# *Meloidogyne paranaensis*

## Scientific Name
*Meliodogyne paranaensis* Carneiro, Carneiro, Abrantes, Santos, & Almeida, 1996

**Synonyms:**
Race 5 of *Meloidogyne incognita, Meloidogyne incognita* biotype IAPAR

## Common Name(s)
Paraná coffee root-knot nematode, coffee root-knot nematode

## Type of Pest
Nematode

## Taxonomic Position
**Class:** Secernentea, **Order:** Tylenchida, **Family:** Heteroderidae

## Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List - 2010

## Pest Description
**From Carneiro et al. (1996):**
Eggs: Length, 82-106 µm (mean 90.5), width, 37-51 µm (mean 43.3); length/width ratio 2.08-2.22. Egg morphology similar to that of other *Meloidogyne* spp. (Fig. 1).

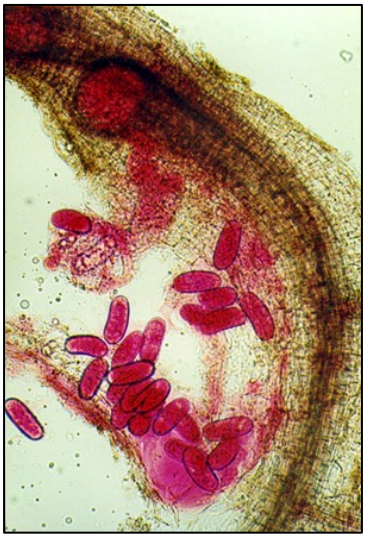

**Figure 1:** Stained egg masses of *Meloidogyne* spp. Photo courtesy of R. Gapasin.

Second-stage juveniles: Body vermiform (Fig. 2), 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 light microscopy, cephalic framework weak, hexaradiate. Vestibule and vestibule extension distinct. In SEM, stoma slit-like, located in oval prestomal 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. Distance from dorsal esophageal gland orifice (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. Tail usually conoid with rounded terminus. Hyaline tail terminus distinct. Rectal dilatation large. Phasmids small, posterior to anus.

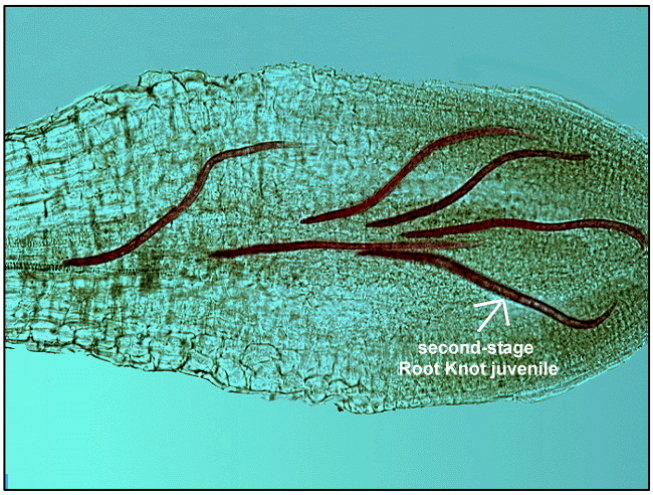


**Figure 2:** Second-stage juveniles inside a host root. Photo courtesy of Eisenback and Ulrich (Nemapix).

Holytype (female in glycerine): Body length, 684 µm; body width, 470 µm, neck length, 185 µm; neck width, 211 µm, body length without neck, 423 µm; stylet length 16.2 µm; stylet knob height, 2.4 µm; stylet knob width, 4.8 µm; DGO to stylet base, 4.2 µm; head end to posterior end of metacorpus, 96.2 µm; metacorpus length, 39 µm; metacorpus width, 33.8 µm; metacarpus valve length, 13.8 µm; metacorpus valve width, 10.4 µm; excretory pore to head end, 32.5 µm.

