Mini Risk Assessment
Coffee Root-knot Nematode: *Meloidogyne paranaensis* Carneiro, Carneiro, Abrantes, Santos, & Almeida
[Nematoda: Meloidogynidae]

Erica E. Davis & Robert C. Venette
Department of Entomology, University of Minnesota
St. Paul, MN 55108
September 29, 2004

Introduction

Meloidogyne paranaensis is one of several significant *Meloidogyne* species that parasitize coffee in Brazil (Carneiro et al. 1996). This nematode was recovered from Paraná State, Brazil for which it is named. The recommended common name for the nematode is Paraná coffee root-knot nematode (Carneiro et al. 1996). Outside of Brazil, this species has also been reported from Guatemala.

Meloidogyne paranaensis is not known to occur in the United States (Inserra et al. 2003), though it has been shown to reproduce on experimental hosts of economic importance that are grown in the US. Consequently, *M. paranaensis* has some chance of becoming established in the United States if accidentally or intentionally introduced. In a previous assessment, the risks associated with *M. paranaensis* were judged to be moderate relative to the risks posed by other exotic plant parasitic nematodes (Inserra et al. 2003). The current document evaluates several factors that influence the degree of risk posed by *M. paranaensis* and applies this information to the refinement of sampling and detection programs.

Figure 1. Symptoms caused by *Meloidogyne paranaensis* on (A) foliage and (B) roots of coffee (*Coffea arrabica*). [Image and legend reproduced from Castro et al (2003).]

1. **Ecological Suitability**. **Rating: Low.** *Meloidogyne paranaensis* is known to occur in tropical coffee growing regions of Central and South America. The known distribution of the nematode is limited to Guatemala and three states in Brazil. Appendix A provides a detailed description of the reported worldwide distribution of this nematode. This distribution suggests that the pest may be most closely associated with biomes characterized as: mangrove; tropical and subtropical coniferous forest; tropical and subtropical dry broadleaf forest; and

tropical and subtropical moist broadleaf forest. Mangroves and tropical and subtropical dry broadleaf forests do not occur in the US. Nevertheless, we estimate that <2% of the continental US could provide a suitable climate for *M. paranaensis* (Fig. 2). See Appendix A for a more complete description of this analysis.

Figure 2. Predicted distribution (shaded red) of *Meloidogyne paranaensis* in the continental US.

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

2. **Host Specificity/Availability. Rating: Low/Moderate.** Table 1 lists host plants reported for *Meloidogyne paranaensis*. The primary and only known host of *M. paranaensis* from the field is coffee, though this nematode has been shown to feed and reproduce on a few other experimental hosts.

Table 1. Host plants of *Meloidogyne paranaensis*:

Host(s)	**Reference(s)**
coffee (*Coffea arabica*)	(Carneiro et al. 1996, Randig et al. 2002, Anthony et al. 2003, Inserra et al. 2003)
*mate (*Ilex paraguariensis*)	(Santiago et al. 2000)
*tobacco (*Nicotiana tabacum*)	(Carneiro et al. 1996, Inserra et al. 2003)
*tomato (*Solanum lycopersicum* =[*Lycopersicon esculentum*])	(Carneiro et al. 1996, Santiago et al. 2002, Inserra et al. 2003)
*watermelon (*Citrullus lanatus*)	(Carneiro et al. 1996, Inserra et al. 2003)

*=experimental hosts:

- *M. paranaensis* can reproduce and complete its life cycle on *Ilex* sp. roots (Santiago et al. 2000) ;
- In host studies, tobacco, tomato and watermelon were reported as suitable hosts (Carneiro et al. 1996);
- Tomato plants have been used to rear populations of nematodes in a study to evaluate the antagonist effect of *Arachis pintoi* on *M. paranaensis* and *M. incognita* (Santiago et al. 2002).

Coffee is not grown in the continental US. See Appendix B for maps showing where experimental hosts are grown commercially within the country.

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.

