Mini Risk Assessment
Asian Citrus Root-knot Nematodes: *Meloidogyne citri* Zhang, Gao, & Weng;
***M. donghaiensis* Zheng, Lin, & Zheng;**
***M. fujianensis* Pan;**
***M. indica* Whitehead;**
***M. jianyangensis* Yang, Hu, Chen, & Zhu;**
***M. kongi* Yang, Wang, & Feng;**
and *M. mingnanica* Zhang
[Nematoda: Meloidogynidae]

Erica E. Davis & Robert C. Venette
Department of Entomology, University of Minnesota
St. Paul, MN 55108
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Introduction

In Asia, a complex of root-knot nematodes [referred to throughout this document as either *Meloidogyne* spp. or Asian citrus root-knot nematodes] attacks the roots of *Citrus* crops. These plant-parasitic nematodes are only known to occur regionally within China and India, in areas where the climate ranges from tropical-subtropical to temperate. Available literature suggests that these nematodes do not occur throughout the citrus production areas of Asia.

The Asian citrus root-knot nematodes addressed in this mini-pest risk assessment are not currently known to occur in the U.S. (Vovlas and Inserra 2000). These nematodes should not be confused with *Meloidogyne indica,* the "citrus root-knot nematodes" that occur in the United States, or *Tylenchulus semipenetrans*, the "citrus root nematodes" that are important pests of *Citrus* in the southern U.S. (Norton et al. 1984). Within the US, few *Meloidogyne* spp. can parasitize and complete development on citrus (Vovlas and Inserra 1996). Because *Meloidogyne* spp. are generally considered among the most economically damaging nematodes, not necessarily to just citrus, these nematodes may have the potential to severely affect citrus production if Asian root-knot nematodes arrived and established in the United States. In a series of assessments, *M. citri*, *M. donghaiensis*, *M. fujianensis, M. indica*, *M. jiangyangensis*, *M. kongi*, and *M mingnanica* received medium risk ratings (Inserra et al. 2003a, b, c, d, e, f, g). The high value of citrus elevated the ratings, but limited information on the potential degree of economic damage and the basic biology of the nematodes tempered the assessments. Unlike most of our other pest risk assessments, we have elected to address the entire complex of nematodes in a single document because information on each species is currently scarce. This document evaluates several factors that influence the degree of risk posed by Asian citrus root-knot nematodes and applies this information to the refinement of sampling and detection programs. Where possible, species specific information is provided.

1. **Ecological Suitability. Rating: Low-Moderate.** Asian citrus root-knot nematodes occur within portions of China and India. Appendix A provides detailed records on the reported worldwide distribution of these nematodes. The

geographic distribution of the nematodes provides insights on the climatic requirements for the species. Table 1 lists the climates with which the nematode(s) are most closely associated. See Appendix A for a more complete description of this analysis.

Table 1. Habitat Zones for Asian Citrus Root-knot Nematodes

Nematode(s)	**Biomes/Habitat Zones**	**Estimated % of the continental U.S. that could provide a suitable climate for establishment**
Meloidogyne citri *M. donghaiensis* *M. fujianensis* *M. mingnanica*	Tropical and subtropical moist broadleaf forests[1]	<1% (See Fig. 2a)
M. indica	Tropical and subtropical moist broadleaf forests[1]; Tropical and subtropical dry broadleaf forests[2]	<1% (See Fig. 2a)
M. jianyangensis	Temperate coniferous forests	19% (See Fig. 2b)
M. kongi	Temperate broadleaf and mixed forests; Tropical and subtropical moist broadleaf forests[1]	28-29% (See Fig. 2c)

1. Collier, Dade and Monroe Counties in southern Florida have this climate zone.
2. This biome does not occur in the continental U.S.

Figure 2a. Predicted regions (shaded green) within the continental United States that are likely to provide a suitable climate for *Meloidogyne citri, M. donghaiensis, M. fujianensis, M. indica,* or *M. mingnanica***.** This map does not account for the availability of hosts.

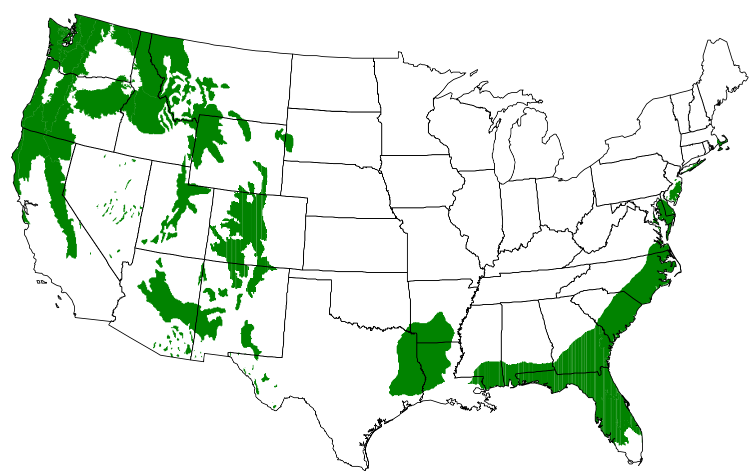


Figure 2b. Predicted regions (shaded green) within the continental United States that are likely to provide a suitable climate for *Meloidogyne jianyangensis*. This map does not account for the availability of hosts.

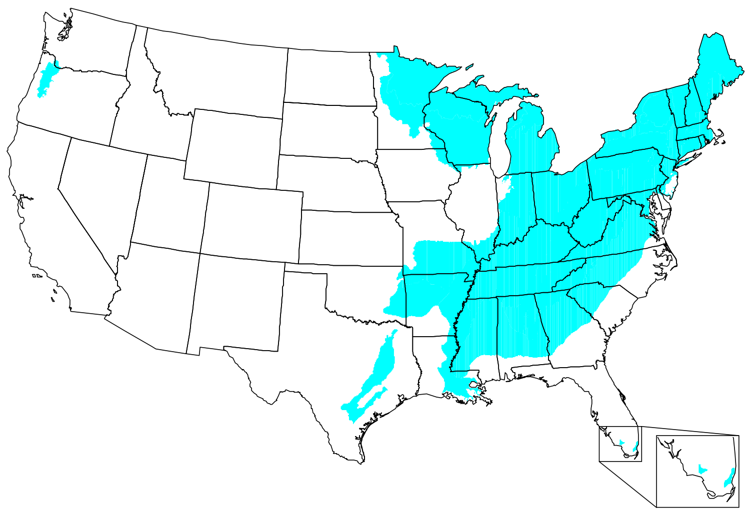


Figure 2c Predicted regions (shaded blue) within the continental United States that are likely to provide a suitable climate for *Meloidogyne kongi*. This map does not account for the availability of hosts.

Figures 2a-c illustrate where the Asian citrus root-knot nematodes are most likely to encounter a suitable climate for establishment within the continental US. These predictions are based only on the known geographic distribution of the species. Because the forecasts are based on coarse information, areas that are not highlighted on the maps may have some chance of supporting populations of these exotic species. However, establishment is less likely in areas that are not highlighted than in those areas that are highlighted. For initial surveys, efforts should be concentrated in the higher risk areas and gradually expanded as needed.

As is evident from Table 1 and Figures 2a-c, not all members of the Asian citrus root-knot nematode complex are equally likely to find a suitable climate in the continental United States. *Meloidogyne citri, M. donghaiensis, M. fujianensis, M. indica,* and *M. mingnanica* are tropical species that are only likely to find a suitable climate in southern Florida. Thus, the overall ecological suitability of the continental United States seems low. In comparison, *M. jianyangensis* and *M. kongi* are more temperate species and are likely to find substantially more of the United States climatically suitable. However, the degree of suitability is only moderate compared with other exotic pests that might invade the US.

