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Meloidogyne javanica Parasitic on Peanut[1]

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Abstract: Peanut fields in four governorates of Egypt were surveyed to identify species of *Meloidogyne* present. Fourteen populations obtained from peanut roots were all identified as *M. javanica* based on perineal patterns, stylet and body lengths of second-stage juveniles, esterase phenotypes, and restriction fragment length polymorphisms of mtDNA. Three of 14 populations, all from contiguous fields in the Behara governorate, had individuals with a unique two-isozyme esterase phenotype. All populations of *M. javanica* tested on peanut had levels of reproduction on the *M. arenaria*-susceptible peanut cultivar Florunner that were not different from *M. arenaria* ($P = 0.05$), and had lower levels of reproduction on the *M. arenaria*-resistant genotype TxAG-7 than on Florunner ($P = 0.05$). Reproduction of the five Egyptian populations of *M. javanica* tested was lower on root-knot nematode resistant tomato cultivars Better Boy and Celebrity than on the root-knot nematode susceptible cultivar Rutgers ($P = 0.05$). These data are evidence that some populations of *M. javanica* are parasitic on peanut and that the peanut and tomato genotypes resistant to *M. arenaria* are also resistant to these populations of *M. javanica*.

Key words: Arachis hypogaea, Egypt, esterase isozyme phenotype, host resistance, *Lycopersicon esculentum, Meloidogyne arenaria, Meloidogyne javanica,* nematode, peanut, restriction fragment length polymorphism, RFLP, root-knot nematode, tomato.

Root-knot nematodes are common parasites of peanut (*Arachis hypogaea* L.) in many areas of the world. *Meloidogyne arenaria* (Neal) Chitwood is most commonly found on peanut in the southern United States (6,19), with *M. hapla* Chitwood being common in the more northern production areas of Virginia, North Carolina (17), and Oklahoma. *Meloidogyne javanica* (Treub) Chitwood also has been reported to parasitize peanut, but definitive data to support most of these reports are lacking. Previous reports of *M. javanica* on peanut were lacking either an adequate description of the species (5,7,8), confirmation of peanut as a host (11,13,15), or both (14). In one survey of over 300 populations of *M. javanica* from a number of different hosts collected worldwide (3), less than 1% of the populations were parasitic on peanut. Collectively, these reports leave considerable doubt as to the importance of *M. javanica* as a parasite of peanut.

Peanut is an increasingly important crop in Egypt, with current production at 23,000 hectares. Much of this production is on recently reclaimed desert soils that has supplemental irrigation. Root-knot symptoms, including heavily galled roots associated with stunted, frequently chlorotic shoot growth, have often been observed on peanut in Egypt, but the *Meloidogyne* species were not identified. We present data on the identity of *M. javanica* isolated from peanut in four governorates of Egypt.

Materials and Methods

Populations of root-knot nematodes were collected from arbitrarily selected fields located in governorates of Ismailia, Behara, Alexandria, and Giza, Egypt (Table 2). Eggs of each population were collected from galled peanut roots with 0.52% NaOCl (4). Individual populations were composed of combined eggs from at least four plants from a single field and were maintained as permanent cultures on *Lycopersicon esculentum* M. cv. Rutgers. Single egg mass subcultures were derived from two populations and are designated with a lowercase letter (e.g., 92–23a, 92–25a, and 92–25c) to distinguish them from the original field populations. Inoculated

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TABLE 1. Mean total body length and stylet length for 20 second-stage juveniles of populations of *Meloidogyne* from peanut in Egypt.

Population number	Body length (μm)	Stylet length (μm)
92-5	384	13.3
92-7	409	14.9
92-13	456	14.3
92-18	456	15.3
92-19	419	14.6
92-23a	431	16.1
92-25	470	15.1
92-25c	458	15.2
Means	433	14.8
LSD 0.05	28	NS

Populations of 92-23a and 92-25c are single-egg-mass subcultures of the original populations (92-23 and 92-25) and have the two esterase isozyme phenotype. Population 92-25 is a mixture of two and three esterase isozyme phenotypes. The remaining populations have three esterase isozyme phenotypes.

tomato was grown in 25-cm-d pots filled with a coarse sand–peat mix (6:1, v/v) and maintained at 26 C with 14 hours light per day.

