Journal of Nematology 37(3):313–322. 2005.

Diversity of *Meloidogyne* spp. on *Musa* in Martinique, Guadeloupe, and French Guiana[1]

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Abstract: Ninety-six isolates of *Meloidogyne* species collected from banana fields from Martinique, Guadeloupe, and French Guiana, were examined using esterase (Est) and malate dehydrogenase (Mdh) phenotypes. Adult females identified as *M. arenaria, M. incognita, M. javanica, M. cruciani, M. hispanica,* and *Meloidogyne sp.* showed species-specific phenotypes only for the esterase enzymes. Intraspecific variability among isolates of *M. arenaria, M. incognita,* and *M. javanica* was detected using Est and Mdh. Perineal patterns were used as a complementary tool together with enzyme characterization and were essential for checking the morphological consistency of the identification. The major species of *M. arenaria* and *M. incognita* were detected at 61.9% and 34.3% of the total number of isolates, respectively, and the other minor species at 3.8%. The mixed *Meloidogyne* species were detected in 45.1% of the samples. Genetic analysis was conducted using RAPD markers, which alone or in combination provided reliable polymorphisms both between and within species. RAPD analysis of the data resulted in clustering of species and isolates congruent with esterase phenotype characterization. The intraspecific variability in *M. incognita* and in *M. arenaria* represented 14.9% and 61.6% of the amplified polymorphic fragments, respectively. This high level of variation in *M. arenaria* isolates may indicate multiple origins for populations classified as *M. arenaria* or more than one species inside the same group, but more detailed morphological and DNA studies will be necessary to test this hypothesis.

Key words: banana, biochemical identification, electrophoresis, *Meloidogyne* spp., *Musa,* RAPD.

In recent years, the root-knot nematodes (RKN), *Meloidogyne* spp., have been found more and more frequently during routine nematode analysis of banana roots in Martinique and Guadeloupe islands (French West Indies) instead of the formerly omnipresent burrowing nematode *Radopholus similis* (Cobb) Thorne. This observation seems primarily linked with the decline in prevalence of *R. similis* correlative to the increased use of nematode-free banana vitroplantlets when old fields are replanted after appropriate fallow and cultivation practices. Different species of *Meloidogyne* may invade banana roots and associated weeds in banana fields (De Waele, 2000; Gowen and Quénéhervé, 1990). The specific identification of these root-knot nematodes is the first requirement for devising better control methods (appropriate fallow, weed management, use of non-host and (or) antagonistic plants, use of tolerant or resistant cultivars) in an integrated pest management program for bananas and other cultivated crops. The biochemical electrophoretic procedure, esterase, and malate dehydrogenase phenotypes are rapid and efficient methods of identification, and many individual females can be compared in the same gel (Esbenshade and Triantaphyllou, 1990). In parallel, egg-masses can be saved and used later as inoculum to obtain progeny of the individual females (Carneiro and Almeida, 2001). These features make this biochemical approach a valuable tool in *Meloidogyne* research and confirm that enzyme phenotype can be used to carry out extensive field surveys to determine the frequency and relative distribution of *Meloidogyne* spp. and to detect atypical phenotypes (Carneiro et al., 1996, 2000; Esbenshade and Triantaphyllou, 1985, 1990).

However, enzymatic phenotypes provide insufficient information at the intraspecific level. Early molecular analysis mostly used Random Amplified Polymorphic DNA (RAPD), a polymerase chain reaction (PCR) amplification of genomic DNA using a single oligonucleotide primer that can reveal considerable genetic variation, even between closely related organisms (Welsh and McClelland, 1990; Williams et al., 1990). As a result, it has been extensively used as a genetic marker for estimating genetic, taxonomic, and phylogenetic relationships among root-knot nematodes (Baum et al., 1994; Blok et al., 1997; Castagnone-Sereno et al., 1994; Cenis, 1993; Chacon et al., 1994; Randig et al., 2002).

The objectives of the present work were to characterize and identify the species of *Meloidogyne* in banana fields in Martinique, Guadeloupe, and French Guiana and to identify the predominant species. We also examined the intraspecific variation and relationships among the isolates using RAPD-PCR.

Materials and Methods

Fifty-five banana fields in Martinique, 28 in Guadeloupe, and 13 in French Guiana, representative of different ecological situations in terms of soil type, climate, and field history, were selected for their known infestation with RKN (Tables 1, 2, 3). In each selected field, root samples were collected at random on different banana plants. *Meloidogyne* spp. females were dissected from roots using a stereomicroscope and directly

Received for publication 27 April 2004.

[1] This research was a part of the PhD thesis of the first author and was supported by Institut de Recherche pour le Développement, Martinique, France; Embrapa Recursos Genéticos e Biotecnologia, DF, Brazil; and Conselho Nacional de Pesquisa, DF, Brazil.

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The authors acknowledge Hervé Mauleon and Dominique Denon from INRA in Guadeloupe, and Jean-Michel Risède and Max Vingadassalon from CIRAD for their technical assistance, and, in French Guiana, Guy Tiego from the Plant Protection Service for its assistance.

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This paper was edited by R. T. Robbins.

TABLE 1. Population code, origin, soil type, enzyme phenotype, and percentage occurrence of *Meloidogyne* spp. on "Cavendish" bananas from Martinique.