2. **Host Specificity/Availability. Rating: Low-Moderate/Moderate-High.** Table 2 lists host plants reported for Asian citrus root-knot nematodes. *Citrus* is the primary host for all of these nematodes; however, in host range studies, pepper and tomato were identified among a few other non-citrus, experimental hosts (Table 2). Some of these nematodes have shown preferences for particular species of *Citrus* over other members of the same genus (Zhang and Xu 1994).

Table 2. Host plants of Asian citrus root-knot nematodes

Hosts	**Reference(s)**
M. citri	
citrus (*Citrus* sp. and/or *Citrus* spp.)	(Zhang et al. 1990, Inserra et al. 2003a){Zhang, 1994 #44 {Vovlas, 1996 #205}
orange, mandarin/tangerine (*C. reticulata*)	(Vovlas and Inserra 2000)
orange, Satsuma (*Citrus unshiu*)	(Zhang and Weng 1991, Vovlas and Inserra 1996, 2000, Inserra et al. 2003a)
*orange, sour (*Citrus aurantium*)	(Zhang and Xu 1994, Inserra et al. 2003a) (Vovlas and Inserra 2000)
orange, trifloliate or hardy (*Poncirus trifoliata*)	(Zhang and Xu 1994, Vovlas and Inserra 2000, Inserra et al. 2003a)
*tomato (*Solanum lycopersicum* =[*Lycopersicon esculentum*])	(Vovlas and Inserra 2000, Inserra et al. 2003a)
M. donghaiensis	
orange, mandarin/tangerine (*Citrus reticulata*)	(Vovlas and Inserra 1996, 2000)
M. fujianensis	
cogongrass (*Imperata cylindrica*)	(Pan et al. 1999)
orange, mandarin/tangerine (*Citrus reticulata*)	(Vovlas and Inserra 1996, 2000)
M. indica	
citrus (*Citrus* sp. and/or *Citrus* spp.)	(Franklin 1978, Vovlas and Inserra 1996, 2000)Whitehead, 1968 #270}
lime (*Citrus aurantifolia*)	(Whitehead 1968, Vovlas and Inserra 2000)
orange (*Citrus sinensis*)	(Vovlas and Inserra 2000)
morinda (*Morinda officianalis*)	(Zhang and Weng 1991)
M. jianyangensis	
citrus (*Citrus* sp.)	(Hu et al. 1991)
orange, mandarin/tangerine (*Citrus reticulata*)	(Yang et al. 1990, Vovlas and Inserra 1996, 2000, Inserra et al. 2003e)
M. kongi	
citrus (*Citrus* sp. and/or *Citrus* spp.)	(Vovlas and Inserra 1996, 2000)
*pepper (*Capsicum* sp.)	(Vovlas and Inserra 2000)

*=indicates an experimental host

Hosts	Reference(s)
M. mingnanica	
citrus (*Citrus* sp. and/or *Citrus* spp.)	(Zhang and Xu 1994, Inserra et al. 2003g)
orange, Satsuma (*Citrus unshiu*)	(Vovlas and Inserra 1996, 2000)
orange, trifloliate or hardy (*Poncirus trifoliata*)	(Zhang and Xu 1994, Vovlas and Inserra 2000)
*orange, sour (*Citrus aurantium*)	(Zhang and Xu 1994)

*=indicates an experimental host

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.

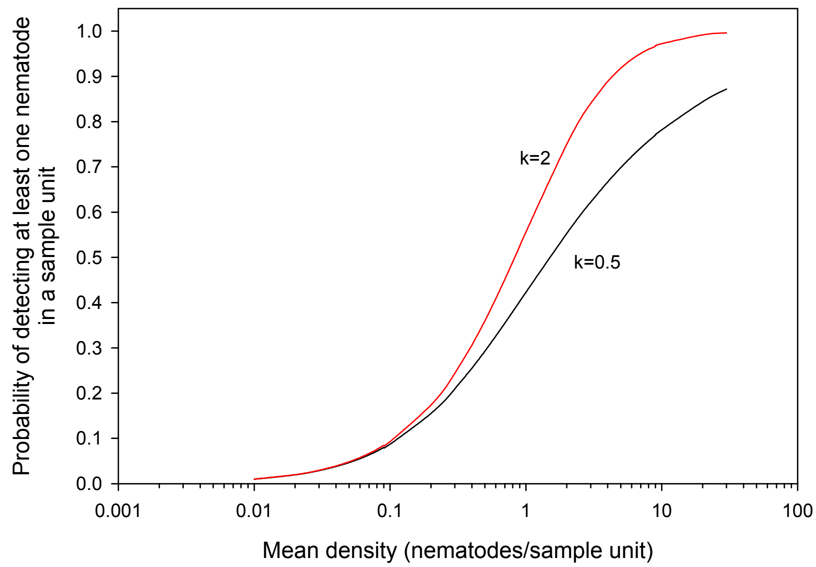


Figure 3. Influence of nematode density and spatial distribution on the likelihood of observing at least one nematode from a soil sample. Lines are based on the negative binomial distribution.

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.

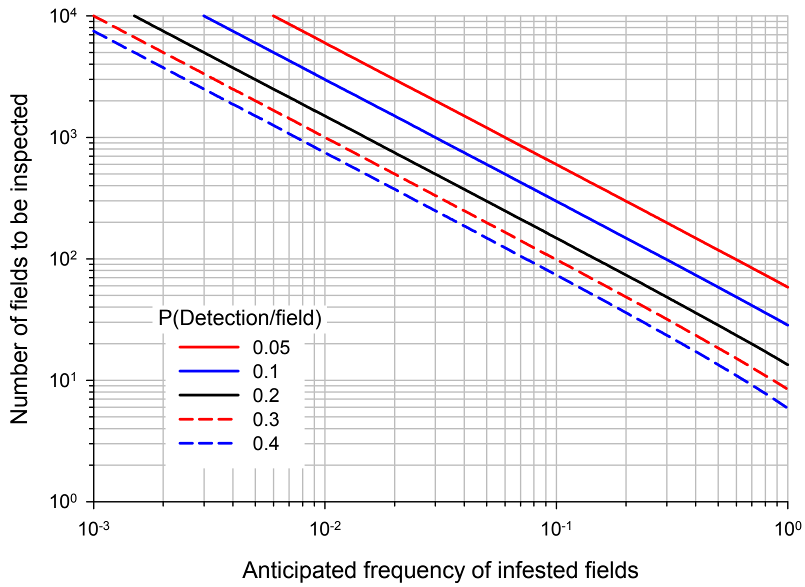


Figure 4. Influence of the frequency of infested fields and the likelihood of detecting an infestation in an individual field on the number of fields that should be inspected to have 95% confidence of detecting at least one exotic nematode within a region.

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.

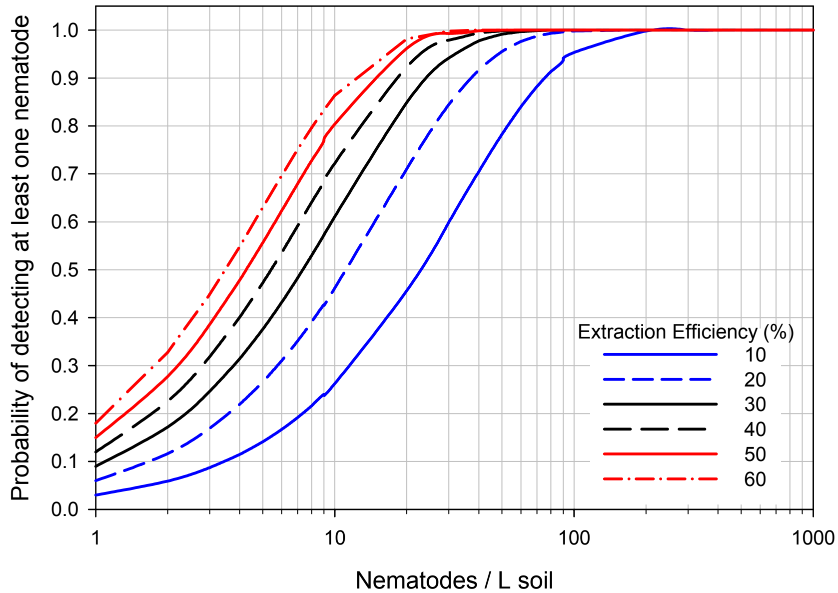


Figure 5. Influence of extraction efficiency and nematode density on the probability of detecting at least one nematode in 300 cc of a well-mixed, 1-liter soil sample.

