

## *Meloidogyne javanica* on Peanut in Florida<sup>1</sup>

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**Abstract:** A mixed population of *Meloidogyne arenaria* race 1 and *M. javanica* race 3 is reported on peanut from a field in Levy County, Florida. Confirmation of *M. javanica* on peanut is based on esterase and malate dehydrogenase isozyme patterns resolved on polyacrylamide slab gels following electrophoresis, and perineal patterns. Up to 29% of 290 individual females collected from peanut roots in the field in autumn 2002 showed a typical esterase J3 phenotype for *M. javanica*. This is the third report of *M. javanica* infecting peanut in the United States.

**Key words:** *Arachis hypogaea*, electrophoresis, esterase phenotype, host race, malate dehydrogenase phenotype, *Meloidogyne arenaria*, *Meloidogyne javanica*, nematode, peanut, root-knot nematode.

Several species of root-knot nematodes are pathogens of peanut, *Arachis hypogaea* L. Worldwide, *Meloidogyne arenaria* (Neal) Chitwood (peanut root-knot nematode) is the most common species infecting peanut (Dickson, 1998). It is found on peanut in the southeastern United States (Ingram and Rodríguez-Kábana, 1980; Powers and Harris, 1993; Sturgeon, 1986), whereas *M. hapla* Chitwood (northern root-knot nematode) is encountered more frequently in the more northern peanut production regions of the United States (Dickson, 1998). In addition, *M. javanica* (Treub) Chitwood (Javanese root-knot nematode) also has been reported to infect peanut in the United States as well as other parts of the world. This nematode was first reported on peanut in Zimbabwe (Martin, 1958). Eleven years later, Minton et al. (1969) reported *M. javanica* on peanut in Georgia. Later, the nematode was reported in Texas (Tomaszewski et al., 1994). Other reports of *M. javanica* on peanut are from Africa (Ibrahim and El-Saedy, 1976; Rammah and Hirschmann, 1990; Tomaszewski et al., 1994), India (Patel et al., 1988; Sakhuja and Sethi, 1985), and Brazil (Lordello and Gerin, 1981).

In 2001, a single root-knot nematode infected peanut plant collected from a commercial production field in Levy County, Florida, was brought to the laboratory for species determination. Biochemical analysis of the females extracted from the roots of this plant showed a typical esterase J3 phenotype for *M. javanica* (Lima et al., 2002). A follow-up was made 1 week later when roots were collected from volunteer peanut plants in an experimental research site located on the same farm. This field had just been harvested, and the only remaining peanut plants were volunteers in the borders. These

samples revealed a mixture of *M. javanica* and *M. arenaria*. The objective of this study was to confirm the occurrence of *M. javanica* on peanut in Florida based on these preliminary observations and to determine the ratio of *M. javanica* to *M. arenaria* race 1 on peanut at the experimental site.

### MATERIALS AND METHODS

The experimental site was located in a production field near Williston, Florida, Levy County. There was a known infestation of *M. arenaria* race 1 and a population of *Pasteuria penetrans* that was specific to and suppressive for the nematode (Dickson et al., 1994). From 1991 to 1999, the site consisted of three treatments, bahiagrass (*Paspalum notatum* Flugge cv. Tifton 9), rhizomal peanut (*Arachis glabrata* Benth. cv. Florigraze), and weed fallow plots, each replicated 10 times in a randomized complete block design.

In 1999, the bahiagrass and weed fallow plots were plowed and disked, and the rhizomal peanut plots were treated with glyphosate. The latter was plowed and disked along with all other plots in winter 2000. In 1999, the bahiagrass and weed fallow plots were planted with peanut (*Arachis hypogaea* L. cv. Florunner). Peanut cv. Southern Runner was planted in 2001, and cv. Georgia Green was planted in 2001 and 2002. Each plot consisted of 10 rows of peanut, each 38 m long. The row spacing was 90 cm.

*Meloidogyne javanica* on peanut based on soil samples: Soil samples were taken from each plot on 4 February 2002. Twelve cores (2.5-cm-diam., 20-cm-deep) were taken per plot in a zigzag pattern with a cone-shaped sampling tube. The soil from each plot was mixed thoroughly and divided into three portions, each of which was placed in a 12-cm-diam. clay pot. Either tomato (*Lycopersicon esculentum* cv. Rutgers) or pepper (*Capsicum annuum* L. cv. California Wonder) seedlings were transplanted into 30 pots each. The remaining 30 pots were seeded with peanut (*A. hypogaea* L. cv. Florunner). The plants were grown in a greenhouse for 105 days, after which they were removed and the roots washed and examined for galling. Thirteen females were extracted from each peanut plant, each from a single gall. The females were preserved individually in 10 µl of an

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extraction buffer (55% deionized water, 12% 0.5 M Tris-HCl, pH 6.8, 30% glycerol, 2% of 0.5% [w/v] bromophenol blue) contained in a cone-shaped microfuge tube and placed in a freezer at  $-5^{\circ}\text{C}$ .