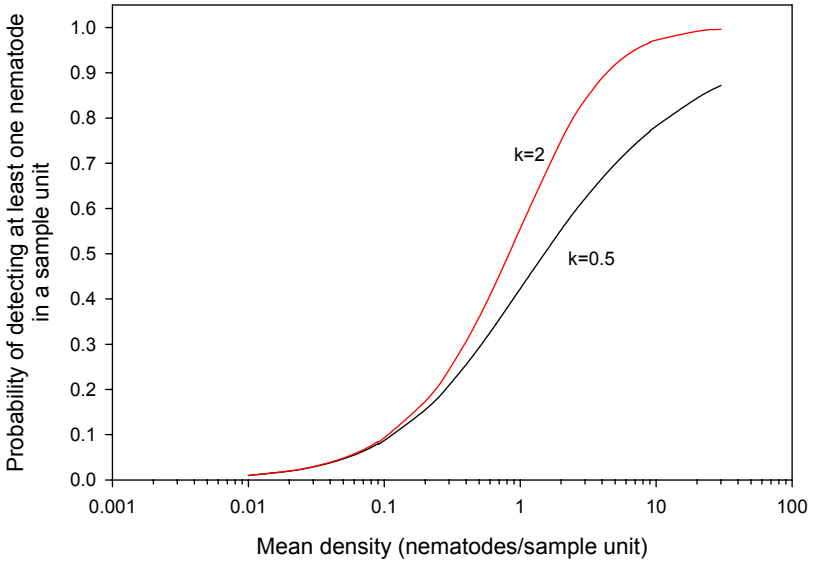


Figure 3. Influence of nematode density and spatial distribution on the likelihood of observing at least one nematode from a soil sample. Lines are based on the negative binomial distribution.

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.

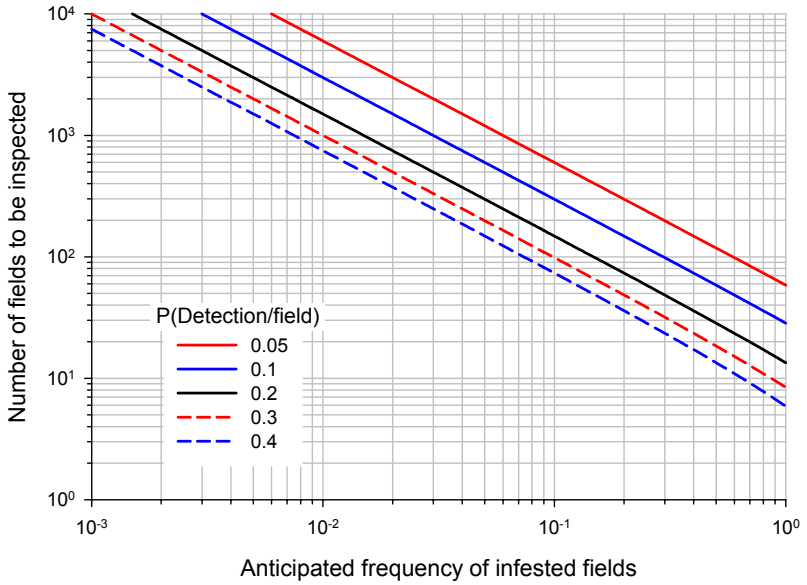


Figure 4. Influence of the frequency of infested fields and the likelihood of detecting an infestation in an individual field on the number of fields that should be inspected to have 95% confidence of detecting at least one exotic nematode within a region.

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.

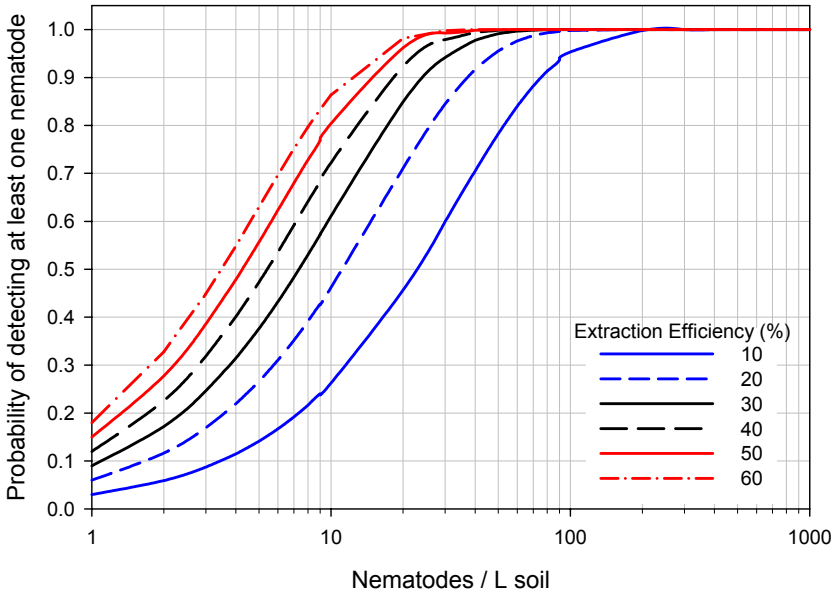


Figure 5. Influence of extraction efficiency and nematode density on the probability of detecting at least one nematode in 300 cc of a well-mixed, 1-liter soil sample.