Population code	Township	Soil type	Species	Enzyme phenotype[b]		Occurrence[c] %
				Est	Mdh	
1	Saint Joseph	Halloysitic	*M. arenaria*	A2	N1	100
2	Saint Joseph	Halloysitic	*M. incognita*	I2	N1	100
3 A	Saint Joseph	Halloysitic	*M. arenaria*	A2	N1	80
3 B		Halloysitic	*M. incognita*	I2	N1	20
4	Carbet	Allophanic	*M. arenaria*	A2	N1	100
5	Carbet	Allophanic	*M. incognita*	I2	N1	100
6 A	Carbet	Allophanic	*M. arenaria*	A2	N1	11.7
6 B		Allophanic	*M. incognita*	I2	N1	88.3
7 A	Carbet	Allophanic	*M. arenaria*	A2	N1	50
7 B		Allophanic	*M. incognita*	I2	N1	50
8 A[a]	Carbet	Allophanic	*M. incognita*	I2	N1	38.4
8 B[a]		Allophanic	*Meloidogyne* sp.	B2	N1	7.8
8 C		Allophanic	*M. hispanica*	Hi3	N3	53.8
9 A	Carbet	Allophanic	*M. arenaria*	A2	N1	45.2
9 B		Allophanic	*M. arenaria*	A2	N3	32.3
9 C		Allophanic	*M. incognita*	I2	N1	22.5
10	Ajoupa Bouillon	Andosol	*M. arenaria*	A2	N1	100
11	Ajoupa Bouillon	Andosol	*M. arenaria*	A2	N1	100
12	Ajoupa Bouillon	Andosol	*M. arenaria*	A2	N1	100
13	Ajoupa Bouillon	Andosol	*M. arenaria*	A2	N1	100
14[a]	Ajoupa Bouillon	Andosol	*M. arenaria*	A2	N1	100
15	Ajoupa Bouillon	Andosol	*M. arenaria*	A2	N1	100
16	Ajoupa Bouillon	Andosol	*M. arenaria*	A2	N1	100
17	Sainte Marie	Ferrisol	*M. arenaria*	A2	N1	100
18	Sainte Marie	Ferrisol	*M. arenaria*	A2	N1	100
19	Sainte Marie	Ferrisol	*M. incognita*	I2	N1	100
20	Gros-Morne	Halloysitic	*M. arenaria*	A2	N1	100
21	Gros-Morne	Halloysitic	*M. arenaria*	A2	N1	100
22	Trinité	Ferrisol	*M. arenaria*	A2	N1	100
23	Trinité	Ferrisol	*M. arenaria*	A2	N1	100
24	Trinité	Ferrisol	*M. incognita*	I2	N1	100
25 A	Trinité	Ferrisol	*M. arenaria*	A2	N1	60
25 B	Trinité	Ferrisol	*M. incognita*	I2	N1	40
26	François	Ferrisol	*M. arenaria*	A2	N1	100
27	François	Ferrisol	*M. arenaria*	A2	N1	100
28	François	Ferrisol	*M. arenaria*	A2	N3	100
29 A	François	Ferrisol	*M. arenaria*	A2	N1	55
29 B		Ferrisol	*M. arenaria*	A2	N3	45
30 A	François	Ferrisol	*M. arenaria*	A2	N1	80
30 B		Ferrisol	*M. incognita*	I2	N1	20
31 A	François	Ferrisol	*M. arenaria*	A2	N1	40
31 B		Ferrisol	*M. incognita*	I2	N1	60
32	Vauclin	Vertisol	*M. arenaria*	A2	N1	100
33	Vauclin	Vertisol	*M. arenaria*	A2	N1	100
34	Vauclin	Vertisol	*M. arenaria*	A2	N1	100
35	Vauclin	Vertisol	*M. incognita*	I2	N1	100
36 A	Vauclin	Vertisol	*M. arenaria*	A2	N1	34.5
36 B		Vertisol	*M. arenaria*	A2	N3	65.5
37 A	Vauclin	Vertisol	*M. arenaria*	A2	N1	89
37 B		Vertisol	*M. incognita*	I2	N1	11
38	Lamentin	Alluvial	*M. arenaria*	A2	N1	100
39 A	Saint-Pierre	Allophanic	*M. incognita*	I2	N1	100
40	Saint-Pierre	Allophanic	*M. incognita*	I2	N1	100
41	Saint-Pierre	Allophanic	*M. incognita*	I2	N1	100
42	Saint-Pierre	Allophanic	*M. incognita*	I2	N1	100
43 A	Saint-Pierre	Allophanic	*M. arenaria*	A2	N1	52.4
43 B		Allophanic	*M. incognita*	I2	N1	47.6
44	Basse Pointe	Allophanic	*M. incognita*	I2	N1	100
45	Basse Pointe	Allophanic	*M. incognita*	I2	N1	100
46	Basse Pointe	Allophanic	*M. incognita*	I2	N1	100
47 A	Basse Pointe	Allophanic	*M. arenaria*	A2	N1	35.7
47 B		Allophanic	*M. incognita*	I2	N1	64.3
48 A	Marigot	Halloysitic	*M. incognita*	I2	N1	100
49 A	Marigot	Halloysitic	*M. arenaria*	A2	N1	50
49 B		Halloysitic	*M. arenaria*	A2	N3	50

