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Meloidogyne partityla on Pecan: Isozyme Phenotypes and Other Hosts

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Abstract: *Meloidogyne* sp. from five pecan (*Carya illinoensis*) orchards in Texas were distinctive in host range and iszoyme profiles from common species of *Meloidogyne* but were morphologically congruent with *Meloidogyne partityla* Kleynhans, a species previously known only in South Africa. In addition to pecan, species of walnut (*Juglans hindsii* and *J. regia*) and hickory (*C. ovata*) also were hosts. No reproduction was observed on 15 other plant species from nine families, including several common hosts of other *Meloidogyne* spp. Three esterase phenotypes and two malate dehydrogenase phenotypes of *M. partityla* were identified by polyacrylamide gel electrophoresis. Each of these isozyme phenotypes was distinct from those of the more common species *M. arenaria, M. hapla, M. incognita,* and *M. javanica.*

Key words: *Carya illinoensis, C. ovata,* detection, esterase, hickory, isozyme phenotype, *Juglans hindsii, J. regia,* malate dehydrogenase, *Meloidogyne partityla,* pecan, root-knot nematode, walnut.

Pecan (*Carya illinoensis*) is an important nut crop throughout much of the southern United States, including Texas. In 1994, Texas was the second leading producer in the United States with a total production of 18,100 metric tons valued at $48 million. Root-knot nematodes are recognized pathogens of pecan (8). Soil samples from 9 of 60 orchards in Georgia were infested with *Meloidogyne incognita* (Kofoid and White) Chitwood (4). *Meloidogyne arenaria* (Neal) Chitwood was confirmed from another Georgia orchard (2). *Meloidogyne javanica* (Treub) Chitwood was parasitic on several cultivars of pecan in Spain; two additional cultivars were resistant to *M. javanica* (9). In addition to these well-known species, *Meloidogyne partityla* Kleynhans has been described from pecan in South Africa (7). Few data are available on the biology or distribution of *M. partityla* as no reports other than the original description are known. This species is believed to have been introduced into South Africa from the United States on pecan seedlings (7). In this report, we tested for the presence of *M. partityla* in Texas. Host range as well as esterase and malate dehydrogenase (MDH) phenotypes are useful in determining if the Texas population is distinct from *Meloidogyne* species previously reported on pecan. Morphological identification tests if the Texas population is cospecific with *M. partityla.* Data on additional hosts are provided also.

MATERIALS AND METHODS

Soil and root samples were collected in 1993 from five pecan orchards distributed in four counties from northeast Texas (Anderson County), central Texas (Comanche County), and southwest Texas (Maverick and Real Counties). Multiple trees were sampled in each orchard. Nematodes were extracted from soil samples by elutriation (1) and centrifugation (6). Feeder roots collected from under the canopy of each tree were rinsed with running water and then treated with 0.05% NaOCl to extract *Meloidogyne* eggs (5). Species identification was based on a comparison of the morphology and morphometrics of 80 second-stage juveniles (J2), 10 males, and 20 females with those of the original description (7).

All eggs collected from a single orchard were combined and used to inoculate replicate 15-cm-diam. pots of cotton (*Gossypium hirsutum* 'Rowden'), pecan (*C. illinoensis* 'Riverside'), sorghum (*Sorghum bicolor* 'TX7000'), soybean (*Glycine max* 'Braxton'), and tomato (*Lycopersicon esculentum* 'Rutgers'). The pots were filled with a coarse

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sand-peat soil mix (6:1, v/v). The inoculum rate varied from 1,000 to 5,000 eggs/pot, based on numbers of eggs available, and there were a minimum of three replications of each host species. Plants were harvested after 3 months, roots examined for evidence of root infection, and then eggs were extracted from pecan roots with 0.05% NaOCl (5). Permanent cultures of each isolate were established and maintained on pecan.

In a second study conducted in 1994, two isolates of the Texas population (nos. 93-6 and 93-7) were inoculated separately onto seedlings of English walnut (*Juglans regia*), California black walnut (*J. hindsii*), and shagbark hickory (*C. ovata*). The seedlings were grown in 4-liter containers filled with a sandy loam soil in a shade house. Each seedling was inoculated with 8,000 eggs obtained from permanent cultures (5). Root samples were collected and examined for evidence of nematode development and reproduction 6 months after inoculation.