4. **Taxonomic Recognition**. **Rating: Medium.** *Meloidogyne paranaensis* may occur by itself or in mixed populations with other *Meloidogyne* spp. (Carneiro et al. 1996). *Meloidogyne paranaensis* has been confused with *M. incognita* (which occurs in the continental US (Norton et al. 1984)) and *M. konaensis*. *Meloidogyne paranaensis* may be differentiated most reliably by biochemical methods (Carneiro et al. 1996). Randig et al. (2002) found that *M. exigua, M. incognita and M. paranaensis* could be identified reliably using a polymerase chain reaction (PCR) technique with sequence-characterized amplified region (SCAR) primers. Biological and morphological studies of *M. paranaensis* have been conducted by Carneiro et al. (1996). For a detailed description of the taxonomy and morphology of *M. paranaensis*, 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 paranaensis is most likely to be transported into the United States in infested plant material or infested soil. Approximately 5% of interceptions of "*Meloidogyne* sp." mention soil (USDA 2004). Infested soil may be associated with some commodities, but the greatest volumes are likely to be moved with international transport of equipment and machinery (Greco et al. 2002). As this nematode feeds strictly on roots, plant material is only likely to be infested if roots remain 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. Consequently, a great deal of uncertainty is associated with our rating.

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

7. **Potential Economic Impact. Rating: Low-Medium.** *Meloidogyne paranaensis* is an economically important pest of coffee, causing severe damage in Brazil (Carneiro et al. 1996). Inserra et al. (2003) suggested that this nematode may lower yield potentials by 50%. This estimate is based on information provided by Carniero et al. (1996), but the original authors only suggested that this particular species may "[account] for approximately 52% of all root-knot nematode infestations in Paraná". Carniero et al. (1996) do not comment on the magnitude of damage when these infestations occur. Historically, economic losses to coffee were attributed primarily to *M. incognita*, though *M. paranaensis* is thought to have been prevalent in this region and was likely mistaken for *M. incognita* for over 20 years (Campos et al. 1990, Carneiro et al. 1996, Inserra et al. 2003).

   The economic impacts of *Meloidogyne* spp. are difficult to measure because it is common for multiple members of the genus to occur in the same fields (Jensen 1972). This is true for *M. paranaensis* in particular (Carneiro et al. 1996). As a result, it is possible to ascribe nematode damage within a field to *Meloidogyne* spp. in general but not *M. paranaensis* alone. *Meloidogyne* species are among the most economically important plant parasitic nematodes (Jensen 1972). Crop

losses resulting from nematode damage have been estimated at an average of 10-11% worldwide (Jensen 1972, Potter and Olthof 1993, Whitehead 1998, Nicol 2002), but the economic impact from nematodes is thought to be grossly underestimated.

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

Specific damage caused by *Meloidogyne paranaensis* on roots of coffee typically does not involve gall formation, which is characteristic of many *Meloidogyne*. Instead, *M. paranaensis* causes the tap root of coffee to crack and split, as well as damage to other root tissue. Necrosis also occurs where females are imbedded and near the giant cells where feeding occurs. Above ground symptoms generally range from chlorosis and reduced plant growth to death (Carneiro et al. 1996).

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

8. **Potential Environmental Impact. Rating: Low.** 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 paranaensis* is likely to stimulate the use of nematicides or biological controls to maintain productivity. However, given the small geographic area over which the nematode is likely to become established, the net increase in control measures is likely to be minimal.

Meloidogyne paranaensis has a narrow known host range. The only known field host for this nematode is coffee (Family: Rubiaceae), though host range tests have demonstrated that plants in other families may be susceptible (see 'Host Specificity'). Appendix D summarizes state- and federally-listed threatened or endangered plant species (USDA NRCS 2004) found within plant genera known to be hosts (or potential hosts) for *M. paranaensis.* Currently, only plants within the genus *Ilex* (Family: Aquifoliaceae) have the potential to be adversely affected by the nematode. Of the species listed in Appendix D, only *I. krugiana* occurs in an area with a climate that may also be suitable for *M. paranaensis*. Thus, the chances for adverse effects on threatened or endangered species seem remote.

9. **Establishment Potential. Rating: Low.** Our initial predictions suggest that only a limited area within the continental US has a climate that could support populations of *M. paranaensis*. The primary host for this nematode is coffee, but this host is not grown in the continental US. However, some experimental hosts (esp. tomatoes and watermelons) are widely grown. Because nematodes move greater distances through passive means than active movement (Potter and Olthof 1993), nematodes must be introduced directly into an area that either currently is suitable for reproduction or eventually will be. Based on current interception records, very few *Meloidogyne* species (or materials that are likely to harbor *M. paranaensis*) are intercepted annually. When *Meloidogyne* spp. were intercepted, those conveyances were not destined for Florida or Arizona. Thus, compared to other exotic pests the relative risks of establishment by this species seem low.