TABLE 1. *Continued*

Population code	Township	Soil type	Species	Enzyme phenotype[b]		Occurrence[c] %
				Est	Mdh	
50	Macouba	Allophanic	*M. incognita*	I2	N1	100
51	Macouba	Allophanic	*M. incognita*	I2	N1	100
52 A	Macouba	Allophanic	*M. arenaria*	A2	N1	28.6
52 B		Allophanic	*M. incognita*	I2	N1	71.4
53 A	Macouba	Allophanic	*M. incognita*	I2	N1	80
53 B		Allophanic	*M. incognita*	I1	N1	20
54 A	Sainte-Anne	Vertisol	*M. arenaria*	A2	N1	20
54 B		Vertisol	*M. incognita*	I2	N1	80
55 A	Sainte-Anne	Vertisol	*M. arenaria*	A2	N1	81.8
55 B	Sainte-Anne	Vertisol	*M. incognita*	I2	N1	18.2

[a] *Meloidogyne* spp. isolates used in morphological and DNA analyses.
[b] Phenotype designations: Est = esterase, Mdh = malate dehydrogenase.
[c] Percentage of occurrence of *Meloidogyne* spp. phenotypes in 10 analyzed females.

analyzed to obtain isozyme profiles of esterase (Est) and malate dehydrogenase (Mdh) (Esbenshade and Triantaphyllous, 1985, 1990). Electrophoresis was performed with PhastGel gradient 10% to 15%, in discontinuous buffer system using an automated system (PhastSystem, GMI, Inc., Ramsey, MN). Ten young females were collected directly from the banana root sample and individually macerated in 0.6 µl of extraction buffer containing 20% sucrose, 2% Triton X-100, and 0.01% Bromophenol blue. The macerate was applied with two combs in the electrophoresis apparatus (Karssen et al., 1995). On each gel slab the protein extracts of two females of *M. javanica* (Treub) Chitwood were included as a reference standard.

Gels were stained for esterase activity for 60 min, and for malate dehydrogenase for 10 min (Esbenshade and Triantaphyllou, 1985). Gels were washed and placed in a solution containing 10% acetic acid, 8% glycerol, and 82% water for 1 day. Gels were pressed between two cellophane sheets and left to dry (Carneiro and Almeida, 2001). Enzyme phenotypes were designated with letter(s) suggestive of the species and the number of bands (Esbenshade and Triantaphyllou, 1985, 1990).

Due to limitations in the biological material available, the RAPD and morphological analyses were performed only in 16 isolates, which were single egg mass isolates and transported to Brazil (Embrapa Recursos Genéticos e Biotecnologia, Brasília, DF) to complete the analyses. These comprised two isolates of *M. javanica,* two isolates of *M. cruciani* Garcia-Martinez, Taylor & Smart, 1982, seven isolates of *M. arenaria* (Neal) Chitwood, three isolates of *M. incognita* (Kofoid & White) Chitwood, and two isolates of an unidentified *Meloidogyne* species. Unfortunately, the isolates of *M. hispanica* Hirschmann, *M. incognita* (I2N3), and the isolate *Meloidogyne* sp. (B0N1) without esterase bands were lost during the transportation to Brazil (Tables 1, 2, 3).

For the morphological studies, perineal patterns were cut from females in 45% lactic acid and mounted in glycerin (Taylor and Netscher, 1974), and at least 30 specimens were examined from each isolate.

For molecular analysis, the eggs of each isolate were extracted using the method of Cofcewicz et al. (2004). Total genomic DNA was extracted from 150 to 200 µl of nematode eggs of each available isolate that had been stored at −80°C. Eggs were placed in liquid nitrogen and ground with a pestle and mortar. The DNA was purified from the resulting powder by a phenol-chloroform extraction (Sambrook et al., 1989). Following ethanol precipitation, DNA was resuspended in TE buffer.

Forty-one random 10-mer primers, purchased from Eurogentec (Herstal, Belgium), were used in RAPD experiments (Table 4). RAPD-PCR was performed in a final volume of 25 µl containing 5 ng of total genomic DNA; 80 pM of primer, dATP, dCTP, dGTP, and dTTP each at 200 µM final concentration; 1xTaq incubation buffer; and 1.25 units Taq polymerase (Phoneutria Biotechnologia & Serviços, SP, Brazil). Amplification was performed on a PTC-100MJ Research thermal cycler (MJ Research Inc., Waltham, MA). The cycling program was 1 min at 94 °C, 40 cycles of 20 sec at 94 °C, 30 sec at 36 °C, and 2 min at 70 °C. Amplification products were separated by electrophoresis in 1.4% agarose gels in TBE buffer at a constant current of 150 mA for approximately 3 hours and visualized with ethidium bromide (0.5 µg ml^{-1}) under UV light.

Observed DNA bands were scored as present or absent directly from the gels. For each isolate, two independent PCR reactions were electrophoresed in the same gel; only DNA fragments consistently present or absent in these replicated samples were scored as binary characters. Each reaction was repeated at least once. DNA fingerprints from each isolate were converted to a 0-1 matrix and a phylogenetic analysis was conducted using the computer program PAUP*4.0 (Swofford, 1998). Distance analysis was performed according to the UPGMA method. One thousand bootstrap replicates were performed to test the node sup-

TABLE 2. Population code, origin, soil type, enzyme phenotype, and percentage occurrence of *Meloidogyne* spp. on "Cavendish" bananas from Guadeloupe.

