 times between 1985 and 2004 from countries with *H. latipons* (USDA 2004). Edible carrots (*Daucus carota* ssp. *sativus*) are closely related to Queen Anne's lace (*Daucus carota*) which is frequently intercepted as a cut flower. We have attempted to remove Queen Anne's lace from our analysis as flowers and foliage are unlikely to harbor the nematode.

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 *Heterodera latipons* or "*Heterodera* sp." were destined for 12 states (including the District of Columbia) (USDA 2004). The most commonly reported destinations were New York (27%), Florida (23%), California (12%), and Louisiana (8%). We note that some portion of each of these states has a climate and hosts that would be suitable for establishment by *H. latipons*.

7. **Potential Economic Impact. Rating: Medium.** *Heterodera latipons* is an economically important pest, particularly of barley and durum wheat, in semi-arid regions of the Mediterranean, North Africa and the Middle East where cereals are grown under intense cropping systems (Sikora 1987b, Swarup and Sosa-Moss 1990, Nicol 2002, Rivoal et al. 2003). Damage caused by cyst nematodes in this region is compounded by heat and drought stress (Bekal et al. 1998). Severe stunting and yield loss of barley and wheat have been reported in Lebanon, Libya and Syria (Franklin 1969, Sikora 1987b, Inserra et al. 2003). In Syria, an estimated 24% yield loss in barley was reported in a field with 28 eggs+second-stage juveniles/g soil (Greco et al. 2002). In semi-arid regions of Cyprus, up to 50% yield reduction in barley has been attributed in part to *H. latipons* (Philis 1988a, b, Rivoal and Cook 1993, Nicol 2002). In Israel, where *H. latipons* occurs in more arid areas, the nematode does not always produce knotted roots; consequently, little or no damage may result (Stoyanov 1982, Mor et al. 1992, Rivoal and Cook 1993, Nicol 2002). Nevertheless, *H. latipons* has caused yield losses in Israeli wheat (Cohn and Ausher 1973).

   Heterodera species are among some of the most economically important plant parasitic nematodes found worldwide. Many are associated with small grains, legumes and root crops. The economic impact caused by nematode damage is thought to be grossly underestimated. Whitehead (1998) reports an estimated 10% crop loss (worldwide) resulting from nematode damage (Nicol 2002). The economic impact of *H. latipons* is not well known, and difficult to measure because this species sometimes occurs in mixed populations. In fact, some historic reports of crop damage by *H. avenae* may have been caused by *H. latipons*. Both *H. avenae* and *H. latipons* can cause similar symptoms of yellowing and stunting on wheat (Kort 1972, Cohn and Ausher 1973, Stoyanov 1982, Sikora 1987b, Baldwin and Mundo-Ocampo 1991, Philis 1997, Nicol 2002).

   Members of the genus *Heterodera* are only known to feed on roots. Cyst nematodes damage host plants directly by drawing photosynthate from the plant, interfering with normal root function, and facilitating infection by plant pathogens (Hesling 1978, Pitcher 1978, Sasser 1987). Nematode infestation of plant roots limits water absorption, which can cause plant wilting or death, and nutrient uptake, which can cause chlorosis or necrosis of photosynthetically active tissues. Impeded root function contributes to poor or stunted growth and ultimately can affect yield. Damage caused by nematodes may be similar to that caused by

nutrient or water deficiency. Symptoms of nematode infestation may not be detected until later stages of plant growth. Much of the visible damage to plant hosts is likely caused by a combination of biotic and abiotic factors (Stoyanov 1982, Sikora 1987b, Swarup and Sosa-Moss 1990, Baldwin and Mundo-Ocampo 1991, Mor et al. 1992, Potter and Olthof 1993, Philis 1995, 1999, Ismail et al. 2000, Greco et al. 2002).