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

References:

Anthony, F., P. Topart, C. Astorga, F. Anzueto, and B. Bertrand. 2003. Genetic resistance to *Meloidogyne* spp. in *Coffea* spp.: identification and utilization for Latin American coffee cultivation. Manejo Integrado de Plagas y Agroecologia 67: 5-12.

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.

Campos, V. P., P. Sivapalan, and N. C. Gnanapragasam. 1990. Nematode Parasites of Coffee, Cocoa and Tea. *In* M. Luc, R. Sikora and J. Bridge [eds.], Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. CAB International Institute of Parasitology, Wallingford, UK.

Carneiro, R. M. D. G., R. G. Carneiro, I. M. O. Abrantes, M. S. N. A. Santos, and M. R. A. Almeida. 1996. *Meloidogyne paranaensis* n. sp. (Nemata:

Meloidogynidae), a root-knot nematode parasitizing coffee in Brazil. Journal of Nematology 28: 177-189.

Castro, J. M. C., V. P. Campos, and R. L. Naves. 2003. Ocorrência de *Meloidogyne paranaensis* em cafeeiros na região do Alto Paranaíba em Minas Gerais. Fitopatologia Brasileira 28: 565.

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.

Greco, N., N. Vovlas, M. DiVito, and R. N. Inserra. 1992. *Meloidogyne artiellia*: a root-knot nematode parasite of cereals and other field crops. Nematology Circular No. 201, pp. 4 pp. Florida Department of Agriculture & Consumer Services, Division of Plant Industry, Gainsville, FL.

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.

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. 2003. Exotic nematode plant pests of agricultural and environmental significance to the United States: *Meloidogyne paranaensis*. 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.

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

Nicol, J. M. 2002. Important nematode pests., pp. 345-366. *In* Anon. [ed.], *Bread Wheat*: improvement and production. FAO, Rome, Italy.

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.

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.

Randig, O., M. Bongiovanni, R. M. D. G. Carneiro, and P. Castagnone-Sereno. 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. Genome 45: 862-870.

Santiago, D. C., A. A. Krzyzanowski, and M. Homechin. 2000. Behavior of *Ilex paraguariensis* St. Hilaire, 1822 to *Meloidogyne incognita* and *M. paranaensis* and their influence on development of plantlets. Brazilian Archives of Biology and Technology 43: 139-142.

Santiago, D. C., M. Homechin, A. A. Krzyzanowski, I. C. d. B. Fonseca, and S. de Carvalho. 2002. Antagonist effect of *Arachis pintoi* on *Meloidogyne paranaensis* and *M. incognita*. Nematologia Mediterranea 30: 147-152.

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.

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.

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

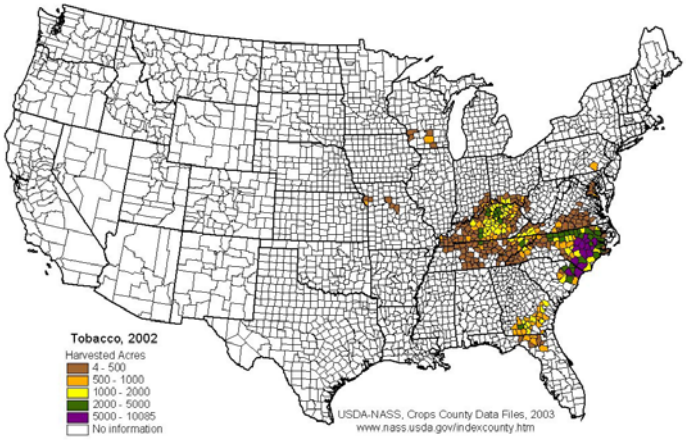
Appendix A. Comparison of climate zones. To determine the potential distribution of a quarantine pest in the US, we first collected information about the worldwide geographic distribution of the species (Table A1). Using a geographic information system (e.g., ArcView 3.2), we then identified which biomes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

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

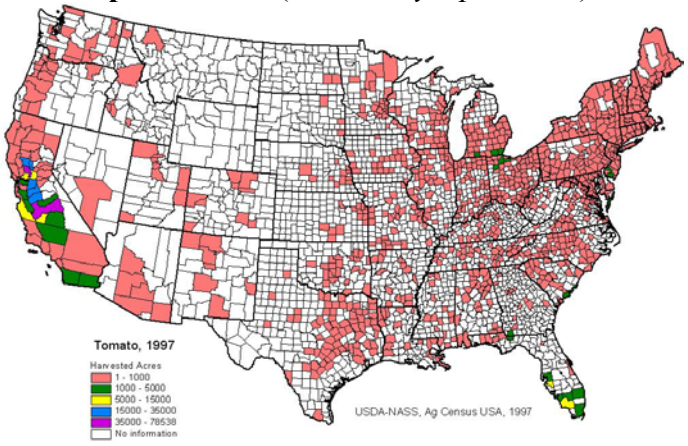
Locations	**Reference(s)**
Brazil (Southern: States of Minas Gerais, Paraná and São Paulo)	(Carneiro et al. 1996, Randig et al. 2002, Castro et al. 2003, Inserra et al. 2003)
Guatemala (Pacific Coast)	(Anthony et al. 2003)