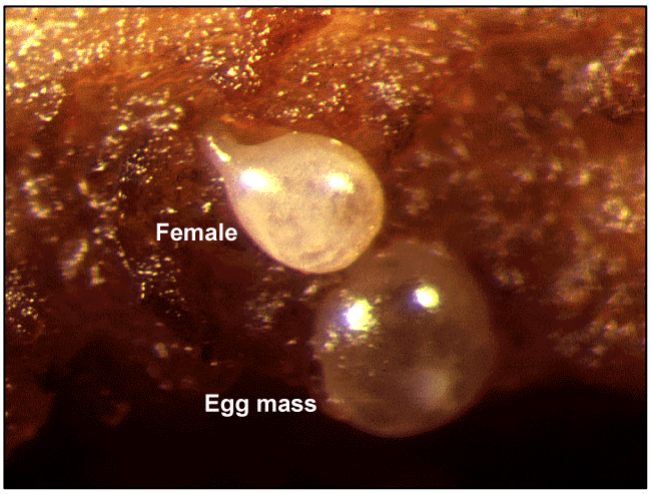


**Figure 3:** Root-knot nematode female and egg mass on a host root. Photo courtesy of Eisenback and Ulrich (Nemapix).

Females: Body translucent-white (Fig. 3), variable in size, elongate, ovoid to pear-shaped. Neck sometimes prominent, cuticular annulations on body finer than that on neck. Body posteriorly rounded, without tail protuberance. Head region not set off from body, not annulated. In SEM, 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 light microscopy, cephalic framework weakly sclerotized, lateral sectors slightly enlarged, vestibule extension distinct. 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. Distance of stylet base to DGO 4.2-5.5 µm. Esophagus with large, rounded metacorpus, valve plates large. 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 metacarpus and intestine. Excretory pore at level of anterior metacorpus.

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.

Allotype (male in glycerine): Body length, 1708 µm; greatest body width, 39 µm; body width at stylet knobs, 19.2 µm; body width at excretory pore, 27.6 µm; stylet length, 22.2 µm; stylet knob width, 4.8 µm; stylet knob height, 2.4 µm; DGO to stylet base, 4.2 µm; head end to metacorpus valve, 86 µm; metacorpus width, 9.8 µm; head end to excretory pore, 157 µm; testis length, 897 µm; spicule length, 26 µm.

Males: Body vermiform, length variable, body tapering anteriorly, bluntly rounded posteriorly, tail arcuate twisting through 90°. Head cap high, rounded, continuous with body contour. In light microscopy, cephalic framework strongly developed, vestibule and extension distinct. 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. In SEM, head cap flat, labial disc fused with medial lips forming elongate, rectangular head cap. Lateral lips absent. Head region usually marked by a short, incomplete annulation in lateral view. Stoma opening slit-like, located in ovoid prestomal 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. 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, gubernaculums distinct. Tail short, phasmids at level of cloaca.

*Meliodogyne paranaensis* biochemically shows a characteristic esterase phenotype with one fast migrating band, $F_1$, and one malate-dehydrogenase phenotype, $N_1$.

**Biology and Ecology**

At this time, little is known about the biology and ecology of this nematode species. Carneiro et al (1996) first described *Meloidogyne paranaensis* as a new species of *Meloidogyne* parasitizing coffee in Brazil. Root knot nematodes have sedentary endoparasitic habits. As far as is known, all coffee root-knot nematodes undergo the basic *Meloidogyne* spp. life cycle (Souza and Bresan-Smith, 2008). After eclosion, second-stage juveniles (J2) in the soil penetrate host roots where they establish a specialized feeding site (giant cells) in the stele (SON, n.d.). As J2s develop, they

sequentially develop into J3, J4, and adult stages. The females become swollen and produce egg masses(Carneiro et al., 1996). Reproduction is by asexual (mitotic parthenogenesis) (Carneiro et al., 1996). Egg masses in the soil and/or within roots are believed to be the nematode's main survival stage (Souza and Bresan-Smith, 2008).


**Figure 4.** Symptoms of *M. paranaensis* on coffee. Inset shows a female nematode on a host root. Photo courtesy of EPAMIG (the agricultural research enterprise of Minas Gerais).

There is no reported temperature threshold for *M. paranaensis*, though populations have been reared and maintained on greenhouse-grown tomatoes at temperatures between 22-28°C (72-82°F) (Carneiro et al., 1996).

Sandy soil and organic matter depletion seem to enhance the damage caused by *M. paranaensis* in Brazil (Campos and Villain, 2005).

## Symptoms/Signs

Foliar chlorosis (Fig. 4), leaf drop, general decline, reduced growth, dieback, and often plant death are observed in infested plants. Splitting and cracking of the cortical root tissue, especially the taproot, is characteristic. Necrotic spots occur along the roots where the females are located (Carnerio et al., 1996). The nematode does not produce typical root-knot galls on coffee (Carneiro et al., 1996). *M. paranaensis* females are mostly found in the older sections of the root (usually with few side rootlets), especially the principal root (Campos and Villain, 2005). Nematode feeding causes the tissues around the giant cells to die. Egg masses are produced in the root tissues (Campos and Villain, 2005).