Severity of damage caused by *Heterodera* can be species specific and may vary by host and soil type (Mor et al. 1992). Cyst nematode damage may occur in light, moderate or heavy soil types, though damage tends to be more severe in lighter soils (Sikora 1987a, b). Economic thresholds have been established for several *Heterodera* species on various hosts and are summarized by Potter and Olthof (1993). For vegetable crops, the threshold is approximately 0.5-2 juveniles g^{-1} of soil. No thresholds have been developed specifically for *H. latipons* (Greco et al. 2002).

8. **Potential Environmental Impact. Rating: Medium.** 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. *Heterodera latipons* 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.

   Heterodera latipons has a narrow host range feeding primarily on graminaceous cereal hosts (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 *H. latipons* Plants listed in Appendix D might be suitable hosts for *H. latipons*, and thus, could be adversely affected by this nematode.

9. **Establishment Potential. Rating: Moderate.** Our initial predictions suggest that much of the US has a climate that could support populations of this nematode. Moreover, potential host plants (esp. wheat) are grown commercially throughout the country. The propensity for the nematode to move after it is introduced seems limited as it has no stage for long-distance active dispersal, though movement of other cyst nematodes by wind and water has been noted (Potter and Olthof 1993). Thus, if introduced into an agricultural area, the

potential for establishment is high. However, interception records suggest that the nematode itself or potential commodities that might be infested with the nematode do not arrive frequently within the US. The lower likelihood of arrival lowers the overall establishment potential to medium. See Appendix E for a more detailed description of the biology of *H. latipons*.

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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 *H. latipons*.

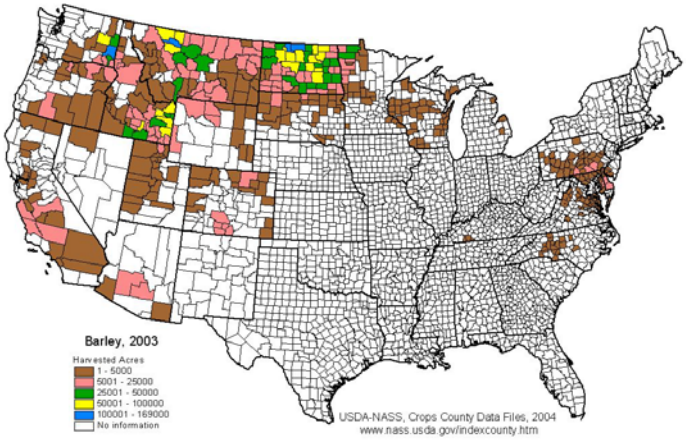
Locations	**Reference(s)**
Algeria	(Mokabli et al. 2002)
Azores	(Mor and Sturhan 2000)
Bulgaria (N, NE, NW and Thracian lowland region)	(Kort 1972, Mulvey 1972, Stoyanov 1982, Mulvey and Golden 1983, USDA 1985, Sabova et al. 1988, Greco et al. 2002)
Canada (Prince Edward Is.)	(Mulvey 1972, Mulvey and Golden 1983, USDA 1985, Ebsary 1986, Sabova et al. 1988, Greco et al. 2002)
Cyprus	(USDA 1985, Sikora 1987b, Philis 1988a, b, Swarup and Sosa-Moss 1990, Rivoal and Cook 1993, Philis 1995, 1997, 1999, Greco et al. 2002, Nicol 2002, Inserra et al. 2003)
Czechoslovakia (formerly Pohled near Havlíčkův Brod district)	(Sabova et al. 1988)
Greece	(Mulvey 1972, Mulvey and Golden 1983, USDA 1985, Sabova et al. 1988, Greco et al. 2002)
Iran (E. Azerbaijan: Ardabil, Marand; W. Azerbaijan: Reza'iyeh or Urmia, Khvoy, Schahpur, Naghadeh, Miandowab; Hamadan: Hamadan, Malayer; Kermanschah: Kerman; Golestan: Agh Ghaleh; Lorestan: Doroud; Zanjan: Abhar)	(Talatschian and Achyani 1976, USDA 1985, Mor and Sturhan 2000, Maafi et al. 2003)
Israel (northern Negev, Sharon regions)	(Franklin 1969, Kort 1972, Mulvey 1972, Cohn and Ausher 1973, USDA 1985, Sabova et al. 1988, Swarup and Sosa-Moss 1990, Mor et al. 1992, Rivoal and Cook 1993, Mor and Sturhan 2000, Rivoal et al. 2001, Nicol 2002, Inserra et al. 2003, Rivoal et al. 2003)