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

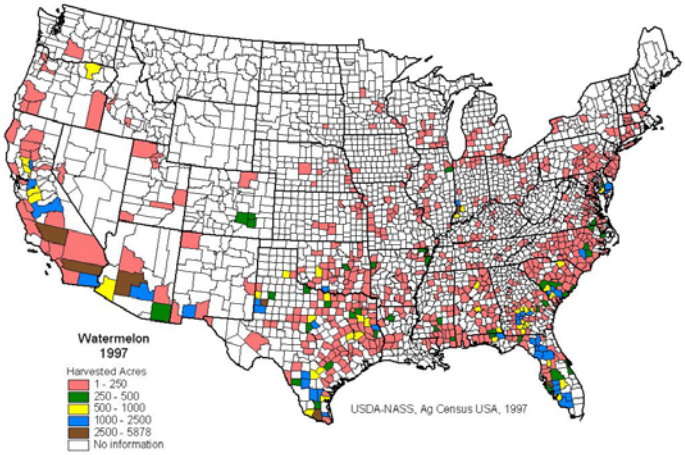
Map 1. Tobacco (*Nicotiana tabacum*)



Map 2. Tomato (*Solanum lycopersicum*)



Map 3. Watermelon (*Citrullus lanatus*)



Appendix C. Taxonomy and Morphology of *Meloidogyne paranaensis*

Meloidogyne paranaensis Carneiro, Carneiro, Abrantes, Santos, & Almeida, 1996

The following excerpts are quoted from the original description of *M. paranaensis* by Carneiro et al. (1996).

Systematics

Meloidogyne paranaensis n. sp. (Figs. C1-C2)

Female

Measurements of 30 females (in formalin) are listed (partial list) in Table C1 below. Body translucent-white, variable in size, elongate, ovoid to pear-shaped. Neck sometimes prominent, cuticular annulation on body finer than that on neck. Body posteriorly rounded, without tail protuberance. Head region not set off from body, not annulated, stoma slit-like, located in ovoid prestomatal cavity, central on labial disc. Pore-like openings of six inner labial sensilla surrounding prestoma. Labial disc and medial lips fused, asymmetric and rectangular, forming two straight lateral edges in face view. Lateral lips small, triangular, fused laterally with head region. Amphidial openings elongated slits between labial disc and lateral lips. In LM, cephalic framework weakly sclerotized, lateral sectors slightly enlarged, vestibule extension distinct (Fig. C1(A, B)). Anterior half of stylet cone pointed and slightly curved dorsally, posterior half conical. Shaft cylindrical, widening slightly near junction with knobs. Three large knobs tapering onto shaft (Fig. C1(C)). Distance of stylet base dorsal esophageal gland orifice (DGO) 4.2-5.5 µm. Esophagus with large, rounded metacorpus, valve plates large (Fig. C1(A)). Esophageal gland with one large dorsal lobe with one nucleus; two small nucleated subventral gland lobes, variable in shape, position, and size, usually posterior to dorsal gland lobe. Two large esophago-intestinal cells near junction of metacorpus and intestine. Excretory pore at level of anterior metacorpus (Fig. C1(A)). Perineal patterns variable, typically rectangular to oval shaped, dorsal arch generally high, squarish, dorsal striae varying from fine to coarse, smooth to wavy. Lateral lines mostly discontinuous, without distinct incisures, sometimes appearing as a discontinuous linear depression faintly marked by breaks and forks. All variants with a triangular postanal whorl. Phasmids distinct (Fig. C1(D-F)).