Population code	Township	Soil type	Species	Enzyme phenotype[b]		Occurrence[c] %
				Est	Mdh	
1	Saint-Caude	Andosol	*M. arenaria*	A2	N1	100
2	Saint-Caude	Andosol	*M. arenaria*	A2	N1	100
3	Saint-Caude	Andosol	*M. arenaria*	A2	N1	100
4	Goyave	Ferrisol	*M. arenaria*	A2	N1	100
5 A	Goyave	Ferrisol	*M. arenaria*	A2	N1	78.9
5 B[a]		Ferrisol	*M. arenaria*	A2	N3	21.1
6 A	Goyave	Ferrisol	*M. arenaria*	A2	N1	82.3
6 B		Ferrisol	*M. incognita*	I2	N1	17.7
7	Trois-Rivières	Andosol	*M. arenaria*	A2	N1	100
8 A	Trois-Rivières	Andosol	*M. arenaria*	A2	N1	81.8
8 B		Andosol	*M. incognita*	I2	N1	18.2
9 A	Trois-Rivières	Andosol	*M. arenaria*	A2	N1	9.9
9 B		Andosol	*M. incognita*	I2	N1	90.1
10	Baie-Mahault	Ferrisol	*M. arenaria*	A2	N1	100
11[a]	Capesterre-Belle-Eau	Halloysitic	*M. arenaria*	A2	N1	100
12	Capesterre-Belle-Eau	Andosol	*M. arenaria*	A2	N1	100
13	Capesterre-Belle-Eau	Andosol	*M. arenaria*	A2	N1	100
14	Capesterre-Belle-Eau	Andosol	*M. arenaria*	A2	N1	100
15	Capesterre-Belle-Eau	Alluvial	*M. arenaria*	A2	N1	100
16	Capesterre-Belle-Eau	Andosol	*M. arenaria*	A2	N1	100
17 A	Capesterre-Belle-Eau	Andosol	*M. arenaria*	A2	N1	70.6
17 B		Andosol	*M. incognita*	I2	N1	29.4
18 A	Capesterre-Belle-Eau	Andosol	*M. arenaria*	A2	N1	68.2
18 B[a]		Andosol	*M. cruciani*	Cr3	N1	31.8
19 A	Capesterre-Belle-Eau	Andosol	*M. arenaria*	A2	N1	72.7
19 B[a]		Andosol	*Meloidogyne* sp.	Ba2	N1	27.3
20	Capesterre-Belle-Eau	Andosol	*Meloidogyne* sp.	B0	N1	100
21	Petit-Canal	Vertisol	*M. arenaria*	A2	N1	100
22	Bouillante	Ferrisol	*M. arenaria*	A2	N1	100
23[a]	Vieux-Habitants	Ferrisol	*M. arenaria*	A2	N1	100
24 A	Vieux-Habitants	Alluvial	*M. arenaria*	A2	N1	66.7
24 B		Alluvial	*M. incognita*	I2	N1	33.3
25 A	Vieux-Habitants	Alluvial	*M. arenaria*	A2	N1	81.8
25 B[a]		Alluvial	*M. incognita*	I2	N1	18.2
26	Saint-François	Vertisol	*M. incognita*	I2	N1	100
27 A	Petit Bourg	Ferrisol	*M. arenaria*	A2	N1	27.3
27 B		Ferrisol	*M. incognita*	I2	N1	72.7
28 A	San Julian	Vertisol	*M. incognita*	I2	N1	13.3
28 B		Vertisol	*M. arenaria*	A2	N1	60
28 C[a]		Vertisol	*M. arenaria*	A1	N1	26.7

[a] *Meloidogyne* spp. isolates used in morphological and DNA analyses.
[b] Phenotype designation: Est = esterase, Mdh = malate dehydrogenase.
[c] Percentage of occurrence of *Meloidogyne* spp. phenotypes in 10 analyzed females.

port (Felsenstein, 1985), and a consensus dendrogram was computed.

RESULTS

Esterase characterization: Eleven bands for Est activity were detected in the 96 *Meloidogyne* populations. A distinct Est-phenotype was associated with the major and minor *Meloidogyne* species (Tables 1, 2, 3, 4; Figs. 1, 2).

The species-specific phenotype J3 (Rm: 1.0, 1.25, 1.4) and phenotype J2 (Rm: 1.0, 1.25) were detected in two *M. javanica* populations from French Guiana (Table 3; Figs. 1, 2).

The phenotype I2 (Rm 1.05), with a minor band (Rm 1.10), and phenotype I1 (Rm 1.0) were detected in 39 and 3 populations of *M. incognita,* respectively, from Martinique (Table 1; Figs. 1, 2) and French Guiana (Table 3; Figs. 1, 2). In Guadeloupe nine populations with the phenotype I2 were detected, but the phenotype I1 was not observed (Table 2; Figs. 1, 2).

The phenotype A2 (Rm 1.2, 1.3) was detected in 41 populations of *M. arenaria* from Martinique (Table 1; Figs. 1, 2), in 27 populations from Guadeloupe (Table 2; Figs. 1, 2), and in nine populations from French Guiana (Table 3; Figs. 1, 2). Only one population with the phenotype A1 (Rm 1.2) was detected from Guadeloupe (Table 2; Figs. 1, 2).