Locations	Reference(s)
Italy (Calabria, Sottommarina, Sardinia, "northern and peninsular Italy")	(Tacconi 1976, Palmisano and Cavalli 1982, USDA 1985, Sabova et al. 1988, Nicol 2002)
Japan (Asahi-shi, Chiiba-ken region)	(Momota 1979, Mulvey and Golden 1983, USDA 1985, Greco et al. 2002)
Jordan	(Greco et al. 2002)
Lebanon (Azzahra, Tripoli)	(Franklin 1969, Mulvey 1972, Mulvey and Golden 1983, Greco et al. 2002, Inserra et al. 2003)
Libya	(Kort 1972, Mulvey and Golden 1983, USDA 1985, Sikora 1987b, Swarup and Sosa-Moss 1990, Mor and Sturhan 2000, Nicol 2002, Inserra et al. 2003)
Poland	(Greco et al. 2002)Mulvey, 1972 #108;Mulvey, 1983 #155}
Russia (Rostov region or Rostovskaya Oblast)	(Subbotin et al. 1999)
Scotland	(Mackintosh 1970, Mulvey 1972, Mulvey and Golden 1983, USDA 1985, Sabova et al. 1988, Greco et al. 2002)
South Africa (Northern Cape)	(Fourie et al. 2001)
Spain (Murcia)	(Romero 1980, USDA 1985, Sabova et al. 1988, Greco et al. 2002)
Syria (Aleppo, Boueidar, Breda, Homs, Tel Hadya)	(Sikora 1987b, Ismail et al. 2000, Rivoal et al. 2000, Ismail et al. 2001, Rivoal et al. 2001, Greco et al. 2002, Mokabli et al. 2002, Nicol 2002, Inserra et al. 2003, Rivoal et al. 2003)
Tunisia	(USDA 1985, Sikora 1987b, Swarup and Sosa-Moss 1990, Inserra et al. 2003)
Turkey (Central Anatolia, Kadinhani, Yunak)	(Rumpenhorst et al. 1996, Greco et al. 2002, Inserra et al. 2003)
USSR (Armenia, Tadzhikistan, Turkmenistan, Ukraine, Eastern Europe)	(Mulvey and Golden 1983, USDA 1985, Sabova et al. 1988, Greco et al. 2002)

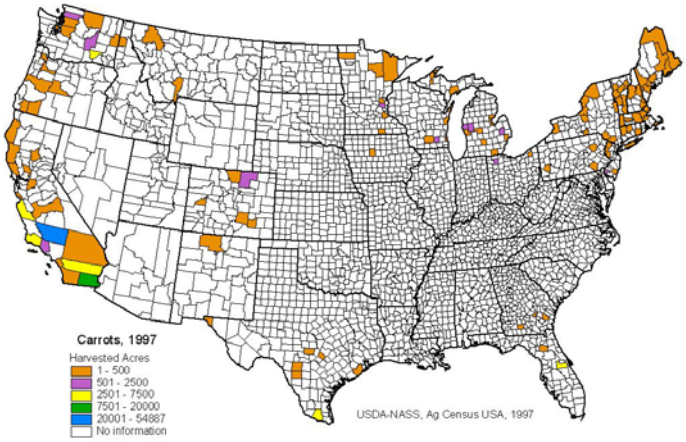
Scotland was not included in our climatic analysis. Many reviews cite the report from Mackintosh (1970) who suggested that *H. latipons* occurred in Scotland. This early report is likely to be in error. The specimens identified as *H. latipons* were more likely *H. hordecalis*; *H. latipons* is not currently known to occur in the United Kingdom (Cook 2004, Hockland 2004).