*Meloidogyne javanica* on peanut roots grown in field site: In 2002, females of root-knot nematodes were extracted from 125-day-old peanut (cv. Georgia Green) roots taken from each plot. Fresh roots were collected randomly from 13 plants in each plot. One female was extracted from each plant and stored in the extraction buffer as stated above.

*Meloidogyne javanica* standard control: Females of a known greenhouse isolate of *M. javanica* were extracted from tomato roots and stored in a freezer in the extraction buffer reported above.

*Electrophoresis, sample preparation, and loading*: A Bio-Rad mini-PROTEIN II (Bio-Rad, Philadelphia, PA) electrophoresis unit was used. Before electrophoresis, the females were thawed and homogenized individually in a microhaematocrite plastic tube in 10  $\mu\text{l}$  of extraction buffer. Each sample then was loaded into a well. Each gel contained 15 wells. The standard *M. javanica* female extract was placed into wells 1 and 14. The remaining 13 wells were loaded with the protein extract from test sample females. Electrophoresis was carried out in a discontinuous buffer system with 8% acrylamide running gel, pH 8.8 and 4% acrylamide stacking gel, pH 6.8. The voltage was maintained at 80 volts for the first 15 minutes and increased to 200 volts for the

remainder of the running period. Following electrophoresis, the gels were removed and placed in an enzyme reaction mixture to determine esterase and malate dehydrogenase activity (Harris and Hopkinson, 1976).

*Preparation of perineal patterns*: Single egg mass cultures of *M. arenaria* and *M. javanica* were prepared from peanut roots. Confirmation of these cultures was based on isozyme phenotypes and perineal patterns. Forty females each from these two cultures were extracted from roots of peanut grown in a greenhouse. Perineal patterns were prepared following procedures of Hartman and Sasser (1985). Morphological observations and photographs of perineal patterns were completed within 12 hours following slide preparation.

*Statistical analysis*: Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC), and mean treatment differences were separated and compared using Duncan's multiple-range test.

## RESULTS

*Meloidogyne javanica* on peanut based on soil samples: Of the plants grown in a greenhouse, tomato and peanut roots were heavily galled, but no galling was observed on the pepper roots. Tomato roots were not processed further because tomato is a known susceptible host for the three most common root-knot nematode species,