The phenotype Cr3 of *M. cruciani* with three bands (Rm 1.05, 1.28, 1.38) was detected in two populations from Guadeloupe and French Guiana.

TABLE 3. Population code, origin, soil type, enzyme phenotype, and percentage occurrence of *Meloidogyne* spp. on "Cavendish" bananas from French Guiana.

Population code	Township	Soil type	Species	Enzyme phenotypes[b] Est	Enzyme phenotypes[b] Mdh	Occurrence[c] %
1 A[a]	Macouria	Alluvial	*M. incognita*	I2	N1	50
1 B[a]		Alluvial	*M. cruciani*	Cr3	N3	40
1 C[a]		Alluvial	*M. javanica*	J3	N1	10
2	Macouria	Alluvial	*M. incognita*	I2	N1	100
3 A	Macouria	Alluvial	*M. incognita*	I2	N1	80
3 B[a]		Alluvial	*M. arenaria*	A2	N3	20
4 A	Macouria	Alluvial	*M. arenaria*	A2	N3	90.9
4 B[a]		Alluvial	*M. javanica*	J2	N1	9.1
5 A	Macouria	Alluvial	*M. arenaria*	A2	N3	93.3
5 B		Alluvial	*M. incognita*	I2	N1	6.7
6 A	Macouria	Alluvial	*M. arenaria*	A2	N1	27.3
6 B		Alluvial	*M. incognita*	I2	N1	72.7
7	Régina	Ferrisol	*M. incognita*	I1	N1	100
8	Régina	Ferrisol	*M. arenaria*	A2	N1	100
9 A	Cacao	Ferrisol	*M. arenaria*	A2	N1	27.3
9 B		Ferrisol	*M. arenaria*	A2	N3	72.7
10 A	Cacao	Ferrisol	*M. incognita*	I2	N1	33.3
10 B		Ferrisol	*M. arenaria*	A2	N1	66.7
11 A	Counamama	Alluvial	*M. incognita*	I1	N1	10
11 B		Alluvial	*M. incognita*	I2	N1	90
12	Counamama	Alluvial	*M. incognita*	I2	N1	100
13[a]	Rocoucoua	Ferrisol	*M. arenaria*	A2	N1	100

[a] *Meloidogyne* spp. isolates used in morphological and DNA analyses.
[b] Phenotype designations: Est = esterase, Mdh = malate dehydrogenase.
[c] Percentage of occurrence of *Meloidogyne* spp. phenotypes in 10 analyzed females.

The phenotype Ba2 (Rm 1.05, 1.38) was detected in one population from Guadeloupe and is a new esterase phenotype.

In Martinique, the phenotype Hi3 (Rm 0.8, 0.9, 1.05) of *M. hispanica* (Hirschmann, 1986) was detected mixed with one population of *Meloidogyne* sp., Est phenotype B2 (Rm 0.9, 1.05). One population (*Meloidogyne* sp.) from Guadeloupe (Table 2) did not give any major esterase bands (B0).

In the banana field conditions of Martinique, *M. arenaria, M. incognita,* and *Meloidogyne* sp., were detected in 58.8%, 40.1%, and 1.1%, respectively. Of the 55 analyzed areas, 34.6% presented multiple species and the other 65.4% individual species (Table 1).

In Guadeloupe, *M. arenaria, M. incognita,* and *Meloidogyne* spp. were detected in 80.9%, 13.4%, and 5.7% of the samples, respectively. Of the 28 locations sampled, 39.3% contained multiple species and the other 60.7% contained an individual species (Table 2).

In French Guiana, *M. arenaria, M. incognita,* and *Meloidogyne* spp. were detected in 46.0%, 49.4%, and 4.6%, respectively. Of the 13 locations sampled, 61.5% contained multiple species and the other 38.5% contained an individual species, with *M. arenaria* and *M. incognita* prevailing (Table 3).

Malate Dehydrogenase (Mdh): Three bands of Mdh activity were detected in the 96 populations of RKN studied (Fig. 1). Two distinct phenotypes were recognized (Fig. 1). However, the phenotypes were not species-specific and were designated by the letter N for nonspecific phenotype and a number indicating the number of bands of activity.

The Mdh N1 (Rm 1.0) and N3 (Rm 1.0, 1.1, 1.2) differentiated two populations of *M. arenaria.* Phenotype N1 was detected in 69, 39, and 17 populations from Martinique, Guadeloupe, and French Guiana, respectively. Phenotype N3 was detected in six, one, and five populations.

The populations of *M. incognita* were differentiated for Mdh phenotypes in French Guiana, where phenotype N1 appeared in 10 populations and N3 in one population. All the minor species, except *M. hispanica* (N3), had the phenotype N1.

Morphological features: The *M. arenaria* perineal patterns as described by Eisenback and Triantaphyllou (1991) were observed in the isolates of phenotypes A2N1, A1N1. The *M. arenaria* isolated with phenotypes A2N3 had the different perineal pattern reported previously by Cliff and Hirschmann (1985). Dorsal arch was usually high and rounded to squarish in shape. Occasionally dorsal striae form shoulders in lateral regions of the pattern. Lateral lines near the tail tip were often widely spaced, with few broken irregular striae between lines. Anteriorly from the tail tip lines became finer. Dorsal and ventral striae were wavy, irregular, or forked near lateral lines but usually smooth thoughout remainder of the pattern, never a forming zigzag pattern (Cliff and Hirschmann, 1985).