## Pest Importance

Root-knot nematodes (*Meloidogyne* spp.) are a major constraint on coffee production in most countries worldwide (Campos and Villain, 2005; Herve et al., 2005; Boisseau et al., 2009). Seventeen species of *Meloidogyne* are acknowledged as pathogens of

coffee (Carneiro and Cofcewicz, 2008). Economic losses due to root-knot nematodes vary considerably depending upon the species involved and its distribution. Some *Meloidogyne* species induce numerous galls but only cause 10 to 20% drop in yield. Other species cause serious damage in plantations, destroying up to 80% of the root system within five years of planting. In the past 20 years the impact of nematodes on coffee has increased due to intensified cultivation through the use of higher-yielding varieties planted at higher densities. This combined with reduced or no shading, makes the trees more susceptible to limiting conditions (Herve et al., 2005).

*Meloidogyne paranaensis* is one of the most destructive root-knot nematode species on coffee (Carneiro et al., 1996; Campos and Villain, 2005). This species induces foliar necrosis, reduces growth, causes leaf drop and general plant decline, and can even cause plant death. *M. paranaensis* is widely distributed in Brazil and Guatemala, where coffee represents an important source of income and employment. In Guatemala, attacks by *M. paranaensis* lead to serious plant mortality on all current *Coffea arabica* (coffee) cultivars from the nursery stage (Campos and Villain, 2005).

Host resistance has been explored for *M. paranaensis* management. Nine wild coffee accessions from Ethiopia were considered resistant to *M. paranaensis* and provide coffee breeders with additional material whose resistance can be transferred to commercial cultivars (Boisseau et al., 2009). *Coffea arabica* and *C. canephora* hybrid progeny showed resistance to *M. paranaensis* and *M. incognita* race 2 when compared with a susceptible cultivar (Ito et al., 2008). Sera et al. (2009) showed that coffee cultivars Tupi IAC-169-33 and IPR 100 presented moderate resistance with inoculum levels of 500 and 1000 eggs, but were classified as susceptible at inoculum levels of 1500 and 2000 eggs.

An antagonistic effect has been observed between root-knot nematodes (*Meloidogyne paranaensis* and *M. incognita*) and *Arachis pintoi* (perennial, pinto peanut) (Santiago et al., 2002). *Meloidogyne paranaensis* juveniles were shown to be unable to penetrate *Arachis pintoi,* thus no galls or egg masses were observed. The incorporation of non-infested *Arachis* tissues into soil significantly reduced the number of *M. incognita* and *M. paranaensis* galls and egg masses in tomato plant root, which suggests that the plant could be used as an intercalated crop or a cover crop to reduce *M. paranaensis* and *M. incognita* populations.

*Paecilomyces lilacinus*, a fungal species used in biocontrol of nematodes, was evaluated for control of *M. paranaensis* (Santiago et al., 2006). All treatments involving *P. lilacinus* incorporation reduced in the population of M. *paranaensis* in tomato roots.

## Known Hosts

Coffee (*Coffea arabica*) is the primary host for this species. Carneiro et al. (1996) report that other hosts include tobacco (*Nicotiana tabacum*), tomato (*Lycopersicon esculentum*), and watermelon (*Citrullus lanatus*), when using the North Carolina host differential of Hartman and Sasser (1985) (Table 1). No reproduction was observed on cotton, pepper, and peanut, however. (Carneiro et al.,1996). With the exception of

cotton being resistant to *M. paranaensis*, the host differential was similar to that of *M. javanica* (Table 1).

In Brazil, soybean (*Glycine max*), *Ilex paraguariensis* (Paraguay tree), *Ageratum conizoides* (Mexican ageratum), and *Emilia sonchifolia* (lilac tasselflower) are hosts of *M. paranaensis* (Santiago et al., 2000; Castro et al., 2003; Roese et al., 2004; Campos and Villain, 2005; Moritz et al., 2008). In Guatemala, *Impatiens balsamino* (garden, rose balsam), a common weed in coffee plantations, is a good host of *M. paranaensis* and has been used successfully for rearing populations of this nematode in pots (Campos and Villain, 2005). Moritz et al (2003b) tested different genotypes of corn (*Zea mays*) for susceptibility to *M. paranaensis*. Most tested genotypes were immune or resistant, with reproduction factors below 1.0. An exception, however, was the susceptible genotype 69X72 (an experimental genotype), with a reproduction factor of 2.17 (Moritz et al., 2003b).