An additional host test was conducted by establishing plots in an infested pecan orchard in Anderson County. The site was the same location from which *Meloidogyne* isolate no. 93-7 was collected. The mean pre-plant population density, determined from composite samples (10 2.5-cm-diam. × 25-cm deep soil cores) and extracted by elutriation (1) and centrifugation (6), collected from each plot was 930 eggs and J2/500 cm^3 soil. Fifteen plant species representing nine families (bean, *Phaseolus vulgaris;* bell pepper, *Capsicum annum;* collard, *Brassica oleracea;* cotton, *Gossypium hirsutum;* cowpea, *Vigna unguiculata;* live oak, *Quercus virginiana;* marigold, *Tagetes erecta;* okra, *Abelmoschus esculentus;* periwinkle, *Catharanthus roseus;* sorghum, *Sorghum bicolor;* soybean, *Glycine max;* squash, *Cucurbita pepa* var. *melpepo;* maize, *Zea mays;* tomato, *Lycopersicon esculentum;* and watermelon, *Citullis lanatus*) were planted in single-row plots 3 m long with 1 m between the rows. Plants were spaced 25 cm apart within the rows. There were three replications of each species. Plots were planted on 25 April 1988 and harvested 18 July 1988. Roots of all plants from each plot were examined for root galling.

Esterase and MDH phenotypes for each root-knot nematode isolate were determined from replicate samples of single females. Females were teased from roots collected from the orchards and from permanent cultures and macerated individually in 0.1 M phosphate buffer (pH 7.4) with 20% sucrose, 2% Triton X-100, and 0.1% bromophenol blue. Separation of proteins was by an automated electrophoresis system (PhastSystem, Pharmacia, Uppsala, Sweden) on 10% to 15% gradient polyacrylamide gels. Gels were subsequently stained for esterase and MDH activity (3).

Results and Discussion

Pecan trees infected with root-knot nematodes in the orchards sampled were characterized by chlorotic foliage with a thinning of the crown as compared to noninfected trees. Galling of the roots was limited, and mature female nematodes were typically exposed on the root surface.

Considering that previous reports indicated that pecans in the southern United States are parasitized by *M. incognita* and *M. arenaria* (2,4), initial observations of a perineal pattern distinctive from these and other common species warranted more rigorous identification procedures, including additional morphology, host differential tests, and biochemical diagnostics. The 20 perineal patterns examined by light and scanning electron microscopy consistently were more coarsely striated and highly angular with a squarish arch, as compared with the more finely striated and rounded patterns of *M. incognita, M. arenaria,* and *M. javanica.* Comparison with additional species of *Meloidogyne* indicated unique similarity to patterns previously described and illustrated (especially figures 10 and 11 in reference 7) for *M. partityla.* Stylets of females, males, and J2 of the Texas population were robust, and knobs were ovoid and longitudinally grooved as described for *M. partityla* (7). Stylet lengths and dis-

tance of the dorsal gland orifice to the stylet knobs of females, males, and J2 of the Texas population were all close to the mean and range of the original description of *M. partityla* (7). Additional morphometrics of females, males, and J2, including body length, width, position of excretory pore, esophagus length, tail length, and spicule length, were close to the mean and range of *M. partityla.* Whereas any given morphometric and morphologic detail is limited in its diagnostic power, the combined complete congruence of the Texas population with the description of *M. partityla* supports the hypothesis of their cospecificity.

The hypothesis that the Texas population is not a common species of *Meloidogyne* is further tested with host range. Host-range tests are not available for the type population of *M. partityla,* but are well known for *M. incognita, M. arenaria,* and *M. javanica* and include parasitism of tomato. The isolates of the Texas population, however, did not reproduce on cotton, sorghum, soybean, or tomato in greenhouse tests. No root galling or evidence of nematode development was observed on any species planted in the infested orchard. Reproduction on walnut and hickory by *M. partityla* isolate nos. 93-6 and 93-7 was confirmed in subsequent tests by observation of mature females with egg masses on feeder roots 6 months after inoculation. Known hosts for *M. partityla* are restricted to the family Juglandaceae (hickory, pecan, and walnut).