TABLE 4. RAPD primers used and number of amplified fragments scored.

Primer	5′ sequence 3′	N° amplified fragments				
		% GC	Minimum/ isolate	Maximum/isolate	Polymorphic	Total
A01	CAG GCC CTT C	70	7	14	28	28
A04	AAT CGG GCT G	60	3	15	22	24
A12	TCG GCG ATA G	60	5	8	17	17
B11	GTA TAC CCG T	50	4	10	13	15
B12	CCT TGA CGC A	60	7	12	13	14
B17	AGG GAA CGA G	60	3	6	9	10
C02	GTG AGG CGT C	70	4	10	20	20
C07	GTC CCG ACG A	70	2	12	22	23
C09	CTC ACC GTC C	70	5	8	9	12
C16	CAC ACT CCA G	60	3	9	17	18
D05	TGA GCG GAC A	60	8	14	16	18
D13	GGG GTG ACG A	70	9	14	17	21
E07	AGA TGC AGC C	60	8	17	17	22
G02	GGC ACT GAG G	70	6	17	21	23
G03	GAG CCC TCC A	70	2	5	8	9
G05	CTG AGA CGG A	60	5	13	23	24
G06	GTG CCT AAC C	60	1	12	20	21
G13	CTC TCC GCC A	70	7	13	18	20
J10	AAG CCC GAG G	70	5	13	24	25
J20	AAG CGG CCT C	70	5	12	14	16
K01	CAT TCG AGC C	60	4	12	17	18
K04	CCG CCC AAA C	70	10	22	38	40
K06	CAC CTT TCC C	60	9	16	23	24
K10	CAC CTT TCC C	60	4	12	17	20
K14	CCC GCT ACA C	70	4	7	15	15
K16	GAG CGT CGA A	60	7	12	19	21
K19	CAC AGG CGG A	70	9	14	18	20
K20	GTG TCG CGA G	70	9	18	29	30
L08	AGC AGG TGG A	60	8	13	18	21
M20	AGG TCT TGG G	60	8	16	27	29
N07	CAG CCC AGA G	70	4	10	11	12
P01	GTA GCA CTC C	60	6	16	24	26
P02	TCG GCA CGC A	70	8	11	12	16
PO5	CCC CGG TAA C	70	5	12	16	17
R05	GAC CTA GTG G	60	9	15	22	23
R07	ACT GGC CTG A	60	6	12	21	21
R08	CCC GTT GCC T	70	11	13	28	29
AB02	GGA AAC CCC T	60	9	14	31	32
AB04	GGC ACG GGT T	70	7	16	22	24
AB06	GTG GCT TGG A	60	6	16	29	30
AB09	GGG CGA CTA C	70	10	22	31	33
Total			-	-	816	881

The phenotype I2N1 had perineal patterns typical of *M. incognita* and the two isolates of *M. javanica,* phenotypes J3N1 and J2N1, had perineal patterns like those described for the species (Eisenback and Triantaphyllou, 1991).

The phenotype Cr3N1 had perineal patterns with subcuticular punctuations surrounding the anus on the lateral and posterior side; striae deep, wavy, sometimes broken; lateral field deep with distinct phasmids; and tail terminus indistinct, as described for *M. cruciani.* (García-Martinez et al., 1982). Some perineal patterns of this species resembled those of *M. javanica* in having distinct lateral lines; however, the lateral lines of *M. cruciani* did not extend as far as those of *M. javanica.*

The phenotype B2N1 had perineal patterns elongated to ovoid with a flattened-to-high, squarish dorsal arch with widely spaced coarse striae. Lateral fields may have wing-like striae on one or both sides, a well-defined tail tip area with striations in most specimens, and an anus distinct and often covered by a thick cuticular fold.

RAPD characterization: With the 41 random primers used, the number of reproducible fragments varied from 1 to 22/isolate, and ranged from ca 200 to ca 4,000 bp in size. One gel containing the 16 isolate patterns obtained with primer E07 is shown in Figure 3. The global results of the RAPD analysis are provided in Table 4. Over the entire experiment, each primer produced from eight to 38 polymorphic bands. Overall, 881 fragments were amplified and scored as RAPD markers, 816 of which were polymorphic, and 65 were found to be amplified in all the isolates tested (i.e., monomorphic).

For the four species for which more than one isolate

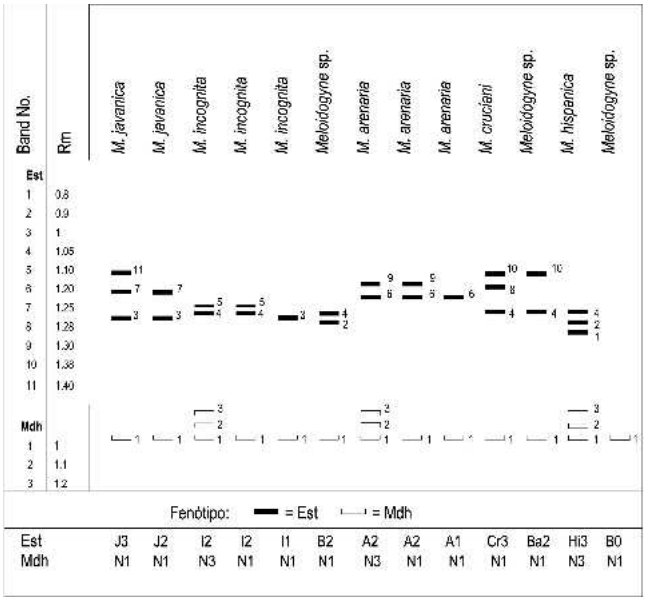

FIG. 1. Phenotypes of esterase (Est) and malate dehydrogenase (Mdh) observed in 96 populations of *Meloidogyne* spp. from *Musa.* (For origin of populations, see Tables 1, 2, and 3.)