4 **Taxonomic Recognition**. **Rating: Medium.** *Meloidogyne* may occur in mixed populations with closely related species (Zhang and Weng 1991). *M. indica* and *M. fujianensis* are best described, though in general little is known about most members of the Asian citrus root-knot nematodes. For a description of the taxonomy and morphology (including some diagnostic characters), 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 spp. are 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). As the complex of nematodes that we are considering in this mini-risk assessment feeds strictly on roots, plant material is only likely to be infested if root remains intact. None of the known or potential hosts are root crops [see 'Host Specificity']. Consequently, the unintentional introduction of this nematode in a commodity shipment or by an international airline passenger seems unlikely.

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. Additionally, no information is available for imported citrus root stocks that are required to pass through quarantine. Vovlas and Inserra (1996) suggest that these nematodes are most likely to be introduced if nurseries do not follow protocols established for nematode certification. Because so little information is available for many pathways, 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 only some of these states have a climate and hosts that may be suitable for establishment by one or more of the Asian citrus root-knot nematodes. The suitability of the climate in each of these states depends on which nematode species is under consideration.

7. **Potential Economic Impact. Rating: High.** The economic impact of the Asian citrus root-knot nematodes is not well known. The impacts of a single species are difficult to measure because these nematodes often occur in mixed populations. However, these nematodes are thought to pose a real economic threat to citrus producing regions, especially China and India, where estimated crop losses have been as high as 20-50% (Pan 1985, Vovlas and Inserra 2000). *Meloidogyne fujianensis*, widespread in the Fuijan region of China after which it is named, reportedly causes more damage than the better known citrus nematode, *Tylenchulus semipenetrans* (Pan et al. 1999). *Meloidogyne* species are among some of the most economically important plant parasitic nematodes found worldwide (DeGiorgi et al. 2002). In general, for a wide variety of crops, losses resulting from nematode damage have been estimated at an average of 10-11% worldwide (Jensen 1972, Potter and Olthof 1993, Whitehead 1998). The economic impact caused by nematode damage is thought to be grossly underestimated.

   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) (Pan et al. 1999). 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). Specific damage to *Citrus* caused by *Meloidogyne* spp. [documented for *M. fujianensis*] includes yellowing of leaves, premature flower drop, early ripening and fruit drop, and reduced fruit quality and yield (Pan et al. 1999, Vovlas and Inserra 2000). Again, because little information is available about the economic impacts of the Asian citrus nematode complex in Asia, a significant degree of uncertainty is associated with our rating of this element.

8. **Potential Environmental Impact. Rating: Low-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. *Meloidogyne* spp. have the potential to affect the environment in some 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.

   Asian citrus root-knot nematodes have a narrow host range, feeding primarily on *Citrus* hosts (see ‘Host Specificity’). There are no native members of the genus citrus in the United States. However, *M. citri* may parasitize some solanaceous crops. Native *Solanum* are found in the United States, and some species are listed as threatened or endangered (USDA NRCS 2004). These native plant species are not found in the continental US. Thus, Asian citrus root-knot nematodes do not pose any additional risks to plants in the continental US that are already listed as threatened or endangered.

9. **Establishment Potential. Rating: Low-Medium.** Not all members of the Asian citrus root-knot nematode complex pose the same degree of risk to agricultural production in the continental US. The relatively restricted distribution of each

nematode species within Asia (well within the bounds of the citrus producing areas) suggests that these nematodes have very specific climatic requirements or are under tight biological controls. A coarse analysis suggests that *M. citri*, *M. kongi* and *M. jianyangensis* are likely to find the greatest area as climatically suitable. *Meloidogyne citri*, *M. kongi*, and *M. fujianensis* have the potential to parasitize hosts other than citrus. Consequently, among the Asian citrus root-knot nematodes, *M. citri* and *M. kongi* pose the greatest risks, but compared to other exotic pests, these two nematodes seem only moderately likely of becoming established. The frequency of nematode introductions based on pest interception records appears to be very low compared to other pests, and this lowers the potential for establishment. No data is available on the frequency of root stock importations from Asia that are infested with root-knot nematodes. Considerable research is needed to better evaluate the risks these species pose.

See Appendix D for a general description of the biology of the Asian citrus root-knot nematodes.

References:

Barker, K. R. 1985. Nematode extraction and bioassays, pp. 19-35. *In* K. R. Barker, C. C. Carter and J. N. Sasser [eds.], An advanced treatise on *Meloidogyne*, Vol II. methodology. North Carolina State University Graphics, Raleigh.

Been, T. H., and C. H. Schomaker. 2000. Development and evaluation of sampling methods for fields with infestation foci of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*). Phytopathology 90: 647-656.

Bunn, D., G. W. Feenstra, L. Lynch, and R. Sommer. 1990. Consumer acceptance of cosmetically imperfect produce. The Journal of Consumer Affairs 24: 268-279.

DeGiorgi, C., P. Veronico, F. DeLuca, A. Natilla, C. Lanave, and G. Pesole. 2002. Structural and evolutionary analysis of the ribosomal genes of the parasitic nematode *Meloidogyne artiellia* suggests its ancient origin. Molecular & Biochemical Parasitology 124: 91-94.

Eisenback, J. D., and H. Hirschmann Triantaphyllou. 1991. Root-knot nematodes: *Meloidogyne* species and races, pp. 191-274. *In* W. R. Nickle [ed.], Manual of Agricultural Nematology. Marcel Dekker, New York.

Franklin, M. T. 1978. *Meloidogyne*, pp. 98-124, Plate VII. *In* J. F. Southey [ed.], Plant Nematology. Ministry of Agriculture Fisheries and Food; Her Majesty's Stationery Office (HMSO), London.

Greco, N., N. Vovlas, A. Troccoli, and R. N. Inserra. 2002. The Mediterranean cereal cyst nematode, *Heterodera latipons*: a menace to cool season cereals of the United States, Nematology Circular 221. Florida Department of Agriculture & Conservation Services Division of Plant Industry.

Hesling, J. J. 1978. Cyst nematodes: morphology and identification of *Heterodera, Globodera* and *Punctodera*, pp. 125-155. *In* J. F. Southey [ed.], Plant Nematology. Ministry of Agriculture Fisheries and Food; Her Majesty's Stationery Office (HMSO), London.

Hu, K. J., B. J. Yang, Q. L. Wang, and W. S. Zhu. 1991. An investigation of plant nematodes around the roots of citrus trees in Sichuan Province. Journal of Southwest Agricultural University 13: 258-264.

Hussey, R. S. 1985. Host-parasite relationships and associated physiological changes, pp. 143-153. *In* J. N. Sasser and C. C. Carter [eds.], An Advanced Treatise on *Meloidogyne*, Volume 1: Biology and Control. North Carolina State University Press, Raleigh. NC.