Species identification was based on body and stylet lengths of second-stage juveniles (J2), perineal patterns of females, esterase phenotypes, and by restriction fragment length polymorphisms (RFLP) of mtDNA sequences amplified by polymerase chain reaction (PCR). Morphometrics of J2 and esterase phenotypes were determined for a minimum of 20 individuals of each population. Perineal patterns from a minimum of five individuals of each population were examined. To determine the esterase phenotype, females were excised from tomato roots and individually macerated in 0.1M phosphate extraction buffer (pH 7.4) with 20% sucrose, 2% Triton X-100, and 0.1% bromophenol blue dye. Electrophoresis of macerates of individual females was accomplished with an automated apparatus (Phastsystem, Pharmacia, Uppsula, Sweden) on 10 to 15% gradient polyacrylamide gels (2). Esterase phenotypes were determined by staining polyacrylamide gels for esterase activity (2). Perineal patterns were cut from recovered carcasses of females following maceration for esterase phenotypes so that the perineal patterns could be matched with esterase phenotypes. Populations of *M. arenaria* (no. 82–4, originally isolated from peanut in Texas) and *M. incognita* (no. 82–2, originally isolated from cotton in Texas), which were previously determined to have esterase phenotypes and perineal patterns typical of the species, were included in these studies as reference standards.

The RFLP from a specific region of the mtDNA was characterized in the laboratory of T. O. Powers at the University of Nebraska (12). Freshly hatched J2 from five populations from peanut in Egypt plus populations of *M. incognita* and *M. javanica* were macerated individually and the macerate added to separate 25-μl reaction mixtures in 0.50-ml microcentrifuge tubes for 45 cycles of amplification of mtDNA using the DNA primers C2F3 and 1108 by PCR. Amplified products were digested separately with the restriction enzyme Hinf 1 and electrophoresed on 2.0% agarose to obtain species-specific RFLP patterns.

To confirm parasitism of peanut, the *M. arenaria*-susceptible peanut cultivar Florunner and the *M. arenaria*-resistant genotype TxAG-7 (formerly TP-135-4, [18]) were each inoculated separately with eggs of *M. javanica* populations from Egypt or with eggs of *M. arenaria* (no. 82–4). Plants were grown separately in 15-cm-d pots filled with the sand–peat mix. For each population tested, eggs were collected from permanent cultures using 0.52% NaOCl (4), and each plant was inoculated with a suspension of 10,000 eggs. Peanut roots were harvested 8 weeks after inoculation, weighed, and eggs collected with NaOCl to determine total eggs produced per gram of roots. In addition to these tests, the ability of five of the Egyptian populations to parasitize the *Meloidogyne*-resistant tomato cultivars Better Boy and Celebrity and the *Meloidogyne*-susceptible cultivar Rutgers was similarly tested. All tests for reproduction on peanut or tomato were conducted in a greenhouse with a completely randomized design with a min-

TABLE 2. Perineal patterns, esterase isozyme phenotypes, and mtDNA RFLP of 14 field populations and three single-egg-mass subcultures of *Meloidogyne javanica* obtained from peanut in four governorates of Egypt.†

Population‡	Origin	Perineal pattern	Esterase isozyme	mtDNA
92-3	Ismailia	Mj	Mj-3	
92-13	Alexandria	Mj	Mj-3	
92-14	Ismailia	Mj	Mj-3	
92-15	Ismailia	unknown	Mj-3	
92-16	Ismailia	Mj	Mj-3	Mj
92-17	Ismailia	Mj	Mj-3	
92-18	Ismailia	Mj	Mj-3	
92-19	Giza	unknown	Mj-3	Mj
92-20	Behara	Mj	Mj-3	
92-21	Behara	unknown	Mj-3	
92-22	Behara	Mj	Mj-3	
92-23	Behara	Mj	Mj-2/3	Mj
92-23a	Behara	Mj	Mj-2	
92-24	Behara	unknown	Mj-2	
92-25	Behara	Mj	Mj-2/3	Mj
92-25a	Behara	Mj	Mj-2	Mj
92-25c	Behara	Mj	Mj-2	

† Mj = character typical of *Meloidogyne javanica;* for esterase phenotype Mj-3 = three-isozyme esterase phenotype, Mj-2 = two-isozyme esterase phenotype, and Mj-2/3 = mixture of individuals with two- and three-isozyme phenotype.
‡ Subcultures 92-23a, 92-25a, and 92-25c are single-egg-mass subcultures derived from 92-23 and 92-25, respectively.

imum of three replications of each nematode population–host combination.

Data on J2 morphometrics and egg production on peanut or tomato were subjected to analysis of variance using the SAS GLM procedure (16) with mean separation by least significant differences.

RESULTS

Mean J2 body length of the eight Egyptian populations examined ranged from 384 μm to 470 μm and differed among the populations ($P = 0.05$). Stylet lengths ranged from 13.3 μm to 16.1 μm but were not different among the populations (Table 1).