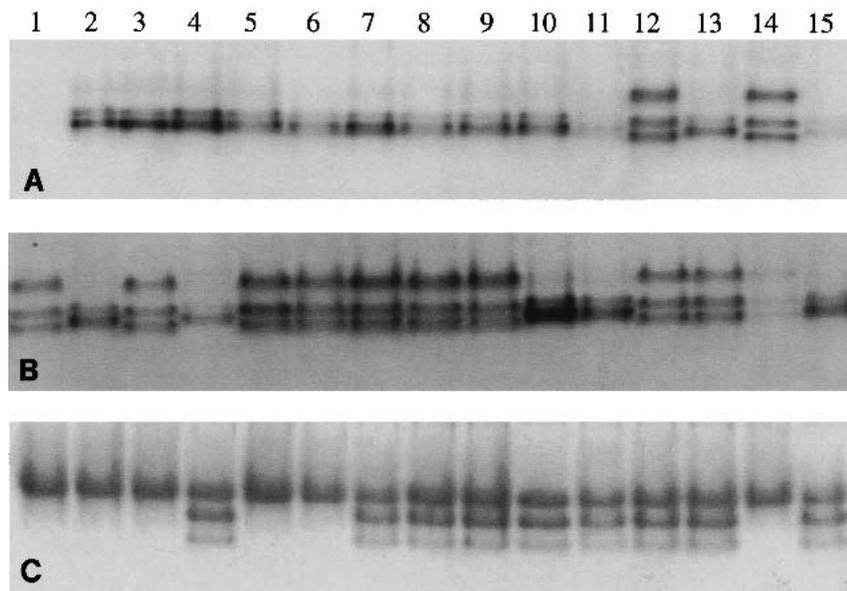


FIG. 1. Esterase (A, B) and malate dehydrogenase (C) (Mdh) isozymes resolved from individual root-knot nematode females following electrophoresis on polyacrylamide slab gels. Shown are the *Meloidogyne javanica* (J3) and *Meloidogyne arenaria* (A2) phenotypes for esterase, and the *M. javanica* (N1) and *M. arenaria* (N3) phenotypes for Mdh. A) Females were extracted from peanut grown in a greenhouse in pots filled with soil collected from a peanut field near Williston, FL. Lanes 1 and 14 = *M. javanica* standard control; lanes 2–11, 13, 15 = *M. arenaria* race 1 A2 esterase phenotype; lane 12 = *M. javanica* J3 esterase phenotype. B) Females were extracted from peanut roots taken at harvest from a field near Williston, FL. Lanes 1 and 14 = *M. javanica* standard control; lanes 2, 4, 10, 11, 15 = *M. arenaria* race 1 A2 esterase phenotype; lanes 3, 5–9, 12, 13 = *M. javanica* esterase phenotypes J3. C) Females were extracted from peanut roots taken at harvest from a peanut field near Williston, FL. Lanes 1, 14 = *M. javanica* standard control; lanes 2, 3, 5, 6 = *M. javanica* N1 Mdh phenotype; lanes 4, 7–13, 15 = *M. arenaria* race 1 N3 Mdh phenotype.

*M. arenaria*, *M. incognita*, and *M. javanica*. Biochemical analysis of the *Meloidogyne* spp. females extracted from peanut roots revealed that 5.7% of 290 individuals had a typical esterase pattern for *M. javanica* J3 phenotype (Fig. 1, A,B). The remaining individuals showed a typical esterase pattern for *M. arenaria* phenotype A2. There was no effect of rhizomal peanut (6.2%), bahiagrass (5.5%), or weed fallow (5.4%) on the frequency of *M. javanica* ( $P > 0.05$ ) (Fig. 2).

*Meloidogyne javanica* on peanut roots grown in the experimental field site: Of the samples collected at harvest in autumn 2002 from roots of peanut grown in field soil, 29% of the 290 individuals collected showed a typical esterase pattern for *M. javanica*. The remaining individuals showed a typical esterase pattern for *M. arenaria* phenotype A2. The weed fallow plots supported the greatest percentage of *M. javanica* (41%), whereas rhizomal peanut plots had 24% and bahiagrass plots had 23% ( $P \leq 0.05$ ) (Fig. 2). The *M. javanica* esterase phenotype was identical with the known greenhouse isolate of *M. javanica*. They both had the typical three-band esterase isozyme phenotype. The malate dehydrogenase phenotype also confirmed the occurrence of *M. javanica* on peanut (Fig. 1, C).

*Perineal patterns*: The observation of perineal patterns cut from single females extracted from peanut roots revealed morphological characters that were typical for *M. javanica* and *M. arenaria* (Fig. 3). *Meloidogyne javanica* perineal patterns had a moderately high dorsal arch and conspicuous lateral lines (Fig. 3, B), whereas *M. arenaria* had a low dorsal arch, irregular striae, and an absence of lateral ridges (Fig. 3, A).

DISCUSSION

This report of *M. javanica* infecting peanut in Florida confirms our earlier finding of *M. javanica* on peanut in

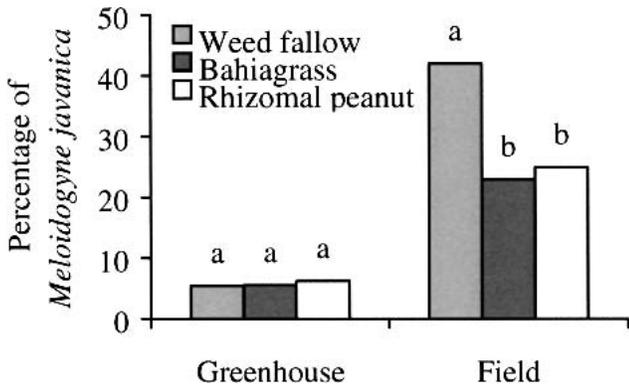


FIG. 2. The percentage of *Meloidogyne javanica* phenotype J3 found in peanut as determined by esterase phenotypes. Each column is an average of 10 replications (130 females), and means with the same letter within each trial are not significantly different according to Duncan's multiple-range test ( $P \leq 0.05$ ). *Greenhouse*: Data are based on females extracted from roots of peanut grown in a greenhouse in pots filled with soil taken from a peanut field near Williston, FL. *Field*: Data are based on females extracted from peanut roots taken at harvest in 2002.