Appendix B. Commercial production of hosts of *Heterodera latipons* in the continental US.

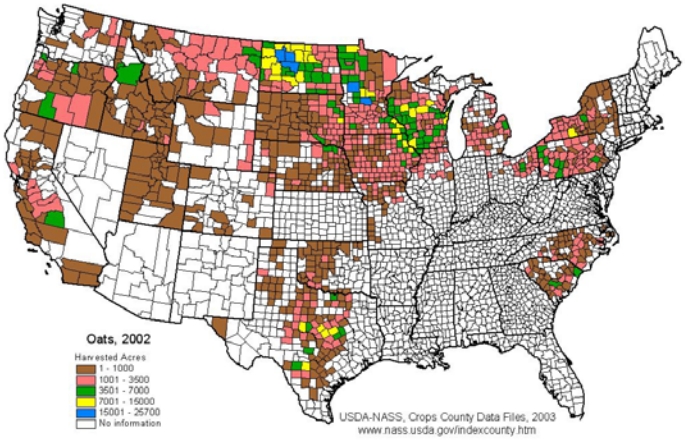
Map 1. Barley (*Hordeum vulgare*)



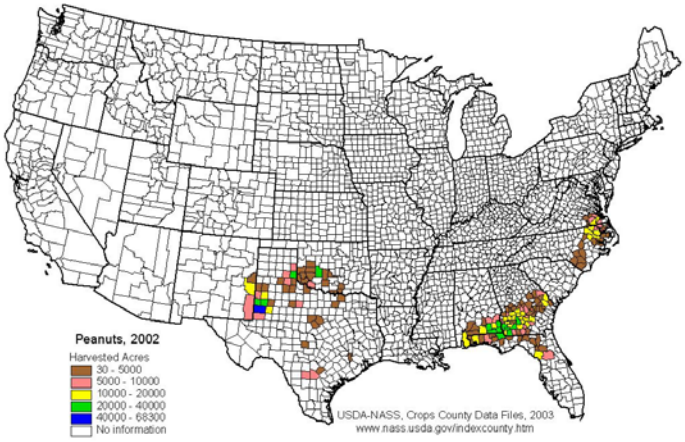
Map 2. Carrot (*Daucus carota*)



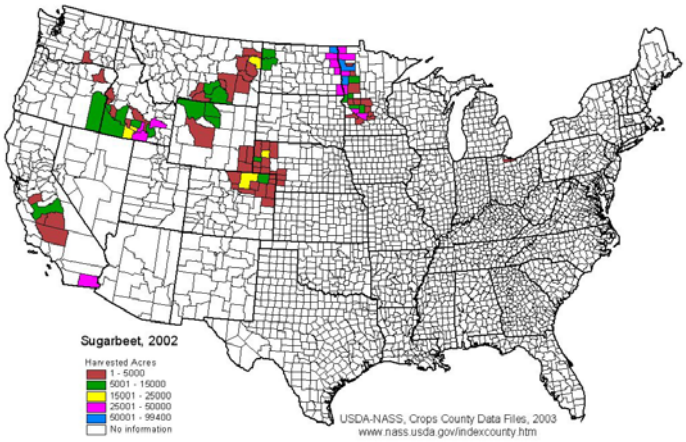
Map 3. Oats (*Avena sativa*)



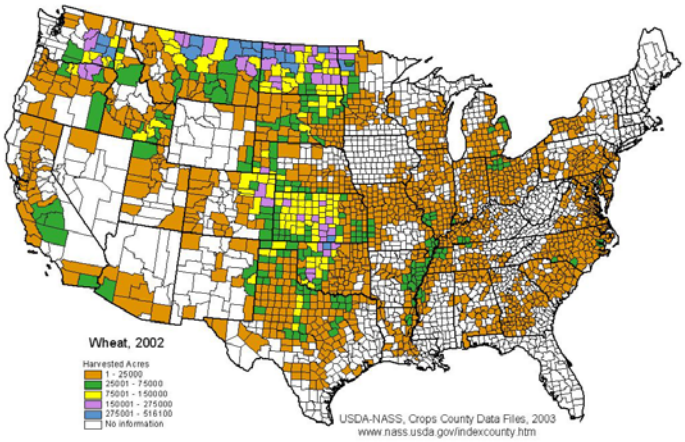
Map 4. Peanut (*Arachis hypogaea*)