Ingham, R. E., and G. S. Santo. 1994a. Factors affecting sampling and extraction methodology, pp. 5-22. *In* G. D. Griffin and P. A. Roberts [eds.], Quantifying nematode control, Research Report 149. Utah Agricultural Experiment Stattion, Logan, UT.

Ingham, R. E., and G. S. Santo. 1994b. Extraction methods, pp. 55-59. *In* G. D. Griffin and P. A. Roberts [eds.], Quantifying nematode control, Research Report 149. Utah Agricultural Experiment Stattion, Logan, UT.

Inserra, R. N., J. A. Brito, K. Dong, Z. A. Handoo, P. S. Lehman, T. Powers, and L. Millar. 2003a. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne citri*. Society of Nematologists. Available on-line at http://nematode.unl.edu/wgroup.htm. Accessed 2004.

Inserra, R. N., J. A. Brito, K. Dong, Z. A. Handoo, P. S. Lehman, T. Powers, and L. Millar. 2003b. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne donghaiensis*. Society of Nematologists. Available on-line at http://nematode.unl.edu/wgroup.htm. Accessed 2004.

Inserra, R. N., J. A. Brito, K. Dong, Z. A. Handoo, P. S. Lehman, T. Powers, and L. Millar. 2003c. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne fujianensis*. Society of Nematologists. Available on-line at http://nematode.unl.edu/wgroup.htm. Accessed 2004.

Inserra, R. N., J. A. Brito, K. Dong, Z. A. Handoo, P. S. Lehman, T. Powers, and L. Millar. 2003d. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne indica*. Society of Nematologists. Available on-line at http://nematode.unl.edu/wgroup.htm. Accessed 2004.

Inserra, R. N., J. A. Brito, K. Dong, Z. A. Handoo, P. S. Lehman, T. Powers, and L. Millar. 2003e. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne jiangyangensis*. Society of Nematologists. Available on-line at http://nematode.unl.edu/wgroup.htm. Accessed 2004.

Inserra, R. N., J. A. Brito, K. Dong, Z. A. Handoo, P. S. Lehman, T. Powers, and L. Millar. 2003f. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne kongi*. Society of Nematologists. Available on-line at http://nematode.unl.edu/wgroup.htm. Accessed 2004.

Inserra, R. N., J. A. Brito, K. Dong, Z. A. Handoo, P. S. Lehman, T. Powers, and L. Millar. 2003g. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne mingnanica*. Society of Nematologists. Available on-line at http://nematode.unl.edu/wgroup.htm. Accessed 2004.

Jensen, H. J. 1972. Nematode Pests of Vegetable and Related Crops, pp. 377-408. *In* J. M. Webster [ed.], Economic Nematology. Academic Press, London.

Jones, F. G. W. 1965. Structure and Classification of Nematodes, pp. 3-29. *In* J. F. Southey [ed.], Plant Nematology, 2nd ed. Her Majesty's Stationery Office, London.

Milne, D. L. 1972. Nematodes of Tobacco, pp. 159-186. *In* J. M. Webster [ed.], Economic Nematology. Academic Press, London.

National Plant Board. 1999. Safeguarding American plant resources: A stakeholder review of the APHIS-PPQ safeguarding system. US Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Riverdale, MD.

Norton, D. C., P. Donald, J. Kimpinski, R. Myers, G. Noel, E. M. Noffsinger, R. T. Robbins, D. P. Schmitt, C. Sosa-Moss, and T. C. Vrain. 1984. Distribution of plant-parasitic nematode species in North America. Society of Nematologists.

Olson, D. M., E. Dinerstein, E. D. Wikramanayake, N. D. Burgess, G. V. N. Powell, E. C. Underwood, J. A. D'Amico, I. Itoua, H. E. Strand, J. C. Morrison, C. J. Loucks, T. F. Allnutt, T. H. Ricketts, Y. Kura, J. F. Lamoreux, W. W. Wettengel, P. Hedao, and K. R. Kassem. 2001. Terrestrial ecoregions of the world: a new map of life on earth. BioScience 51: 933-938.

Pan, C., X. Hu, and J. Lin. 1999. Temporal fluctuations in *Meloidogyne fujianensis* parasitizing *Citrus reticulata* in Nanjing, China. Nematologia Mediterranea 27: 327-330.

Pan, C. S. 1985. Studies on plant-parasitic nematodes on economically important crops in Fujian III. Description of *Meloidogyne fujianensis* n.sp. (Nematoda: Meloidogynidae) infesting *Citrus* in Nanjing County. Acta Zoologica Sinica 31: 263-268.

Pan, C. S., J. Ling, and S. Wang. 1988. Studies on parasitic nematodes in crops of economical importance in Fujian. IV. *Meloidogyne fujianensis*. Acta Zoologica Sinica 34: 305-309.

Pan, C. S., and J. Lin. 1998. A new record of host plants of *Meloidogyne fujianensis* and their observation by scanning electron microscope. Acta Parasitologica et Medica Entomologica Sinica 5: 125-126.

Pitcher, R. S. 1978. Interactions of Nematodes with other Pathogens, pp. 63-77. *In* J. F. Southey [ed.], Plant Nematology. Ministry of Agriculture Fisheries and Food; Her Majesty's Stationery Office (HMSO), London.

Potter, J. W., and T. H. A. Olthof. 1993. Nematode Pests of Vegetable Crops, pp. 171-207. *In* K. Evans, D. L. Trudgill and J. M. Webster [eds.], Plant Parasitic Nematodes in Temperate Agriculture. CAB International, Wallingford, UK.

Prot, J. C., and H. Ferris. 1992. Sampling approaches for extensive surveys in nematology. Journal of Nematology, Supplement 24: 757-764.

Sasser, J. N. 1987. A perspective on nematode problems worldwide. pp. 1-12. *In* Proceedings, Nematodes Parasitic to Cereals and Legumes in Temperate Semi-arid Regions, Larnaca, Cyprus. ICARDA, Aleppo, Syria.

Swarup, G., and C. Sosa-Moss. 1990. Nematode Parasites of Cereals, pp. 109-136. *In* M. Luc, R. A. Sikora and J. Bridge [eds.], Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International, Wallingford.

Tastet, C., F. Val, M. Lesage, L. Renault, L. Marche, M. Bossis, and D. Mugniery. 2001. Application of a putative fatty-acid binding protein to discriminate serologically the two European quarantine root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax*, from other *Meloidogyne* species. European Journal of Plant Pathology 107: 821-832.

Taylor, A. L. 1987. Identification and Estimation of Root-knot Nematode Species in Mixed Populations, Bulletin 12 No. 335. Florida Department of Agriculture & Consumer Services, Gainesville, FL.

USDA. 2004. Port Information Network (PIN-309): quarantine status database. US Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Riverdale, MD. Available on-line at restricted access database. Accessed 2004.

USDA NRCS. 2004. The PLANTS database. National Plant Data Center, Baton Rouge, LA. Available on-line at http://plants.usda.gov. Accessed 2004.

Vovlas, N., and R. N. Inserra. 1996. Distribution and parasitism of root-knot nematodes on *Citrus*, Nematology Circular No. 217. Florida Department of Agriculture & Consumer Services Division of Plant Industry.

Vovlas, N., and R. N. Inserra. 2000. Root-knot nematodes as parasites of *Citrus*. Proceedings of the International Society of Citriculture, Vol. II 2: 812-817.

Whitehead, A. G. 1968. Taxonomy of *Meloidogyne* (Nematodea: Heteroderidae) with descriptions of four new species. Transactions of the Zoological Society of London 31: 263-401.

Whitehead, A. G. 1998. Plant nematode control. CAB International, Wallingford, UK.