Roese and Oliveira (2004) found that the following weeds commonly found in soybean fields were susceptible hosts of *M. paranaensis*: *Ipomea grandifolia* (morning glory), *Cyperus rotundus* (nutgrass), *Solanum americanum* (American black nightshade), *Echinochloa colonum* (junglerice), *Raphanus raphanistrum* (wild radish), *Sorghum halepense* (Johnsongrass), *Galinsoga ciliata* (shaggy soldier), and *Eleusine indica* (Indian goosegrass).

Monaco et al. (2008) confirmed the status of *Raphanus raphanistrum* and *Eleusine indica* as hosts of *M. paranaensis*. They also found that the following weeds were susceptible to *M. paranaensis* using experimental inoculation: *Ageratum conyzoides* (tropical whiteweed), *Amaranthus deflexus* (large fruit amaranth), *Amaranthus hybridus* (slim amaranth), *Amaranthus viridis* (slender amaranth), *Bidens subalternans* (beggartick), *Chenopodium album* (lambsquarters), *Chenopodium carinatum* (clammy goosefoot), *Cleome affinis* (spiderwisp), *Digitaria horizontalis (*Jamaican crabgrass), *Hyptis lophanta* (catirinha), *Ipomoea triloba* (littlebell), *Lepidium pseudodidymum* (mentruz), *Momordica charantia* (balsampear), *Physalis angulata* (cutleaf groundcherry) *Polygonum persicaria* (spotted ladysthumb), *Portulaca oleracea* (little hogweed), *Setaria geniculata* (marsh bristlegrass), *Talinum paniculatum* (jewels of Opar), and *Verbena litoralis* (seashore vervain).

Roese et al. (2007) studied the pathogenicity of two isolates of *M. paranaensis*, one from soybean (Mp-s) and one from coffee (Mp-c). Mp-s was able to reproduce more than Mp-c on tomato and two soybean cultivars, but Mp-c showed a higher reproduction factor on coffee. Resistant and susceptible cultivars of soybean were susceptible to *M. paranaensis* in Brazil (Roese et al., 2007; Moritz et al., 2008). Moritz et al. (2008) noted, however, that fewer J2s established in the resistant cultivar, which resulted in low egg production 45 days after the inoculation.

*Avena* spp. (oats), *Panicum* (=*Urochloa*) *maximum* (guinea grass), *Brachiaria* (=*Urochloa*) *humidicola* (koronivia grass), *B. decumbens* (spreading liverseed grass), *B. brizantha* (palisadegrass), *B. plantaginea* (plantain signalgrass), *Lolium multiflorum*

(Italian ryegrass), *Triticum avenum* x *Secale cereale* (triticale), *Medicago sativa* (alfalfa), and *Manihot esculenta* (cassava) were found to be resistant to *M. paranaensis* (Moritz et al., 2003a; Carneiro et al., 2006a,b,c).

**Table 1: Usual response of the four common *Meloidogyne species* and their races to the North Carolina Differential Host Test from Hartman and Sasser (1985). The *Meloidogyne paranaensis* reaction is extrapolated from Carneiro et al (1996).**

| ***Meloidogyne* species and physiological races** | **Differential Host Plants [a]** | | | | | |
|---|---|---|---|---|---|---|
| | Cotton | Tobacco | Pepper | Watermelon | Peanut | Tomato |
| ***M. incognita*** | | | | | | |
| **Race 1** | - | - | + | + | - | + |
| **Race 2** | - | + | + | + | - | + |
| **Race 3** | + | - | + | + | - | + |
| **Race 4** | + | + | + | + | - | + |
| ***M. arenaria*** | | | | | | |
| **Race 1** | - | + | + | + | + | + |
| **Race 2** | - | + | - | + | - | + |
| ***M. javanica*** | + | + | - | + | - | + |
| ***M. hapla*** | - | + | + | - | + | + |
| | | | | | | |
| ***M. paranaensis*** | - | + | - | + | - | + |

[a] Cotton, Deltapine 61; tobacco, NC 95; pepper, Early California Wonder; watermelon, Charleston Gray; peanut, Florunner; tomato, Rutgers; (-) indicates a resistant host; (+) indicates a susceptible host.

## Known Vectors (or associated insects)

*Meloidogyne paranaensis* is not known to be a vector and does not have any associated organisms.

## Known Distribution

This nematode is known to occur in Brazil and Guatemala (Carneiro et al., 2004; Herve et al., 2005). Carneiro et al. (2004) report that an isolate of *Meloidogyne konaensis* from Hawaii was actually *M. paranaensis* when using biochemical and molecular analyses. Nematologists from the University of Hawaii, however, disagree with this conclusion and do not believe that *M. paranaensis* is present in Hawaii (Sipes, 2010).