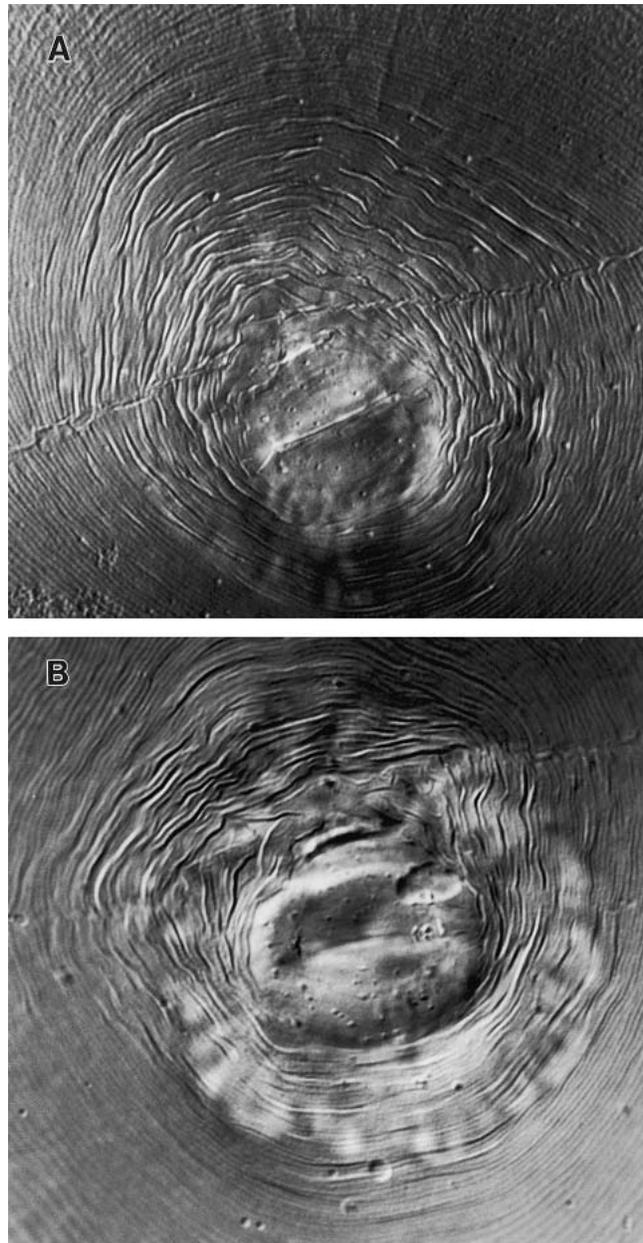


FIG. 3. A) Perineal pattern of *Meloidogyne javanica*. B) Perineal pattern of *Meloidogyne arenaria*. Each perineal pattern was derived from a single egg mass isolate of the two nematode species grown on peanut in the greenhouse and examined using differential interference contrast optics. The high dorsal arch and lateral lines of *M. javanica* and the deep shoulders and low dorsal arch of *M. arenaria* are visible.

a commercial peanut field (Lima et al., 2002). The ratio of *M. javanica* esterase phenotype to the *M. arenaria* race 1 phenotype increased from 5.7% (based on peanut plants grown in soil collected from the field) in February 2002 to 29% (based on peanut plants collected at harvest from the field) in October 2002. The greatest increase of *M. javanica* occurred in the weed fallow plots, which likely resulted from the maintenance of the nematode on weeds over the 10-year period of the experiment.

Because there was infection by *M. javanica* on peanut but none on pepper we conclude that the population is race 3 (Dickson, 1998; Rammah and Hirshmann, 1990). Despite being rare in most parts of the world (Hartman and Sasser, 1985), *M. javanica* populations capable of parasitizing peanut appear to be mostly distributed in Africa and Asia (Dickson, 1998; Tomaszewski et al., 1994), whereas their occurrence on peanut in North America appears to be relatively infrequent (Tomaszewski et al., 1994). As Minton et al. (1969) stated, however, both *M. arenaria* and *M. javanica* cause similar symptoms on peanut. This may result in the false assumption that all heavily galled peanut roots, pods, and pegs are caused by *M. arenaria*. A simple analysis of individual females by electrophoresis and staining for esterase and Mdh activity would make clear the presence of *M. javanica*. It is unclear at this time whether *M. javanica* infects peanut in other production regions in Florida.

This is the third state in the United States in which *M. javanica* has been found infecting peanut, with the first and second states being Georgia and Texas, respectively. Although this study of *M. javanica* on peanut was in an experimental research field, the nematode also was found on peanut in the grower's production field ca. 5 km from the experimental research site (Lima et al., 2002). The research site was originally designed for studying the persistence of *P. penetrans* on *M. arenaria* race 1. It is unclear at this time whether the bacterium also parasitizes *M. javanica* race 3; however, when the isolate of *P. penetrans* that developed in *M. arenaria* was exposed to an *M. javanica* population, only a very low rate of attachment was attained (Oostendorp et al., 1990). Development inside the females of *M. javanica* was not determined nor was the race of *M. javanica* used in this experiment known. This is especially interesting in that if this population of *P. penetrans* is specific and suppressive to *M. arenaria* only (Dickson et al., 1994), then we might speculate that *M. javanica* will eventually replace *M. arenaria* as the dominant species on peanut in this field.

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