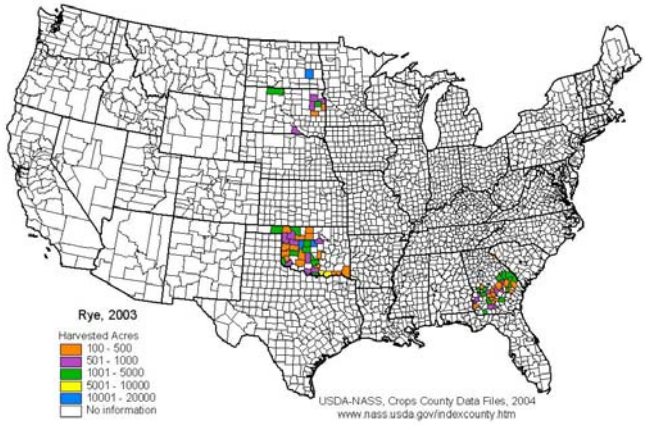
Map 5. Sugarbeet (*Beta vulgaris* subsp. *vulgaris*)



Map 7. Wheat (*Triticum* spp.)



Map 6. Rye (*Secale cereale*)



Appendix C. Taxonomy and Morphology of *Heterodera latipons*

There has been considerable disagreement with respect to taxonomic classification of *Heterodera latipons* and closely associated species referred to as the "*H. avenae* group". According to Handoo (2002), "*H. avenae*, together with other bifenestrate cyst nematodes having a short vulval slit, were placed in the genus *Bidera* (Krall and Krall, 1978), but Mulvey and Golden (1983) synonymized *Bidera* with *Heterodera*". This synonomy was not universally accepted (Baldwin and Mundo-Ocampo 1991). Handoo (2002) provides a key to the species within the *H. avenae* group as well as a thorough review of morphological studies to date. A morphological study by Hesling (1978) also compares cyst nematodes within three genera (*Heterodera*, *Globodera*, and *Punctodera*).

Heterodera latipons Franklin, 1969

Synonyms

Bidera latipons (Franklin, 1969) Krall and Krall, 1978

Ephippiodera latipons (Franklin, 1969) Shagalina and Krall, 1981

This description of *H. latipons* is quoted from Franklin (1969). Greco et al. (2002) provide a more detailed comparison of morphological characteristics of *H. latipons* and closely related species, *H. hordecalis* and *H. turcomanica*. Handoo (2002) provides a detailed key to species within the *H. avenae* group for identification purposes.

Cyst

Measurements of 10 specimens:

fenestral length 58-76 µm;
fenestral width 15-27 µm;
semi-fenestral length 13-19 µm;
vulval slit length 6-9 µm;
vulval bridge length 18-39 µm;
underbridge length 80-125 µm;
underbridge width 7-14 µm.

Dark to mid-brown beneath the white subcrysatalline layer, fully exposed on the roots or slightly embedded, leaving a small "crater" in the root when they are dislodged. Fenestration different from that of all other described species in that the semi-fenestrae are separated by a distance greater than the fenestral width, and the vulval slit is short. There is a strong underbridge with a pronounced thickening in the middle and the ends splayed (Fig. C1-G). Bullae usually absent, but a few sometimes present at the level of the underbridge.