Vergel-Colon et al. (2000) report a root-knot nematode population from coffee in Colombia with a perineal pattern similar to *M. incognita* (as in *M. paranaensis*) that produced a response to the North Carolina differential host test similar to that reported for *M. paranaensis* (Campos and Villain, 2005; De Waele and Elsen, 2007). It is unknown at this time if the population is *M. paranaensis* or another *Meloidogyne* species.

## Potential Distribution within the United States

The area most at risk for introduction and establishment of *M. paranaensis* would be coffee production areas within Hawaii and/or Puerto Rico due to the presence of coffee (the primary host) and having a tropical climate. Coffee is not grown in the continental United States. Secondary hosts are grown or occur (weedy hosts) throughout the continental United States.

## Survey

**CAPS-Approved Method:**

1. Soil sample: send sample to nematology diagnostic lab where nematodes will be extracted from the soil and identified.

2. Collect host roots: *M. paranaensis* does not produce typical root-knot nematode galls on coffee. *M. paranaensis* females are mostly found in the older sections of the root (usually with few side rootlets), especially the principal root. Necrotic spots occur along the roots where the females are located.

**Literature-Based Methods:**

Vovlas and Inserra (1996) outline general considerations for conducting a survey for a new *Meloidogyne* spp. in citrus orchards. In general, they recommend sampling root tissues to inspect for the presence of galled roots. **Due to the lack of gall production with *M. paranaensis*, however, roots should be examined for the presence of female nematodes**. The authors 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. Thus, careful examination of individuals will be necessary to confirm species identity. 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), biochemical, or molecular techniques.

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). The efficiency of the nematode extraction is influenced by the amount of soil that is processed at one time. Extraction efficiencies are greatest when 100 g to 450 g of soil are processed. Extraction efficiencies for *Meloidogyne* spp. are frequently low and can vary between 13 and 45% (Davis and Venette, 2004).

Soil and root sampling: Oliveira et al. (2005) collected soil and root samples from four points, to a depth of 30 cm from the under the canopy of selected symptomatic coffee plants and pooled the samples. The composite sample of approximately 500 g soil and 200 g roots was placed into plastic bags, labeled, and transported to the nematology

laboratory. The eggs were extracted from the roots according to Boneti and Ferraz (1981) and used to inoculate coffee seedlings for nematode multiplication in the greenhouse. Perineal pattern, isozyme characterization, and differential host inoculations then occurred to characterize the species present. The Boneti and Ferraz (1981) method, which extracts a greater number of eggs without affecting infectivity, is a modification of the Hussey and Barker (1973) method. Instead of manual shaking, roots are chopped in a blender in 0.5% sodium hypochlorite (bleach) for 20 seconds.

Host root collection: Herve et al. (2005) sampled roots in Guatemala and Costa Rica for *Meloidogyne* spp. and *Pratylenchus coffea*. The plot, in Guatemala, consisted of 10-year old coffee plants that had been severely attacked by *M. paranaensis* and *Pratylenchus* spp. Samples were taken from a 1-hectare plot comprising 50 rows of 98 coffee trees. The trees were spaced 1 m apart along the planting row, and 2 m between rows. Two coffee trees were chosen at random in each row and the roots of 100 trees were sampled in this way. Root samples of about 100 g were taken 30 cm from the base of the trunk in May-June. An aliquot of 25 g of nonlignified or slightly lignified roots per tree were carefully washed, liquidized in a blender, and poured through sieves (850, 150, 45, and 38 µm mesh aperture). The material remaining in the last two sieves was recovered and processed by centrifugation/flotation. Nematodes were collected in 100 ml of water and individuals (adults and juveniles) of the two genera were counted in three aliquots of 1 ml on a counting slide.

In Costa Rica, where *M. exigua* and *Pratylenchus* spp. were known to occur, a plot of 14-year old coffee was chosen that had been attacked by *M. exigua* and *Pratylenchus* spp. In May-June, samples were taken in a half-hectare plot comprising 33 rows of 97 coffee trees. The trees were spaced 0.44 m apart along the row and 1.87 m between rows. Ten coffee trees were chosen at random in each row, and roots of 327 trees were sampled as described for the Guatemalan site. Nematodes were extracted from each sample using 10 g of roots in a mist chamber, with a 90 s mist cycle at 37°C (99°F) every 10 minutes. The nematodes were recovered and counted on the seventh and 13$^{th}$ days.