Yang, B., K. Hu, H. Chen, and W. Zhu. 1990. A new species of root-knot nematode *Meloidogyne jianyangensis* n. sp. parasitizing mandarin orange. Acta Phytopathologica Sinica 20: 259-264.

Zhang, S., R. Gao, and Z. Wheng. 1990. *Meloidogyne citri* n.sp. (Meloidogynidae), a new root-knot nematode parasitizing citrus in China. Journal of Fujian Agricultural College 19: 311-320.

Zhang, S., and Z. Weng. 1991. Identification of root-knot nematode species in Fujian. Journal of Fujian Agricultural College 20: 158-164.

Zhang, S. S., and M. Y. Xu. 1994. Identification of citrus root-knot nematodes (*Meloidogyne* spp.) in Fujian and their control. China Citrus 23: 9-11.

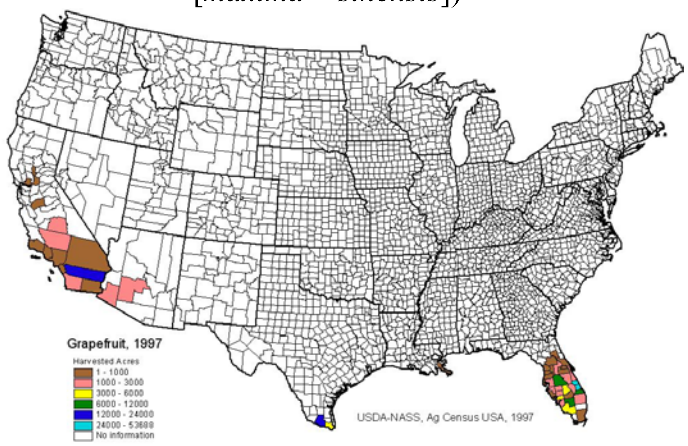
Appendix A. Comparison of climate zones. To determine the potential distribution of a quarantine pest in the US, we first collected information about the worldwide geographic distribution of the species (Table A1). Using a geographic information system (e.g., ArcView 3.2), we then identified which biomes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

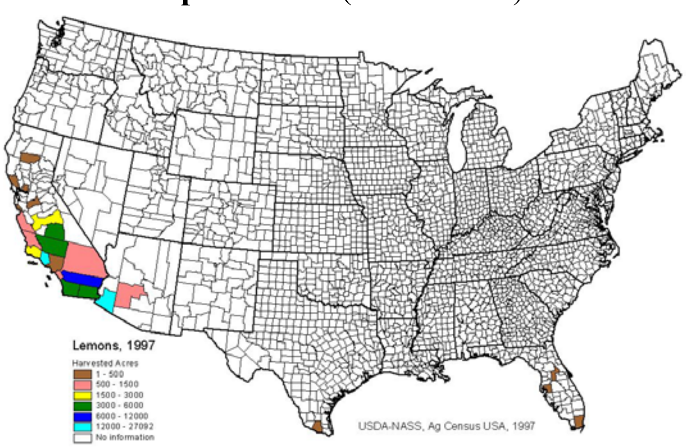
Locations	**Reference(s)**
***Meloidogyne* spp.:**	
M. citri	
China (Shuinan, Shunchang, Xiasha orchard locations in Fujian Province)	(Zhang et al. 1990, Zhang and Weng 1991, Zhang and Xu 1994, Vovlas and Inserra 1996, 2000, Inserra et al. 2003a)
M. donghaiensis	
China (Fujian Province)	(Vovlas and Inserra 1996, 2000, Inserra et al. 2003b)
M. fujianensis	
China (Fujian Province: Nanjing County)	(Pan 1985, Pan et al. 1988, Vovlas and Inserra 1996, Pan and Lin 1998, Pan et al. 1999, Inserra et al. 2003c)
M. indica	
China (Fujian Province: Wuping)	(Zhang and Weng 1991)
India (Delhi)	(Whitehead 1968, Vovlas and Inserra 1996, 2000, Inserra et al. 2003d)
M. jianyangensis	
China (Sichuan Province: Jianyang County)	(Yang et al. 1990, Hu et al. 1991, Vovlas and Inserra 1996, 2000, Inserra et al. 2003e)
M. kongi	
China (Guangxi Province)	(Vovlas and Inserra 1996, 2000, Inserra et al. 2003f)
M. mingnanica	
China (Fujian Province)	(Zhang and Xu 1994, Vovlas and Inserra 1996, 2000, Inserra et al. 2003g)

Appendix B. Commercial production of hosts and potential hosts of Asian Citrus Root-knot Nematodes, *Meloidogyne* spp., in the continental US (see 'Host Specificity' section for crops categorized by nematode).

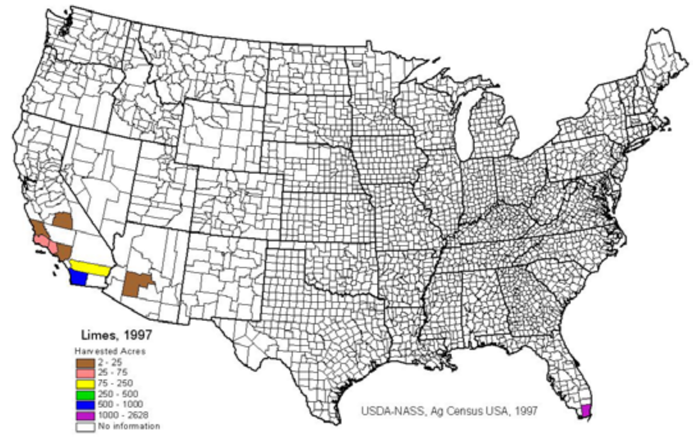
Map 1. Grapefruit (*Citrus* ×*paradisi* (pro sp.) [*maxima* × *sinensis*])



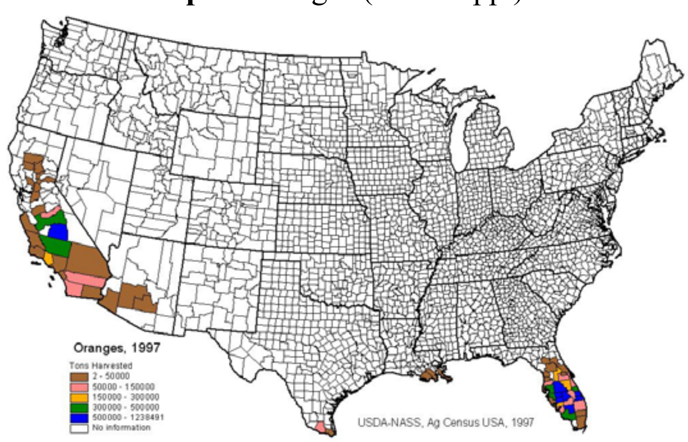
Map 2. Lemon (*Citrus limon*)



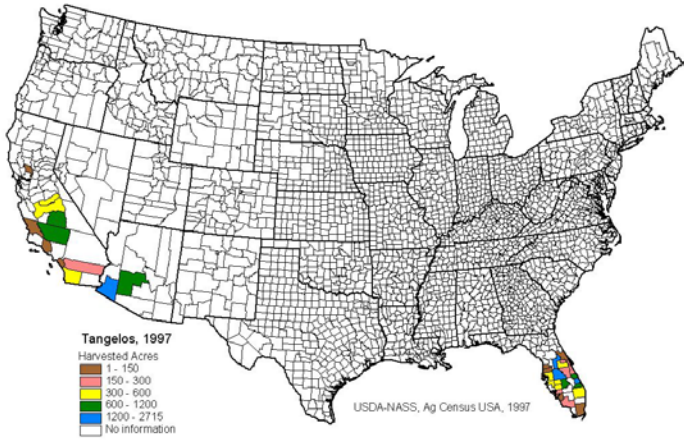
Map 3. Limes (*Citrus aurantifolia*)