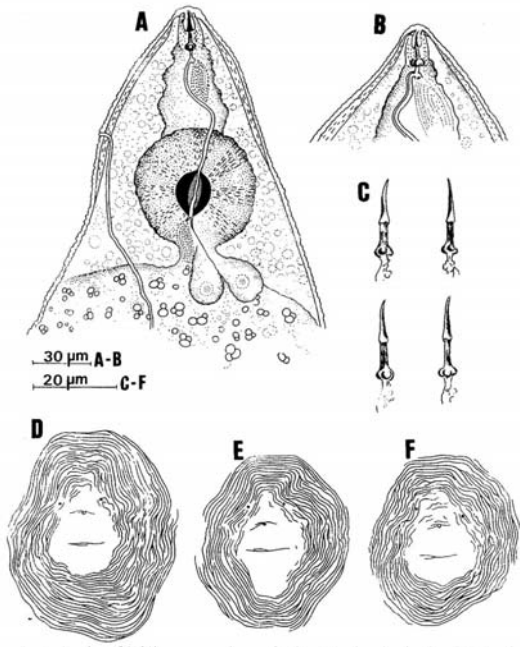


Figure C1. Drawings of *Meloidogyne paranaensis* n. sp. females. **A)** Esophageal region, lateral. **B)** Anterior region, lateral view. **C)** Stylets. **D-F)** Perineal patterns [Quoted and reproduced from (Carneiro et al. 1996)].

Table C1 **Measurements of 30 *M. paranaensis* females:**				
Character	**Range** (µm)	**Mean± Std. errors (SE)** (µm)	**Standard deviation (SD)**	**Coefficient of variability (CV)**
body length (L)	512-780	681±12.5	66.5	9.8
body width (W)	320-532	428±11.7	61.8	9.8
Stylet length	15.0-17.5	16.1±0.1	0.6	3.9
Anus to vulva (center) distance	15-25	20.8±0.5	2.6	12.3
body L/W ratio	1.1-2.1	1.6±0.10.01	0.2	12.7

Male

Measurements of 30 males (in formalin) are listed (partial list) in Table C2 below. Body vermiform, length variable, body tapering anteriorly, bluntly rounded posteriorly, tail arcuate, twisting through 90º. Head cap high, rounded, continuous with body contour. In LM, cephalic framework strongly developed, vestibule and extension distinct (Fig. C2(A-C)). Stylet robust, large, cone straight, pointed, gradually increasing in diameter posteriorly, stylet opening marked by slight protuberance several micrometers from stylet tip, shaft cylindrical, sometimes with one or two large projections, knobs large, rounded, set off from shaft. Distance from stylet base to DGO 3.5-5.0 µm. Procorpus distinct, median bulb ovoid, sometimes covered by intestinal caecum extending anteriorly. Esophago-intestinal junction at level of nerve ring, indistinct (Fig. C2(A)). Lateral lips absent. Head region usually marked by a short, incomplete annulation in lateral view. Stoma opening slit-like, located in ovoid prestomatal cavity, surrounded by pit-like openings of six inner labial sensilla. Four cephalic sensilla marked by distinct cuticular depressions on medial lips. Amphidial apertures elongate slits between labial disc and lateral sectors of head region. Hemizonid distinct, three or four annules anterior to excretory pore (Fig. C2(A)). Body annules large, distinct. Areolated lateral field beginning near level of stylet base, usually with four incisures. Most males sex reversed with two testes, some normal with one testis. Testis(es) outstretched or distally reflexed. Spicules arcuate, gubernaculum distinct. Tail short, phasmids at level of cloaca (Fig. C2(D)).

Table C2 **Measurements of 30 *M. paranaensis* males:**				
Character	**Range** (µm)	**Mean± Std. errors (SE)** (µm)	**Standard deviation (SD)**	**Coefficient of variability (CV)**
body length (L)	983-2284	1868±52.8	284.7	15.2
greatest body width (W)	31-46	40.3±0.7	3.6	8.8
stylet length	20-27	24.7±0.6	1.25	5.0
stylet knob width	4.5-7.0	5.8±0.06	0.32	5.5
stylet knob height	2.0-4.5	3.8±0.06	0.33	8.7
spicule length	22-35	26±0.5	2.9	8.2
body L/W ratio[a]	23.4-53.5	46.4±1.2	6.4	13.9
body length/tail length[c]	58-154	116±4.4	23.9	20.7

Second-stage juvenile

Measurements of 30 second-stage juveniles (in formalin) are listed (partial list) in Table C3 below. Body vermiform, tapering more posteriorly than anteriorly, tail region distinctly narrowing. Body annules distinct, increasing in size and becoming irregular in posterior tail region. Lateral field with four incisures. In LM, cephalic framework weak, hexaradiate. Vestibule and vestibule extension distinct (Fig. C2(F)). In SEM, stoma slit-like, located in oval prestomatal cavity, surrounded by pit-like openings of six inner labial sensilla. Labial disc and medial lips fused, forming a dumbbell-shaped structure. Labial disc rounded, slightly elevated above medial lips. Lateral lip sectors distinct, sometimes fused with head region and labial disc at right angle. Head region smooth, frequently with short broken annulations. Amphid openings slit-like, located between labial disc and lateral lips, often covered by exudate. Stylet 13-14 µm long, delicate. Stylet cone increasing in width gradually, shaft cylindrical, knobs rounded and set off from shaft (Figs. C2(F)). Distance of DGO to stylet base 4.0-4.5 µm, orifice branched into channels. Median bulb oval. Esophago-intestinal junction obscure. Gland lobe overlapping intestine ventrally, with three nuclei; hemizonid 1-2 annules anterior to excretory pore (Fig. C2(F)). Tail usually conoid with rounded terminus. Hyaline tail terminus distinct. Rectal dilatation large (Fig. C2(G, H)). Phasmids small, posterior to anus.