Baiting: Moritz et al. (2008) used a baiting technique. The population *of M. paranaensis* used for testing was from infested coffee plantations. For this, 'Rutgers' tomato was transplanted into pots containing soil and roots collected in the field. After 28 days of transplanting, the population was characterized by electrophoretic isoenzyme profiles.

## Key Diagnostics/Identification

### CAPS-Approved Method:

Morphology, coupled with differential host testing, and biochemical methods (esterase and one malate-dehydrogenase phenotypes) have been used to distinguish *M. paranaensis* as a separate species (Carneiro *et al.*, 1996; 2000).

A combination of methods is recommended, because Sipes et al. (2005) found that esterase phenotypes can be polymorphic. *Meloidogyne konaensis*, originally isolated from coffee, grown for a long-period of time on tomato showed a slow (I1) esterase

band or a combination of F1-I1. Only the F1 isolate parasitized coffee; while the F1-I1 isolate had greater reproduction on tomato and cucumber.

**Literature-Based Methods:**

Carneiro and Cofcewicz (2008) provide a summary of identification procedures and issues for *Meloidogyne* spp. that are parasitic on coffee.

Morphology: *M. paranaensis* can be distinguished from other species in the genus by combinations of the following characteristics. Reliable identification of *Meloidogyne* spp. based on morphology, however, is a formidable task, even for well qualified taxonomists with expertise in the genus (Carneiro and Cofcewicz, 2008). Females with labial disc and medial lips fused, asymmetric and rectangular; stylet 15.0-17.5 µm 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 caps continuous with the body contour; labial disc fused with the 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-state juveniles with stylet 13-14 µm long, distance from the DGO to the stylet base 4.0-4.5 µm, and the tail length 48-51 µm long (Carneiro et al., 1996).

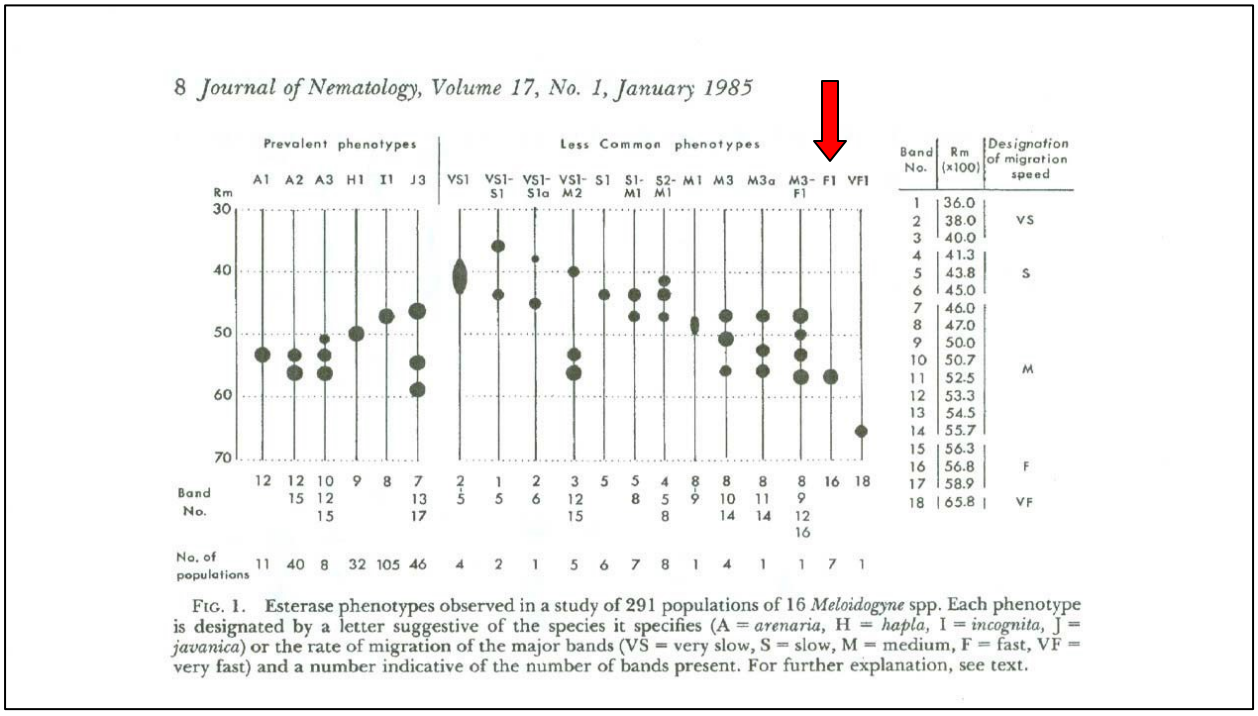


FIG. 1. Esterase phenotypes observed in a study of 291 populations of 16 *Meloidogyne* spp. Each phenotype is designated by a letter suggestive of the species it specifies (A = *arenaria*, H = *hapla*, I = *incognita*, J = *javanica*) or the rate of migration of the major bands (VS = very slow, S = slow, M = medium, F = fast, VF = very fast) and a number indicative of the number of bands present. For further explanation, see text.