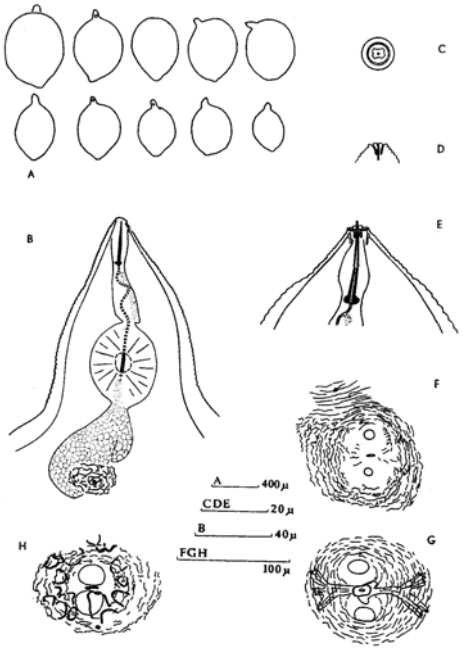


Figure C1. *Heterodera latipons* n sp. Female. A. Mature females. B. Anterior end with excretory pore. C. Face view. D. Head, dorso, ventral. E. Head and stylet, lateral. F. Cuticle of vulval region and anus in white cyst. G. Fenestralia and underbridge in brown cyst. H. Fenestralia and bullae in *H. avenae* brown cyst [Quoted and reproduced from Franklin (1969)].

Male

Measurements for 10-25 specimens:
- length 960-1406 µ
- width 25-32.5 µ
- a=32-51
- b=8.9-11.3 (oesophagus measured to base of median bulb)
  - [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)]
- stylet length 22-29 µ
- stylet knobs 4.2-5 µ across; 1.7-2.3 µ high
- spicules 32-36µ measured along arc

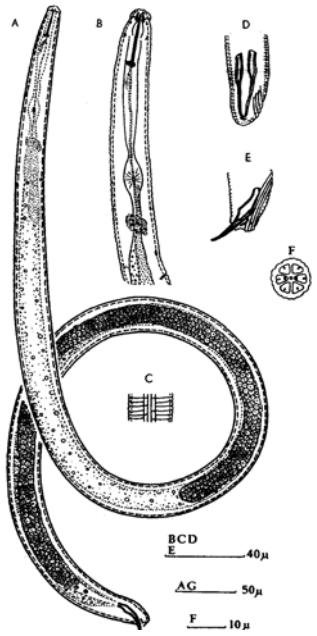


Figure C2. H*eterodera latipons* n sp. Male. A. Whole male. B. Male oesophageal region. C. Lateral field of male at mid body. D. Spicules, ventral. E. Spicules, lateral. F. Face view of male head at level of fourth annule. [Quoted and reproduced with modification from Franklin (1969)].

Head offset, 11.6 µ wide, 6.2 µ high (mean of 10), with four post labial annules. Amphid openings small, lateral head sectors slightly narrower than the others with a small papilla on each sector. Basal annule with 18-19 longitudinal grooves (Fig. C2-F). Four longitudinal incisures on lateral field; outer bands irregularly areolated throughout the body (Fig. C2-C). Hind part of body always twisted in dead specimens, as shown by the direction of the lateral field (Fig. C2-E). Mouth spear with well-defined knobs, which are concave anteriorly:anteriorconical part about equal in length to shaft and knobs together.

Anterior cephalids at the level of the second or third neck annule and posterior ones at mid-stylet level. In three specimens the dorsaloesophageal gland duct opened 3-5 µ behind the stylet knobs. Hemizonid about three annuleswide and at two body widths behind the median bulb; excretory pore 3-6 annules behind it (Fig. C2-B). No hemizonian seen.... Spicules slightly bow-shaped, with a broad anterior end, but narrow and apparently twisted in the posterior part (Fig. C2-D,E). Gubernaculum trough-shaped, about 8 µ long. Phasmids ad-anal and tail less than one anal body-width long.

Egg

Measurement for 25 embryonated eggs from mature females:
- length 100-124 µm;
- breadth 44-56 µm.

Larva

Measurement for 25 specimens
body length 401-478 µ;
body breadth 19-22 µ;
tail length 42-54 µm;
length of hyaline tail tip 20-31 µm;
stylet length 23-25 µm.