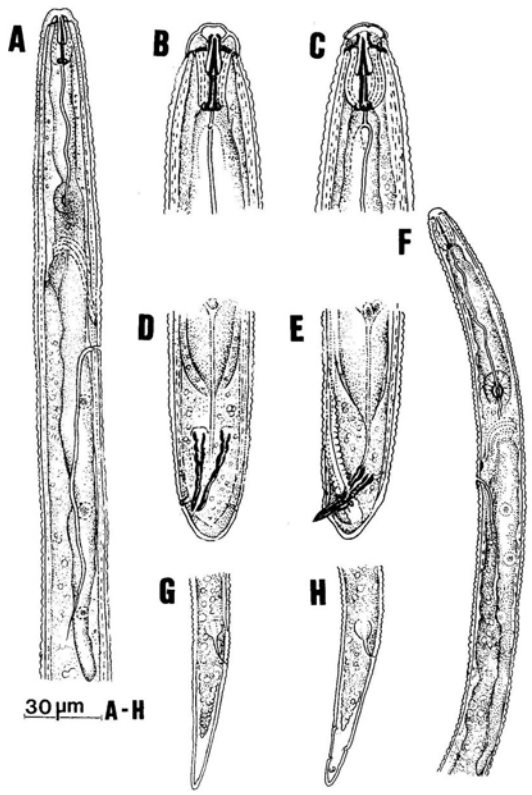


Figure C2. Drawings of males and second-stage juveniles of *Meloidogyne paranaensis* n. sp. **A)** Anterior portion of male. **B)** Male cephalic region, dorsal view. **C)** Male cephalic region, lateral view. **D)** Male tail, ventral view. E**)** Male tail, lateral view. **F)** Anterior portion of second-stage juvenile, lateral view. **G, H)** Second-stage juvenile tail, lateral view [Quoted and reproduced from (Carneiro et al. 1996)]

Table C3 **Measurements of 30 *M. paranaensis* second-stage juveniles:**

Character	**Range** (µm)	**Mean± Std. errors (SE)** (µm)	**Standard deviation (SD)**	**Coefficient of variability (CV)**
body length (L)	389-513	458±5.1	27.9	6.1
greatest body width (W)	15-20	15.9±0.2	1.1	6.6
stylet length	13-14	13.5±0.2	0.9	5.3
tail length	48-51	49.0±0.15	0.8	1.7
tail terminus length	9-10	10.1±0.1	0.7	7.3

Egg
Measurements of 30 eggs (in 2% formalin) are listed in Table C4 below. Eggs morphologically similar to that of other *Meloidogyne* spp.

Table C4 **Measurements of 30 *M. paranaensis* eggs:**

Character	**Range** (µm)	**Mean± Std. errors (SE)** (µm)	**Standard deviation (SD)**	**Coefficient of variability (CV)**
length (L)	82-106 µm	90.5 ±0.82	5.32	5.6%
width (W)	37-51 µm	43.3±0.82	4.6	9.0%
L/W ratio	2.08-2.22	2.09±0.02	0.18	6%

Differential diagnosis
Meloidogyne paranaensis n. sp. can be distinguished from other species in the genus by combinations of the following characteristics. Females with labial disc and medial lips fused, asymmetric and rectangular; stylet 15.0-17.5 µ long, with broad distinctly set off knobs; distance from the DGO to stylet base 4.2-5.5 µm; perineal pattern similar to that of *M. incognita*. Males with high, round head cap continuous with the body contour; labial disc fused with medial lips to form an elongate lip structure; head region frequently marked by an incomplete annulation; stylet robust, 20-27 µm long, usually with rounded to transversely elongate knobs, sometimes with one or two projections protruding from the shaft. Second-stage juveniles with stylet 13-14 µm long, distance of the DGO to the stylet base 4.0-4.5 µm, and the tail length 48-51 µm long. Esterase pattern (F_1) is the most useful character for differentiating this new species from other species in coffee plantation surveys in Brazil.