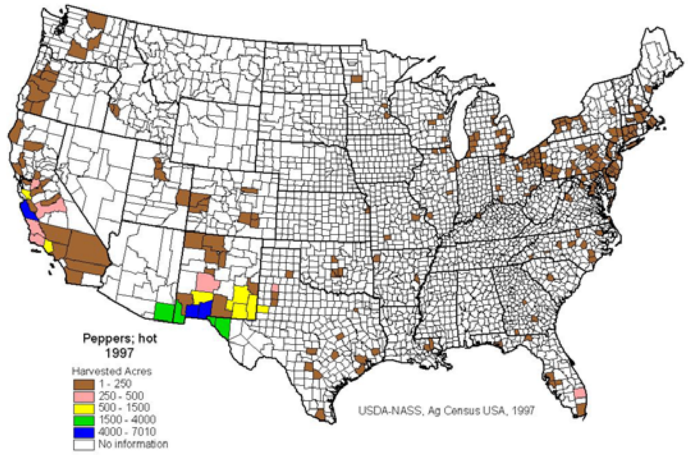
Map 4. Oranges (*Citrus* spp.)



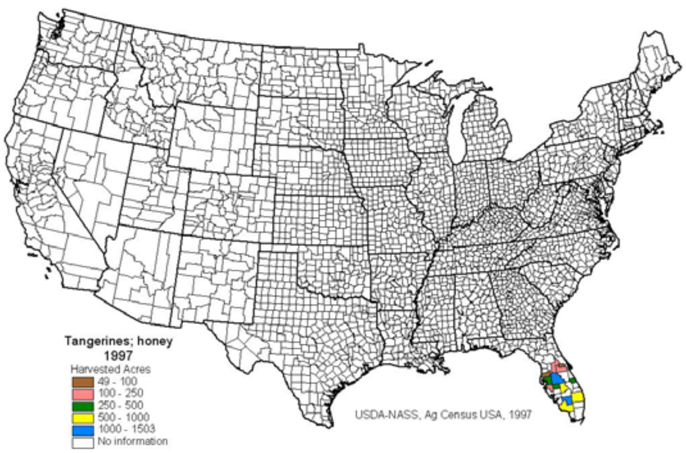
Map 5. Tangelos (*Citrus* ×*tangelo* [*paradisi* × *reticulata*])



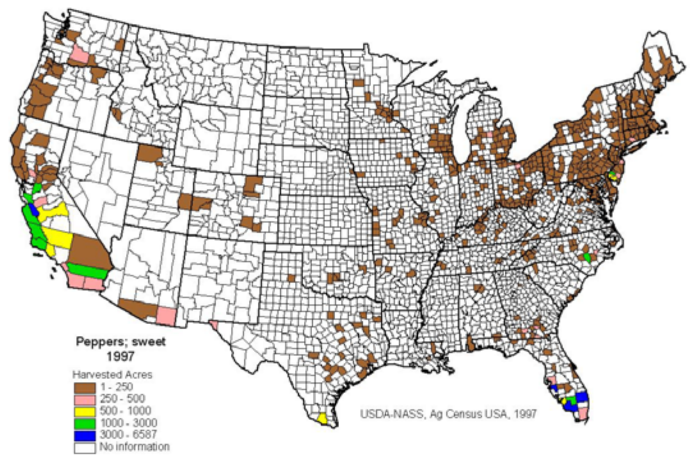
Map 8. Peppers, hot (*Capsicum* spp.)



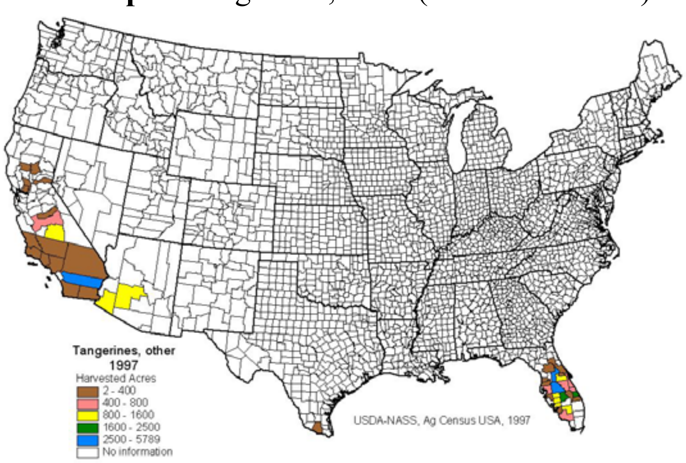
Map 6. Tangerines, honey (*Citrus reticulata*)



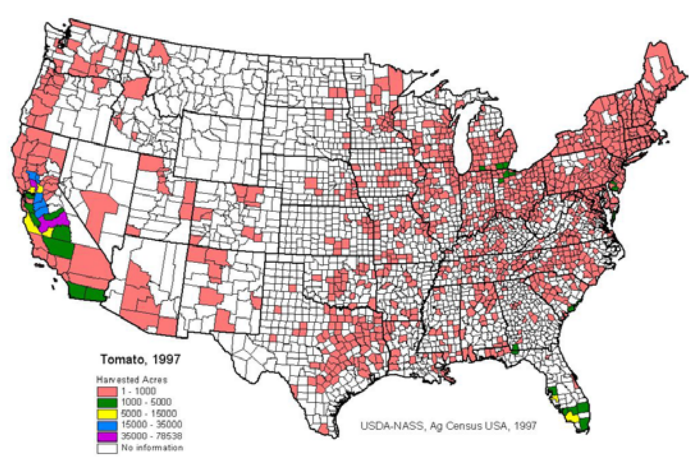
Map 9. Peppers, sweet (*Capsicum annuum*)



Map 7. Tangerines, other (*Citrus reticulata*)



Map 10. Tomato (*Solanum lycopersicum*)



Appendix C. Taxonomy and Morphology of Asian Citrus Root-knot Nematodes,
<u>*Meloidogyne* spp.:</u>
***M. citri* Zhang, Gao, & Weng;**
***M. donghaiensis* Zheng, Lin, & Zheng;**
***M. fujianensis* Pan;**
***M. indica* Whitehead;**
***M. jianyangensis* Yang, Hu, Chen, & Zhu;**
***M. kongi* Yang, Wang, & Feng;** and
***M. mingnanica* Zhang**

Taxonomy and Morphology of *Meloidogyne citri*
Meloidogyne citri Zhang, Gao, and Weng, 1990

The following description of *M. citri* is quoted from Zhang et al. (1990). Specimens (number not specified in translated abstract) were obtained from roots of *Citrus unshiu*.

Female
Body white and globular to pear-shaped with slightly posterior protuberance. The stylet cone exhibits dorsal-curvature; labial disc is "X"-shaped and elevated; perineal pattern is rounded. The dorsal arch is low and flat; inner striae are cheek-shaped in the ventro-lateral areas; inner cuticle is raised.

Male
Labial disc is rounded and slightly elevated; stylet length is 25 µm; stylet knobs are transversely ovoid and set off from shaft; spicule length is 38.6 µm. There are four incisures and areolae in the lateral field; head region is annulated.

Second-stage Juvenile
Mean body lengthis 465 µm; stylet length 11.5 µm; tail length 46.5 µm; and hyaline tail terminus length 16.1 µm.

Diagnosis
Meloidogyne citri n. sp. is distinct from *M. fujianensis* which perineal pattern has a small swell outside the right edge of the vulva.

The following description of *M. citri* is quoted from Inserra et al. (Inserra et al. 2003a).