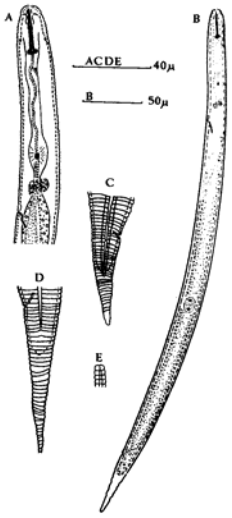


Figure C3. H*eterodera latipons* n sp. A. Larval oesophageal region. B. Whole larva. C. *H latipons* larval tail. D. *H avenae* larval tail. E. Anterior end of lateral field in *H latipons* larva. [Quoted and reproduced with modification from Franklin (1969)].

Body slightly curved dorso ventrally when killed by heat. Offset head with three post-labial annules. In many specimens, the cuticle in the neck region appears slightly inflated for a distance of 7-8 annules behind the head. Lateral field 1/4 to 1/5 width of body with four incisures, starting at about mid-stylet level an ending mid-way along the tail; outer bands areolated (Fig.C3-C,E). Phasmids 2-3 annules behind anus. Stylet with well-developed, anteriorly concave knobs. oesophageal gland lobe overlying intestine latero-ventrally for a distance equal to about 39% of body length; dorsal gland anterior to and appearing more finely granular than the two sub-ventrals. Hemizonid distinct but no hemizonion seen. Excretory pore opening immediately behind or apparently at same level as hemizonid. Posterior cephalids obscure but probably at the eightth neck annule; anterior ones not seen. Rectum nearly as long as anal body width. Gonad initial consisting of two cells, situated at about 60% of body length from anterior end.

Differential diagnosis

H. latipons n. sp. differs from all known species of the genus, except *H. turcomanica* Kirjanova & Shagalina, 1965, in having a strong underbridge and almost circular semi-fenestrae separated by a distance greater than the diameter of a single semi-fenestra. The vulval slit is also shorter than that of any other species except *H. turcomanica*, in which it is 9-14 µ long, and *H. avenae* in which it is 12 µ. The cysts of the new species differ from those of *H. turcomanica* in the absence of the small gland-like sacs beneath the cuticle that are described for that species, and in the frequent absence of bullae....

The cysts of *H. latipons* resemble those of *H. avenae* more than any species, except *H. turcomanica*, but in *H. avenae* the semi-fenestrae are closer together, there is no underbridge and there are always prominant bullae crowded into the cone (Fig. C1-H). Larvae of *H. avenae* are longer (575 µ) and have a narrow lateral field with only two longitudinal incisures (Fig.C3-D).

Appendix D. Threatened or endangered plants potentially affected by *Heterodera latipons*.

Heterodera latipons has the potential to adversely affect threatened and endangered plant species. However, because *H. latipons* 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, *H. latipons* is known to feed primarily on species within the family 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. Note that, as discussed under 'Host Specificity/Availability,' though *H. latipons* may be found on grasses within the geographic range of marram grass (*Ammophila arenaria*), it is not known to reproduce on this host.

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

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
Ammophila arenaria	*A. breviligulata*	American beachgrass		IL (E) MN (T) OH (T) PA (T)
	A. champlainensis	Champlain beachgrass		NY (E) VT (E)
Phalaris minor, P. paradoxa	*P. caroliniana*	Carolina canarygrass		MD (E)

1. E= Endangered; T=Threatened

Appendix E. Biology of *Heterodera latipons*

Population phenology

Like several nematodes, *H. latipons* has the ability to survive unfavorable environmental stresses (Greco et al. 2002). Protected in membranes within the cyst, eggs may remain viable for several years under adverse conditions (Williams 1978, Baldwin and Mundo-Ocampo 1991, Potter and Olthof 1993). In climates with cool, wet winters and warm, dry summers, *H. latipons* is active from November through February. Typically one generation is completed in the Mediterranean region (Mor et al. 1992, Greco et al. 2002).