Relationships
Meloidogyne paranaensis n. sp. is most similar to *M. konaensis* but differs from it in several morphological features. Females of *M. paranaensis* n. sp. have labial disc and medial lips fused, asymmetric and rectangular, forming straight lateral edges; in *M. konaensis* the labial disc is often rectangular and fused with medial lips to form a medial lip divided into distinct pairs. Males of *Meloidogyne paranaensis* n. sp. differ from males of *M. konaensis* in body length (983-2,284 vs. 1,149-1,872 µm), stylet length (20-27 vs. 20-24 µm), stylet knob height (2.0-4.5 vs. 3.4-4.2 µm), stylet knob width (4.5-7.0 vs. 3.4-5.0 µm), head end to excretory pore (130-205 vs. 134-178 µm) and DGO to stylet base (3.5-5.0 vs. 5.9-8.4 µm). Male head cap of the two species are similar, but the medial lip of *M. konaensis* is often divided into distinct medial lip pairs. Male stylets of the two species are also different: *M. paranaensis* n. sp. has stylet knobs transversely elongate, broad and set off from the shaft, sometimes with one or two large projections surrounding the shaft, whereas *M. konaensis* has knobs not set off, backward sloping, merging with shaft, 6-12 large projections surrounding the shaft. The second-stage juveniles of *M. paranaensis* n. sp. differ from *M. konaensis* in body length (389-513 vs. 468-530 µm), stylet base to head end (14-16 vs. 17-19 µ), DGO to stylet base (4.0-4.5 vs. 4.2-5.9 µm, head end to metacorpus valve (53-67 vs. 65-75 µm), excretory pore to head end (85-98 vs. 89-111 µm), and tail length (48-51 vs. 49-73 µm).

Meloidogyne paranaensis n. sp. is distinct from all other described species in the genus, including *M. incognita* with which it was previously confused; however, these earlier comparisons were based only on observations of perineal patterns. *Meloidogyne paranaensis* n. sp. has a characteristic esterase phenotype (one fast migrating band, F_1), which is different from *M. incognita* (one slow band, I_1) but identical to that of *M. konaensis* and *M. querciana*; however *M. paranaensis* can be differentiated biochemically from the latter by MDH pattern, N_1. No MDH pattern was reported for *M. konaensis*. *Meloidogyne paranaensis* n. sp. has the same differntial host response as *M. javanica*.

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

Meloidogyne paranaensis has the potential to adversely affect threatened and endangered plant species. However, because *M. paranaensis* only occurs outside the US and threatened and endangered plant species under consideration only occur within the US, it is not possible to confirm the host status of these rare plants from the scientific literature. From available host records, *M. paranaensis* is known to feed on species within the families Solanaceae and Cucurbitaceae, and also on an experimental host within the family Aquifoliaceae. From these host records, we infer that threatened or endangered plant species which are closely related to known host plants might also be suitable hosts (Table D1). For our purposes closely related plant species belong to the same genus.

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

Documented/Reported Host(s)	Threatened and/or Endangered Plant		Protected Status[1]	
	Scientific Name	Common Name	Federal	State
**Ilex paraguariensis*	*I. collina*	Longstock holly		NC (T) VA (E)
	I. glabra	inkberry		CT (T) ME (T)
	I. krugiana	tawnyberry holly		FL (E)
	I. montana	mountain holly		MA (T) NJ (E)
	I. opaca	American holly		PA (T)
	I. verticillata	common winterberry		AR (T) IA (E)

E= Endangered; T=Threatened

* experimental host

Appendix E. Biology of *Meloidogyne paranaensis*

Population phenology

This nematode is not well-studied and has only been distinguished from *M. incognita* since 1996 (Carneiro et al. 1996). Stage specific development is described below for *Meloidogyne* spp. in general.

Stage specific biology

There is no reported threshold temperature for *M. paranaensis*, though populations have been reared and maintained on greenhouse-grown tomatoes at temperatures between 22-28 °C (Carneiro et al. 1996).

Adult

This species typically reproduces asexually (mitotic parthenogenesis) (Carneiro et al. 1996), though some sexual reproduction must occur as males are observed in the population. The egg sac is deposited on either root surfaces or inside the root (Hussey 1985). Gall formation, a characteristic host response to *Meloidogyne* infection, does not occur on coffee roots (Carneiro et al. 1996).

Egg

Egg hatch may or may not involve stimulation from the host root (Hussey 1985). Eggs will not hatch under extended dry periods and may persist in soil or dry roots awaiting more favorable moist soil conditions.

Larva

Emergence occurs under moist soil conditions; juveniles may become inactive under dry conditions. *Meloidogyne* larvae can be easily distributed by irrigation ditches. 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 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 (Jensen 1972, Hussey 1985). When giant cell formation occurs, tissues surrounding the feeding nematode begin transforming at approximately the same time, producing a gall within 1-2 days following root penetration (Hussey 1985). The

larva will swell as it feeds until development is completed. Total development time varies depending on temperature, host quality, and other factors.