Although esterase and MDH phenotypes are not available for the type population of *M. partityla,* they are useful to confirm the hypothesis that the Texas population is unique from common species of *Meloidogyne.* Three esterase phenotypes were determined from 143 individuals and two MDH phenotypes from 100 individuals (Figs. 1,2). Each of these isozyme phenotypes was distinct from the reported phenotypes characteristic of *M. arenaria, M. hapla, M. incognita,* and *M. javanica* (3). The three esterase phenotypes were detected with approximately equal frequencies, whereas MDH phenotype A was predominant and observed from 76% of the individuals tested (Table 1). Of the 92 females for which both esterase and MDH phenotypes were determined, 42.4% had the combination of esterase phenotype A and MDH phenotype A, 29.4% had esterase B with MDH B, 22.8% had esterase

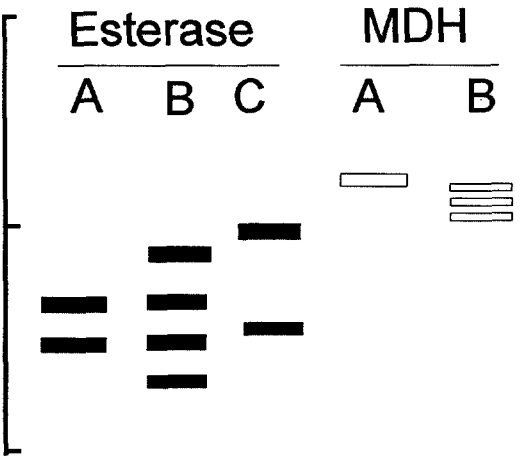

FIG. 1. Schematic diagram of esterase and malate dehydrogenase (MDH) phenotypes observed from *Meloidogyne partityla* by polyacrylamide gel electrophoresis.

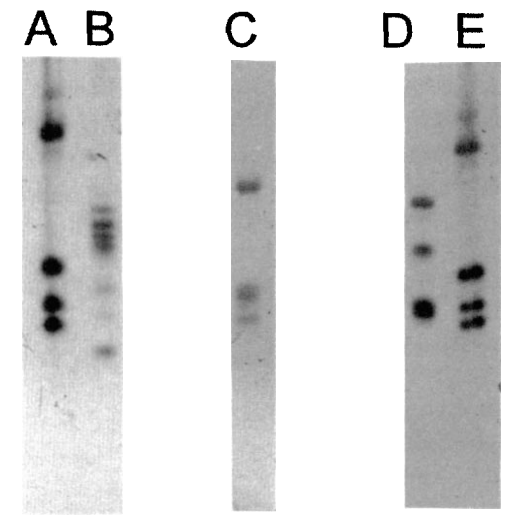

FIG. 2. Comparison of esterase and malate dehydrogenase phenotypes of *Meloidogyne partityla* with that of *M. javanica.* A and E) Esterase (lower three bands) and MDH (upper band) phenotype for *M. javanica;* B) *M. partityla* MDH phenotype B with esterase phenotype B; C) *M. partityla* MDH phenotype A with esterase phenotype A; D) *M. partityla* MDH phenotype A with esterase phenotype C.

TABLE 1. Frequencies of esterase and malate dehydrogenase (MDH) phenotypes in isolates of *Meloidogyne partityla* from pecan in Texas.

Isolate	Source (County)	Number	Esterase (%)			Number	MDH (%)	
			A	B	C		A	B
93-4	Comanche	38	100	0	0	29	100	0
93-5	Maverick	4	0	100	0	0	—	—
93-6	Real	25	0	100	0	25	100	0
93-7	Anderson	54	11	39	50	34	34	66
93-8	Comanche	22	45	0	55	10	100	0
	Total	143	37	35	28	100	76	24

See Fig. 1 for an example of the esterase and MDH phenotypes.

C with MDH A, and 5.4% had esterase C with MDH B.

The presence of *M. partityla* on pecan in Texas is consistent with the hypothesis that this species was introduced into South Africa from the United States. Kleynhans (7), citing personal communication from P. Willers, reported that "the nematode probably entered South Africa together with pecan seedlings from the U. S. A. in 1912, 1939, and 1940."

Although this survey was not extensive, it was surprising no other *Meloidogyne* spp. was associated with pecan in Texas, especially considering the reports that *M. arenaria* and *M. incognita* are parasitic on pecan (2,4) and the widespread distribution of these two species in Texas. We suspect that *M. partityla* is present also in Georgia. A *Meloidogyne* isolate with an esterase phenotype identical to esterase phenotype B from this study was observed on pecan in Georgia (R. S. Hussey, pers. comm.).

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