was available (i.e., *M. arenaria, M. incognita, M. javanica,* and *M. cruciani*), 307 to 576 reproducible fragments were amplified (Table 5). For *M. incognita, M. javanica,* and *M. cruciani,* fewer RAPD bands were polymorphic (14.9%, 16.6%, and 16.1%, respectively). For *M. arenaria,* if all esterase phenotypes are placed in a single group (Table 5), 61.6% of the amplified bands were polymorphic. If the different esterase phenotypes were separated into sub-groups (A2N1, A1N1 + A2N1 and A2N3), then 29%, 37.3% and 23.2%, respectively, of the bands were polymorphic.

In the dendrogram of relationships between the 16 isolates based on the RAPD polymorphisms, the isolates belonging to a given species always clustered together (Fig. 4). These results confirmed the existence of three separate groups corresponding to the different species: (i) the two isolates of *M. javanica* clustered together with a bootstrap support of 100% in the analysis; (ii) the two isolates of *M. cruciani* clustered together (bootstrap support of 100%); (iii) *M. javanica* and *M. cruciani* clustered together with 62% of bootstrap support and 57.4% of polymorphism; (iv) all isolates of *M. arenaria* (A2N1, A1N1, and A2N3) were clustered together, but the support for their clustering was much lower than that for the other species (52% in the bootstrap); (v) *M. arenaria* isolates phenotypes (A2N1 and A1N1) clustered together with a bootstrap of 100%; (vi) the atypical isolate phenotype Ba2N1 (19 B Gua) clustered together with *M. arenaria* (A2N1, A1N1) with a bootstrap of 92%, but with polymorphism of 53.7%; (vi) *M. arenaria* isolates A2N3 clustered together with a bootstrap of 100%; (vii) all isolates of *M. incognita* clustered together (100% of bootstrap) and with the other atypical isolate phenotype B2N1 with a high confidence level (100% of bootstrap), but with a polymorphism of 62.4%.

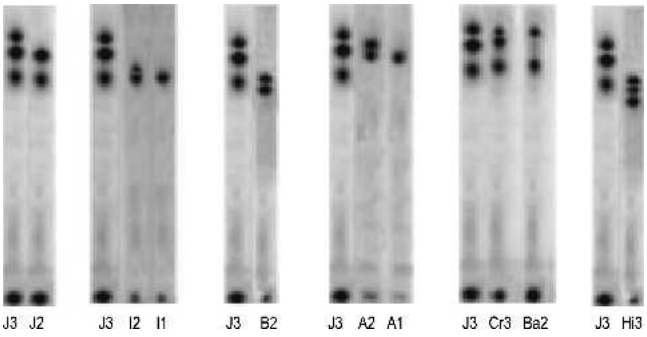

FIG. 2. Eleven esterase phenotypes found in 96 populations of *Meloidogyne* spp. (For explanation of phenotype designations, see Fig. 1.)

DISCUSSION

This study presents a characterization of *Meloidogyne* species collected on banana fields in Martinique, Guadeloupe, and French Guiana, using isozyme phenotype (Est and Mdh), morphology, and DNA approaches. A total of 96 banana fields were sampled and analyzed. This was the most detailed and extensive RKN survey to have been carried out in the French Overseas Departments.

Results confirm that the use of esterase phenotypes is a rapid and efficient method to i) characterize *Meloidogyne* species and detect atypical forms, ii) determine the frequency and relative distribution of *Meloidogyne* spp. in field surveys, and iii) detect mixed species populations prior to conducting subsequent studies such as DNA analyses and morphological characterization (Carneiro et al., 1996, 2000; Cofcewiz et al., 2004; Esbenshade and Triantaphyllou, 1985, 1990).

Malate dehydrogenase phenotypes detected intraspecific variability only among isolates of *M. arenaria* and *M. incognita.* A similar result was observed among populations of *Meloidogyne* from Portugal (Pais and Abrantes, 1989).

The perineal pattern is often an unreliable character for making diagnostic conclusions when used alone but, when used as a complement to isozyme phenotypes, species identification is more robust (Carneiro et al., 2004). The RAPD polymorphism data were consistent with isozyme phenotype data and morphology for identification of species and estimating genetic relationship and diversity among isolates (Carneiro et al., 2004; Cofcewicz et al. 2004).