Eleven of 14 Egyptian field populations had three esterase isozymes detected on the polyacrylamide gels and were phenotypically identical to *M. javanica* (Table 2; Fig. 1) (1). Two other populations that were collected from contiguous fields in the Behara governorate were a mixture of two distinct esterase phenotypes. Some individuals of populations 92–23 and 92–25 had the three-isozyme esterase phenotype characteristic of *M. javanica,* whereas other individuals had only two esterase isozymes; these individuals lacked the fastest migrating isozyme present in most *M. javanica* individuals (Fig. 2). Population 92–24, obtained from a field adjacent to the fields from which 92–23 and 92–25 were collected, contained only individuals of the two-isozyme esterase phenotype. Both *M. javanica* esterase phenotypes were distinct from the esterase phenotype of *M. are-*

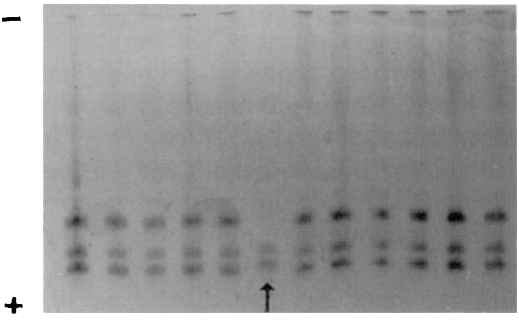

FIG. 1. Esterase isozymes identified from individual females following electrophoresis on polyacrylamide gels. Shown are the three-isozyme esterase phenotype from 11 individuals of Egyptian population 92–14 that is characteristic of *Meloidogyne javanica* and the two-isozyme esterase phenotype characteristic of *M. arenaria* (arrow).

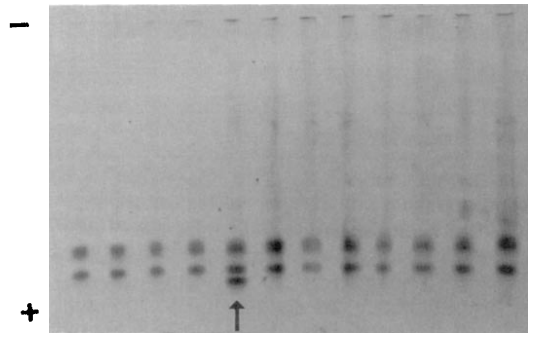

FIG. 2. Two-isozyme esterase phenotype of some populations of *Meloidogyne javanica* from the Behara governorate of Egypt (shown is subculture 92–25a) compared to the three-isozyme esterase phenotype characteristic of most populations of *M. javanica* (arrow).

naria. When single-egg mass subcultures were established from individuals with either the three-isozyme phenotype or the two-isozyme phenotype (92–23a, 92–25a, and 92–25c), esterase phenotypes of the progeny were identical with the parental type.

Perineal patterns were made from approximately 50% of the individuals macerated for esterase phenotype determination. Greater than 90% of the perineal patterns from 10 of 14 field populations had the distinct lateral fields that are characteristic of *M. javanica*, regardless of whether the individual had the two- or three-isozyme esterase phenotype (data not shown). Perineal patterns from populations 92–15, 92–19, 92–21, and 92–24 lacked distinct lateral fields.

A 1.7 kb fragment of DNA was amplified from macerates of all Egyptian populations by the polymerase chain reaction. No population produced the 1.1 kb PCR amplification product characteristic of *M. arenaria* or the 0.52 kb product characteristic of *M. hapla* (12). Digestion of the 1.7 kb fragment with Hinf 1 resulted in identical patterns of 1.0 kb and 0.7 kb fragments for the control population of *M. javanica* and populations from peanut in Egypt (Fig. 3). Digestion of the 1.7 kb amplification product from the control population of *M. incognita* resulted in fragments of 1.0 kb, 0.4 kb, and 0.3 kb (Fig. 3). The RFLP of the control populations of *M. javanica* and *M. incognita* were identical to predicted patterns (12).

All populations tested reproduced on *M. arenaria*-susceptible peanut. Although there was variation ($P = 0.05$) in reproduction among populations, none had a lower level of reproduction on Florunner than did *M. arenaria*. All populations had lower reproduction ($P = 0.05$) on the *M. arenaria*-resistant TxAG-7 than on the susceptible Florunner (Table 3). Additionally, all of the five populations of *M. javanica* tested had lower reproduction ($P = 0.05$) on the two root-knot nematode resistant tomato genotypes than on the susceptible Rutgers (Table 3).