**Figure 5.** Esterase phenotypes observed by Esbenshade and Triantaphyllou (1985). Red arrow shows the F1 esterase phenotype typical of *M. paranaensis.*

Biochemical: Esbenshade and Triantaphyllou (1985) discuss the use of enzyme phenotypes for the identification of *Meloidogyne* species. Figures 5 and 6 show the typical enzyme phenotypes used to characterize *Meloidogyne* spp. by Esbenshade and

Triantaphyllou (1985). According to Carneiro et al. (1996), 'the unidentified *Meloidogyne* population from coffee from Brazil, referenced in the Esbenshade and Triantaphyllou (1985) paper, is most likely *M. paranaensis'*. This nematode had a $F_1$ esterase phenotype and a $N_1$ methyl dehydrogenase phenotype, which is typical of *M. paranaensis*. This nematode also showed an $N_2$ superoxide dismutase phenotype and a $N_1$ glutamate-oxaloacetate transaminase phenotype. According to Carneiro et al. (1996), the esterase pattern (F1) is the most useful character for differentiating this new species from other species in coffee plantation surveys in Brazil. Carneiro et al. (2000) use $P_1$ instead of $F_1$, but they are deemed equivalent.

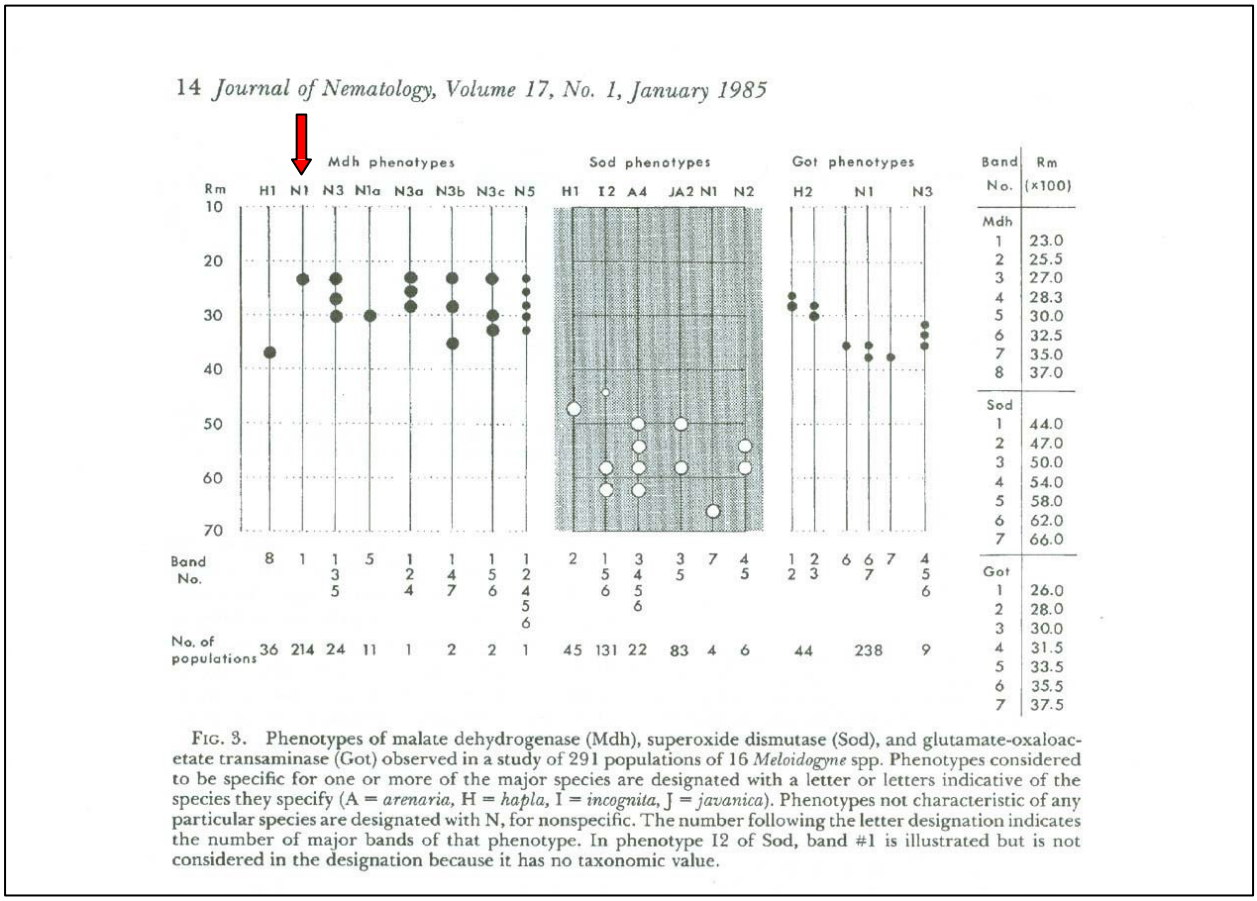


**Figure 6.** Malate dehydrogenase, superoxide dismutase, and glutamate-oxaloacetate transaminase phenotypes observed by Esbenshade and Triantaphyllou (1985). Red arrow shows the N1 malate dehydrogenase phenotype typical of *M. paranaensis.*

Carneiro et al. (2004) found two esterase phenotypes (P1 (=F1) and P2) for *M. paranaensis* when using isolates from Central America, Brazil, and Hawaii. The P2 phenotype was identified from some Guatemalan isolates; while isolates from Brazil, Hawaii, and another isolate from Guatemala showed the P1 phenotype. Although the populations *of M. paranaensis* from Brazil and from Guatemala presented different esterase phenotypes (P1 and P2), respectively, they were very closely related in molecular and morphological approaches. Nevertheless, these two populations

presented different physiological behavior in relations to resistant tomato with *Mi* gene. *M. paranaensis* with the P2 phenotype parasitized resistant tomato; *M. paranaensis* with the P1 phenotype did not (Boisseau et al., 2009).