Female
Female *M. citri* have an oval cuticular perineal pattern marked by coarse and fine striae. Dorsal arch is low or moderately high. Lateral lines indistinct.

Taxonomy and Morphology of *Meloidogyne donghaiensis*

Meloidogyne donghaiensis Zheng, Lin, and Zheng, 1990

The following description of *M. donghaiensis* is quoted from Inserra et al. (Inserra et al. 2003b).

Female *M. donghaiensis* have a rounded cuticular perineal pattern marked by coarse and fine striae. Dorsal arch is low or moderately high. Lateral lines are indistinct. [originally described by Zheng et al., 1990; paper not accessible].

Taxonomy and Morphology of *Meloidogyne fujianensis*

Meloidogyne fujianensis Pan, 1985

The following description of *M. fujianensis* is quoted from Pan et al. (1985, 1988) from specimens obtained from *Citrus reticulata* located in Nanjing County, Fujian, China. Pan et al. (1985, 1988) also provide a comparison of morphology and a key to distinguish between *M. fujianensis* and *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*.

Female (Fig. C1(A))
Female stylet 11.4-12.6 µm long; dorsal gland opening 3.6-5.4 µm from stylet base. Excretory pore situated 2-4 stylet lengths behind the head apex. Distance from anus to an imaginary line joining the phasmids is 7-8.4 µm. Posterior cuticular pattern oval in shape. Arch moderate in height. A small swelling with a central pit outside the right edge of the vulva. Labial disc separated from the medial lips by a ditch. Labial disc X-shaped in face view. Medial lips wider than labial disc. Labial disc slightly raised above lips. Labial lips large, separated from medial lips and head region, but fused with ventral lips. Head region often marked with an incomplete annulation.

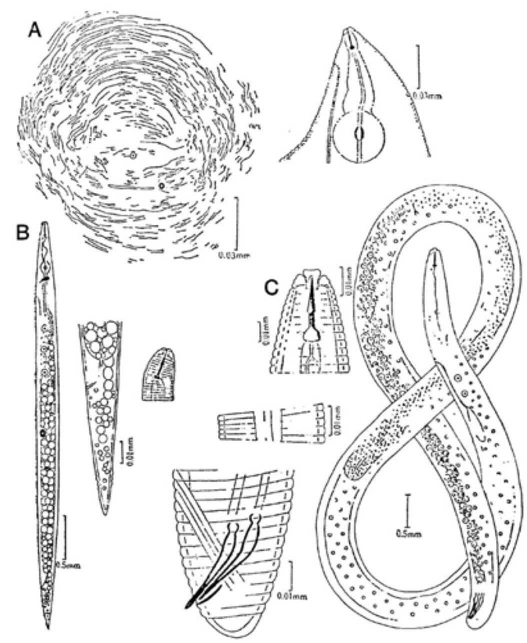


Figure C1. *Meloidogyne fujianensis*.
A. Female: perineal pattern and anterior.
B. Juvenile: full body, posterior and anterior. **C.** Male: anterior (ventral), lateral field, posterior and full body [Quoted from (Pan 1985) and reproduced from (Taylor 1987)].

Male (Fig. C1(C))
Dorsal gland duct opening 5.4-7.2 µm behind stylet base. Lateral field consisted of 3 unareolated bands (4 incisures). Labial disc and medial lips fused, both at contour line. Head cap low and as wide as head region. Head region not annulated.

Second-stage Juvenile (Fig. C1(B))
Measurements:
Length 348-464 µm
a= 20-26
c=7.8-10.1
[DeMan's indices (modified) from Jones (1965):
a=length/greater diameter; c=length/length of tail (anus to tip)]
Stylet length 13-14 µm.
Hemizonid situated at same level of the excretory pore. Rectum undilated. Medial lips and labial disc dumbbell-shaped in face view. Labial disc rounded, raised slightly above medial lips. Labial lips and head region at contour line. Head region generally smooth, occasionally with one short annulation.

Diagnosis
The most important distinguishing feature of *M. fujianensis* n. sp. is that the female has a small swelling with a central pit outside the edge of the vulva. The observation on scanning electron microscope further indicates that there are important differences in morphology between *M. fujianensis* and the four most common species root-knot nematodes (*M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*). This species also differs from *M. enterolobii* Yang (1983) and *M. pinj* Eisenback (1985). Therefore the new species is valid.

Taxonomy and Morphology of *Meloidogyne indica*
Meloidogyne indica Whitehead, 1968

The following description (excerpts) of *M. indica* is quoted from Whitehead (1968). Specimens were obtained from roots of *Citrus aurantifolia*.

Systematics
Meloidogyne indica sp. n. (Fig. C2)

Female (Fig. C2(A))
Measurements of 3-8 females are listed (partial list) in Table C1 below. Body saccate with fairly thick cuticle, neck short; head end-on divided into six sectors, laterals only slightly larger than subdorsals and subventrals, laterals characteristically subdivided and subdorsals and subventrals each with a 'papilla'; head with one annule behind head-cap; excretory pore (5) about 20 annules (16-22) behind head; anterior margins of spear knobs either concave or backward sloping; posterior cuticular pattern faint, composed of closely spaced, usually smooth, striae forming a distinct tail whorl with low, rounded dorsal arch, phasmids not as close to tail terminus as in *M. africana* and lateral field usually absent; posterior cuticular pattern resembles that of *M. decalineata*, *M. coffeicola* and to a lesser extent *M africana*.

Table C1 **Measurements of 3-8 *M. indica* females:**		
Character	**Range** (µm)	**Mean** (µm)
Stylet	12-16	14
Width stylet base	4-5	5
DGO to stylet base	2-4	3
Length median bulb	31-43	38
Width median bulb	33-46	39
Length median bulb valves	13-16	14
Width median bulb valves	7-11	9

Male ((Fig. C2(B))
[No measurements given; only 3 specimens]
Head in lateral view hemispherical or truncate-cone shape, not offset, with two annules behind head-cap, anterior annule longer than posterior, which appeared to be 'tilted' in incident illumination; basal plate fairly thick; anterior part of stylet longer than shaft, stylet knobs small and rounded triangular shape, anterior margins well swept back; posterior cephalid about seven annules behind head; oesophagus overlaps intestine ventrally; excretory pore near anterior end of posterior oesophageal region and two to five annules behind hemizonid; hemizonid one annule long; one testis; lateral field with four incisures for greater length of body, outer bands fully areolated, inner band occasionally cross-striated towards posterior end of body, where lateralfield fully areolated; tail rather long and apparently concave ventrally; striae pass around terminus; phasmids opposite cloaca.

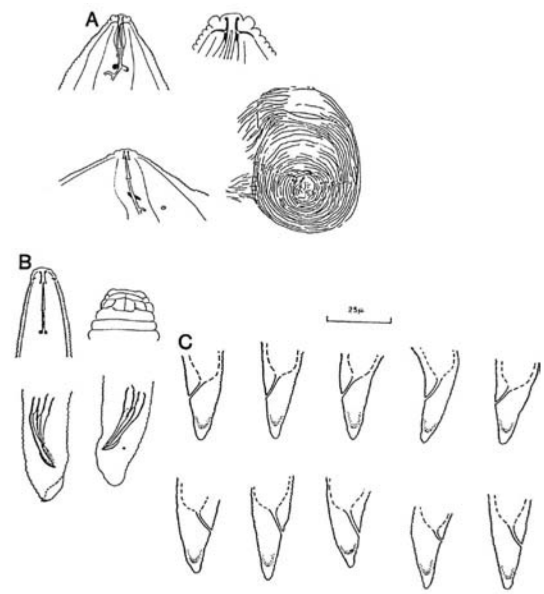


Figure C2. *Meloidogyne indica*.
A. Female: stylet, lip region, anterior and perineal pattern. **B.** Male: stylet, anterior and two posterior. **C.** Juvenile tails [Quoted from (Whitehead 1968) and reproduced from (Taylor 1987)].