Stage specific biology

Development time varies depending on climate (particularly temperature) and is closely tied to host plant phenology (Mor et al. 1992). Development time can range from up to 4 months at 6ºC to about 40 days at 18ºC. Warmer temperatures over 24ºC are unfavorable for root penetration and development (Mor et al. 1992). Root penetration reportedly does not occur at temperatures below 4ºC (Potter and Olthof 1993).

Adult

Female development occurs approximately 215 degree days above 7ºC, and cysts with embryos occur at approximately 386 degree days (Greco et al. 2002). Females are white, lemon-shaped to almost spherical and sedentary, and may be found on the host root before flowering occurs (Greco et al. 2002) (Baldwin and Mundo-Ocampo 1991). Females swell, producing large gelatinous sacs containing several hundred eggs (Stoyanov 1982). Eggs are predominantly held in the female body; occasionally they may be deposited inside a gelatinous matrix outside of the forming cyst (Baldwin and Mundo-Ocampo 1991). Once the life cycle is completed, the female dies leaving a protective egg-containing cyst (Greco et al. 2002).

Males are vermiform. They migrate in order to mate and then die. Many do not feed or if they do, they will feed only to survive while mating. In sizable populations, males will tolerate crowding, where females may die if food becomes scarce. Several males may be attracted to pheromones secreted by a female and mating may occur multiple times (Baldwin and Mundo-Ocampo 1991).

Egg

Egg hatch and attraction to a feeding site may involve a stimus from the host root (Baldwin and Mundo-Ocampo 1991). Hatching can occur for an extended period (even over a period of several years) at cooler temperatures between 5-15ºC (Stoyanov 1982, Potter and Olthof 1993, Greco et al. 2002). In laboratory experiments, greater egg hatch has been observed in 4-5 month-old cysts that were exposed to temperatures between 5-10ºC compared to 1-2 month-old cysts (Greco et al. 2002). In a Syrian laboratory study using barley exudates and water, egg hatch was reportedly greater in the exudates following an incubation period at 5ºC. Dormancy was also reportedly broken at 27ºC (Ismail et al. 2000). Eggs will not hatch under extended dry periods or at warmer temperatures between 20-25ºC, and may persist within protected cysts in soil or dry roots

awaiting more favorable conditions (Greco et al. 2002). Eggs may persist and hatch over a period of 5-6 years (Baldwin and Mundo-Ocampo 1991).

Juvenile

Consistent with the life cycle of Heteroderinae, there are four juvenile stages. Larvae moult four times before developing into adults (Baldwin and Mundo-Ocampo 1991). All of the first and a portion of the second juvenile stages occur inside the egg. Females complete three moults following root penetration and establishment of a feeding site (Baldwin and Mundo-Ocampo 1991). Emergence from eggs may involve several factors including diapause, soil temperature, moisture and aeration. Juveniles emerge in moist soil and may become inactive under dry conditions (Baldwin and Mundo-Ocampo 1991). Second stage juveniles emerge moving toward the host roots. When a suitable site is selected the larva will penetrate the root usually at a lateral root near the root tip, or at a wound site (Williams 1978, Baldwin and Mundo-Ocampo 1991). The larva then moves through the root to the region of cell differentiation, settles, and becomes inactive while feeding. Feeding induces cellular changes in the primary phloem or parenchyma, changing them into large, nutrient-rich cells from which juveniles feed until development is completed (Williams 1978). If, after egg hatch, a larva cannot find a suitable feeding site on a host, it will continue searching until its energy is depleted. If large, specialized cell formation does not happen as a result of host infection, the larva may not complete its development (Baldwin and Mundo-Ocampo 1991). Development of second-third stage juveniles can be completed in 7-11 days, depending on temperature. In the Mediterranean region, peak activity of second stage juveniles coincides with host plant emergence (Greco et al. 2002). Maturation of females is completed within 30 days, resulting in an egg-filled cyst (Williams 1978).