Molecular: RAPD markers have been used to confirm species identification and for estimating the genetic diversity among species and isolates (Carneiro et al., 2004). RAPD markers were also identified and transformed into SCAR makers and allow the detection of *M. exigua*, *M. incognita*, and *M. paranaensis* eggs and egg masses in a multiplex PCR (Randig et al., 2002; Randig et al., 2004). The PCR resulted in the amplification of a specific size fragment for each species: 562 bp for *M. exigua*, 399 bp for *M. incognita*, and 208 bp for *M. paranaensis*. Carneiro et al. (2005) further evaluated the Randig et al. (2002, 2004) SCAR primers in conjunction with esterase phenotype. The authors observed, that the multiplex PCR allowed for the unambiguous differentiation of the *M. exigua*, *M. incognita*, and *M. paranaensis* alone or in mixtures and its potential for application in routine diagnostic protocols was confirmed.

## Easily Confused Pests

*Meloidogyne paranaensi*s was mistaken for *M. incognita* for more than 20 years. Female *M. paranaensis* have a rectangular or oval cuticular perineal pattern with high dorsal arch similar to that of *M. incognita*. *M. paranaensis* has a characteristic esterase phenotype (one fast migrating band, $F_1$), which is different from *M. incognita* (one slow band, I1) but identical to that of *M. konaensis* (known to occur in Hawaii) and *M. querciana*. *M. paranaensis*, however, can be differentiated biochemically from *M. querciana* by the MDH (malate-dehydrogenase) pattern $N_1$. No MDH pattern was reported for *M. konaensis*. *M. paranaensis* also has a similar differential host response as *M. javanica,* but does not infect cotton (Hartman and Sasser, 1985; Carneiro et al., 1996).

*Meloidogyne paranaensis* is most similar to *M. konaensis* but differs from it in several morphological features. Females of *M. paranaensis* have labial disc and medial lips fused, asymmetric and rectangular, forming straight lateral edges, but in *M. konaensis* the labial disc is often rectangular and fused with medial lips to form a medial lip divided into distinct lip pairs. Males of *M. paranaensis* differs from males of M. konaensis in body length (983-2284 vs. 1149-1872 µm), stylet length (20-27 vs. 20-24 µm), stylet knob height (2.0-4.5 vs. 4.4-4.2 µm), stylet knobs 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* 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* differ from *M. konaensis* in body length (389-513 vs. 468-530 µm), stylet base to head end (14-16 vs. 17-19 µm), 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 port to head end (85-98 vs. 89-111 µm), and tail length (48-51 vs. 49-73 µm) (Carneiro et al., 1996).

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