Larva (Fig. C2(C))
Measurements of 20-25 juveniles are listed (partial list) in Table C2 below. Head not offset, low truncate-cone shape, in lateral view of head head-cap outline slightly posterior to general contour of head, two, sometimes three annules behind head-cap; head annules appear marked by longitudinal striae; distal sclerotisation of head thicker than proximal; cephalids not seen; stylet knobs fairly prominent rounded, anterior margins often backsloped; rectum not inflated; tail conoid, terminus usually blunt, unstriated.

Table C2 **Measurements of 30 *M. indica* larvae:**		
Character	**Range** (μm)	**Mean± Std. error (SE)** (μm)
body length (L)	381-448	415±4.5
stylet	10-14	12±0.9
tail length	13-20	16.8±1.88
body length:tail length ratio	21.2-31.0	24.9±1.36
tail length:body width (seen laterally at level of anus)	1.06-1.78	1.57±0.012

Egg
Measurements of 20 fixed eggs are listed in Table C3 below. Eggs morphologically similar to that of other *Meloidogyne* spp.

Table C3 **Measurements of 20 *M. indica* (unembryonated) eggs:**		
Character	**Range** (μm)	**Mean± SE** (μm)
length (L)	71-88	77 ±4.7
width (W)	26-35	30±1.9
L/W ratio	--	2.6

Diagnosis
Meloidogyne with above measurements and description. Diagnosed by the wide stylet base, the saccate body and the posterior cuticular pattern of the female, thelow rounded head with two annules behind the head-cap on the sublateral head sectors, the anterior longer than the posterior, the position of the cephalids, the well swept back stylet knobs and the fairly long ventrally concave tail of the male and the short bluntly rounded conoid tail of the larva.

Taxonomy and Morphology of *Meloidogyne jianyangensis*

Meloidogyne jianyangensis Yang, Hu, Chen, and Zhu, 1990

The following description of *M. jianyangensis* is quoted from Yang et al. (1990) and Inserra et al. (2003e). Specimens were obtained from roots of *Citrus* in Jianyang County, Sichuan, China.

Female
Usually rounded cuticular perineal pattern; striae are smooth, fine and continuous. Some spine-like striae stretch radially from tail terminus to lateral regions and are variable in number. Sometimes the striae among the radial striae are broken like punctuations. Two phasmids are quite near.

Male
Head cap is high. Labial disc fused with medial lips, lateral lip is absent. Stylet length is 19.1-26.0 (mean 21.8) μm; stylet knobs distinctly set off from the shaft. Distance from the dorsal esophageal gland orifice to stylet base is 2.5-3.5 (mean 3.0) μm. Four lateral lines present in lateral field, so the field is divided into three regions. Two regions in both sides of the lateral field are areolated while the region in the center is usually not.

Second-stage Juvenile
Second-stage juveniles total lengths are 387.6-483.3 (mean 423.2) µm. The head cap is high, labial disc fused with medial lips, like a dumbbell. The stylet length is 13.0-16.8 (mean 15.1)µm. Distance from dorsal esophageal gland orifice to stylet base is 2.1-3.7 (mean 2.5) µm. Tail tapers gradually with a bluntly rounded tip.

Diagnosis
Biochemically, this new species has three major bands of esterase activity at Rf= .41, .45, and .48.

Taxonomy and Morphology of *Meloidogyne kongi*

Meloidogyne kongi Yang, Wang, and Feng, 1988

The following description of *M. kongi* is quoted from Inserra et al. (2003f). Specimens were obtained from roots of *Citrus*.

Female *M. kongi* have an ovoid cuticular perineal pattern marked by fine striae. Dorsal arch is low or moderately high. Lateral lines are indistinct [originally described by Yang et al., 1988; paper not accessible].

Taxonomy and Morphology of *Meloidogyne mingnanica*

Meloidogyne mingnanica Zhang, 1993

The following description of *M. mingnanica* is quoted from Inserra et al. (2003g). Specimens were obtained from roots of *Citrus*.

M. mingnanica females have an ovoid cuticular perineal pattern with a very distinct inner area, which contains vulva and anus. This area is marked by a few coarse striae in an eight-shaped figure with a large base and a small top marked by fine striae. Dorsal arch is low or moderately high. Lateral lines are indistinct [originally described by Zhang, 1993; paper not accessible].

Appendix D. Biology of Asian Citrus Root-knot Nematodes: *Meloidogyne* spp.:

M. citri Zhang, Gao, & Weng;
M. donghaiensis Zheng, Lin, & Zheng;
M. fujianensis Pan;
M. indica Whitehead;
M. jianyangensis Yang, Hu, Chen, & Zhu;
M. kongi Yang, Wang, & Feng; and
M. mingnanica Zhang

Population phenology

Collectively, little is known about the biology of Asian citrus root-knot nematodes. Here, *M. fujianensis* is best described. Pan et al. (1999) have investigated the phenology and biology of *M. fujianensis* on *Citrus reticulata* in Nanjing County (Fujian Province), China. *Meloidogyne fujianensis* is active year round in this region, with peak infection occurring between September-October and then again in the following season between March-April (Pan et al. 1999). During these periods, juveniles of various stages may be found in the soil. The life span of *M. fujianensis* lasts 55-60 days at 25°C, with 30-35 days spent from root infection by second stage juveniles to egg production by mature females. In a separate, differential host study by Vovlas and Inserra (2000), *M. citri* egg masses were initially found on satsuma, sour orange and tomato 28-35 days following root penetration.

Stage specific biology

Development time varies depending on temperature, host availability and other biotic and abiotic factors. Population density appears to be correlated to increasing air temperature and peaks have been observed during periods of active *Citrus* root growth in the spring and fall (Pan et al. 1999).

Adult

According to Pan et al. (1999) two months after a reported minimum of 308.4 degree days (°C), a population density peak of 117.6 females/2g roots occurred, compared to 94.3 females/2g roots observed in December, two months following a maximum of 891.3 degree days. Females swell, producing large gelatinous egg masses or sacs, containing very few eggs compared to other *Meloidogyne*. In December and January during peak oviposition, 87.2 egg-sacs/2g roots and 150.2 egg-sacs/2 g of roots were reported, respectively. Only 5 of the 10 egg-sacs examined contained eggs; relatively few eggs were observed (1.2 eggs/female) considering the size of the egg sac matrix is 3-5 times the size of the female (Pan et al. 1999). The egg mass also serves as a protective padding. The egg sac is deposited on either galled root surfaces or inside root galls (Hussey 1985). *M. fujianensis* primarily reproduces asexually, though some sexual reproduction must occur because males are present in the population. More males are observed during periods of drought stress, while more females are observed during more favorable conditions when food supply is abundant (Pan et al. 1999).

Egg
Egg hatch may or may not involve stimulation from the host root (Hussey 1985). Hatching can occur for an extended period depending on temperature. *Meloidogyne* eggs will not hatch under extended dry periods and may persist in soil or dry roots awaiting more favorable moist soil conditions.

Larva
For *Meloidogyne* in general, emergence occurs under moist soil conditions; juveniles may become inactive under dry conditions. *Meloidogyne* larvae 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). 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, after egg hatch, 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 pentrate 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 cellular changes in the primary phloem or parenchyma, changing them into large, nutrient-rich cells from which juveniles feed until development is completed (Hussey 1985). If large, specialized cell (gall) formation does not happen as a result of host infection, the larva may not complete its development and leave in search of another root, or die of starvation in the process. 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). The larva will swell as it feeds until development is completed. Total development time varies depending largely on temperature.