DISCUSSION

All of the 14 field populations of root-knot recovered from peanut roots from four governorates of Egypt were identified as *M. javanica* based on morphometrics of

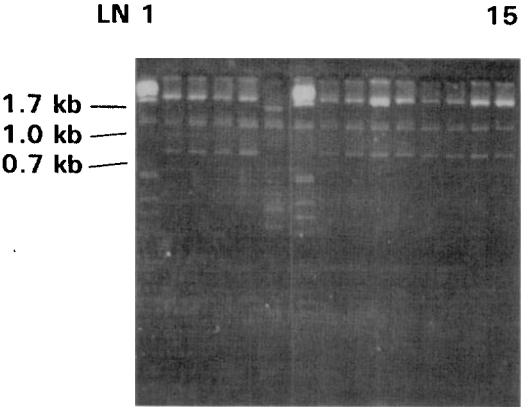

FIG. 3. Electrophoresis of products of Hinf 1 digestion of polymerase chain reaction amplification products of macerates of *Meloidogyne* second-stage juveniles. Lanes 1 and 7 are DNA size markers, lanes 2–3 are population 92–19 from Giza governorate, lanes 4–5 are 92–16 from Ismailia governorate, lane 6 is *M. incognita*, lanes 8–9 are *M. javanica* from University of Nebraska Nematology Laboratory, lanes 10–11 are 92–25, lanes 12–13 are 92–25a, and lanes 14–15 are 92–23, all from Behara governorate. Populations 92–23 and 92–25 are mixtures of two- and three-isozyme esterase phenotypes. Population 92–25a is a single-egg-mass subculture from 92–25 with the two-isozyme esterase phenotype.

TABLE 3. Reproduction (eggs per gram of roots) of populations of *Meloidogyne javanica* from peanut in Egypt and *M. arenaria* (population 82-4) on susceptible and resistant genotypes of peanut and tomato.

	Peanut		Tomato		
Population	Florunner	TxAG-7	Rutgers	Better Boy	Celebrity
82-4	2,040	40*	4,280	240*	120*
92-3	1,990	0*			
92-13	820	0*			
92-14	730	20*	19,220	10*	250*
92-15	3,770	0*			
92-17	7,550	30*			
92-18	1,580	10*	9,280	600*	1,260*
92-20	670	10*	56,520	2,300*	1,540*
92-23a	4,560	70*			
92-24	340	10*	20,030	420*	420*
92-25	1,590	300*	22,030	1,180*	2,980*
LSD 0.05	3,330	60	22,700	1,160	NS

Population 92-23a and 92-24 have two esterase isozyme phenotypes and population 92-25 is a mixture of two and three isozyme phenotypes. All other Egyptian populations have three esterase isozyme phenotypes.

* Indicates significantly lower ($P = 0.05$) egg production on resistant than on paired susceptible host genotypes. Florunner and Rutgers are susceptible genotypes.

J2 (14), perineal patterns, esterase phenotypes (1), and presence of species-specific RFLP in mtDNA (12). Since no variation in esterase phenotypes has been reported from the several hundred populations of *M. javanica* that have been examined previously, variation in this character among populations collected from three contiguous fields in the Behara governorate was unexpected. Other populations from Behara did not exhibit this variation. Because the variation appears to be due to the loss of a single, common isozyme and because all other characters were consistent with these populations being *M. javanica,* we have concluded that individuals with the two-isozyme phenotype are also *M. javanica.*

This is the first comprehensive report of *M. javanica* parasitizing peanut. As most of the previous reports were from Africa (5, 7,14) and India (11,13,15) it is possible that populations of *M. javanica* able to parasitize peanut are more common in these regions than in other parts of the world. Previously, there was a single report of *M. javanica* parasitizing peanut from the United States (8). We have recently found a population of *M. javanica* in Texas that is parasitic on peanut (unpubl. data). This population of *M. javanica* was obtained from potato grown in a field that had been in a potato–peanut rotation system and parasitized peanut in greenhouse tests. Thus, even though populations of *M. javanica* parasitic on peanut may be rare in most parts of the world (3), they are distributed on three continents (Africa, Asia, and North America).

An important finding is that the resistance to *M. arenaria* being developed in peanut (18), which is derived from three wild *Arachis* species (9), also was effective against populations of *M. javanica* parasitic on peanut. Because this resistance is from three different sources and appears to involve more than one mechanism (10) and probably different genes, it is not possible to determine from current data whether genes conferring resistance to *M. arenaria* also confer resistance to *M. javanica* or whether resistance to each nematode species is conferred by different genes.

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