Meloidogyne arenaria was the most common species on banana in Martinique, Guadeloupe, and French Guiana. The phenotypes A3, A2, and A1 were species-specific for *M. arenaria* (Esbenshade and Triantaphyllou, 1985, 1990), with the A2N1 phenotype being most common. The phenotype A1N1 detected only once in Guadeloupe and the A2N3 phenotype was intermediate

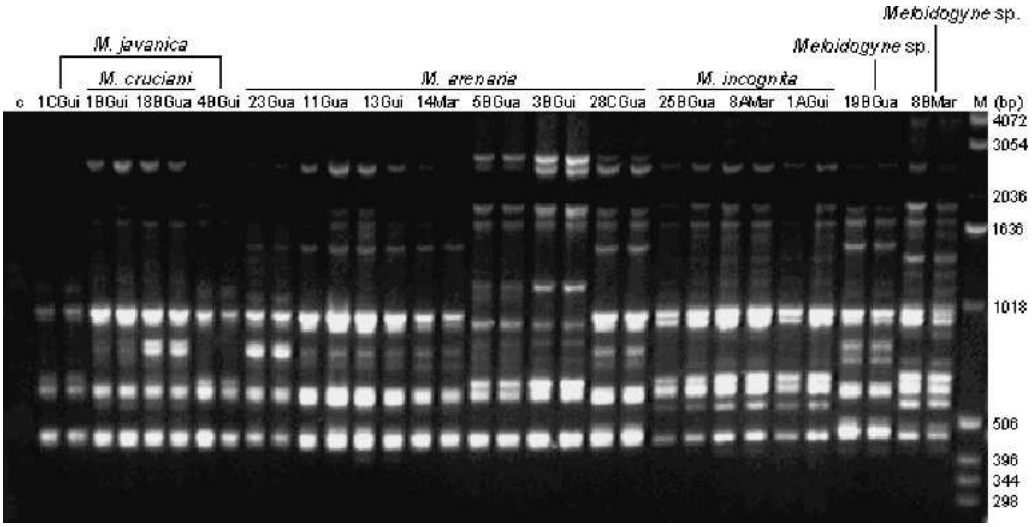

FIG. 3. RAPD patterns for 16 *Meloidogyne* spp. isolates generated with primer E07. For each isolate, two duplicate amplifications were loaded side by side on the gel. (C: control reaction without template DNA. M: Nucleic acid marker in bp. Population codes are given in Tables 1, 2, and 3.)

in occurence and was detected in Martinique, Guadeloupe, and French Guiana. The A3N1 phenotypes did not appear during this survey but were detected from banana in Brazil (Cofcewicz et al., 2004). The *M. arenaria* isolates with phenotype A2N1 clustered with 99% of bootstrap and separated from the isolates A1N1 and A2N3, showing a very high intraspecific variability based on DNA analysis and congruent with isozyme phenotypes. These results agree with the previous molecular analysis on the genome (Castagnone—Sereno et al., 1994; Cofcewicz et al., 2004; Randig et al., 2002; Semblat et al., 1998). The isolates A2N1 and A1N1 presented perineal patterns like those described for *M. arenaria,* but the isolates with phenotype A2N3 had different perineal patterns, as previously demonstrated (Cliff and Hirschmann, 1985). *Meloidogyne arenaria* is known to be morphologically, physiologically, and cytogenetically variable (Cliff and Hirschmann, 1985; Triantaphyllou, 1985). This variation may indicate multiple origins for populations classified as *M. arenaria* or possibly a species complex. Alternatively, these variations may indicate that *M. arenaria* is an old species that has diverged considerably through multiple mutations and adaptations to different environments (Esbenshade and Triantaphyllou, 1987).

The atypical population with the Ba2N1 had a new esterase phenotype not previously detected. This population also had a perineal pattern similar to those of *M. arenaria* and in RAPD analysis clustered with the group *M. arenaria* with high polymorphism. Additional studies will be necessary to identify this isolate.

The Caribbean populations of *M. incognita* had two esterase phenotypes: I2 and occasionally I1. The same phenotypes were observed by Pais and Abrantes (1989) and Carneiro et al. (1996, 2000). Phenotype I1 was also rarely observed among populations from soybean (Castro et al., 2003) or banana (Cofcewicz et al., 2004) in Brazil. As observed in this study, Carneiro et al. (2004), using RAPD analysis, showed that I1 and I2 clustered together and had relatively low polymor-

TABLE 5. Rate of RAPD polymorphisms observed at species level.

Species	RAPD fragments	
	Amplified	Polymorphic (%)
M. javanica (J3N1, J2N1) (Isolates: 1C Gui, 4B Gui)	307	51 (16.6%)
M. cruciani (Cr3N1) (Isolates: 1B Gui, 18B Gua)	378	61 (16.1%)
M. javanica + *M. cruciani* (1C Gui, 4B Gui, 1B Gui, 18B Gua)	467	268 (57.4%)
M. incognita (I2N1) (Isolates: 25 B Gua, 8A Mar, 1 A Gui)	401	60 (14.9%)
M. incognita + *Meloidogyne* sp. (8B Mar)	556	347 (62.4%)
M. arenaria (A2N1) (Isolates: 23 Gua, 11 Gua, 13 Gui, 14 Mar)	382	111 (29.0%)
M. arenaria (A2N3) 5B Gua, 3B Gui	379	88 (23.2%)
M. arenaria (A2N1 + A1N1) (Isolates: 23 Gua, 11 Gua, 13 Gui, 14 Mar, 28C Gua)	421	157 (37.3%)
M. arenaria (A2N1 + A1N1) + *Meloidogyne* sp. (Ba2N1) (Isolate: 19B Gua)	497	267 (53.7%)
M. arenaria group (A2N1 + A1N1 + A2N3) (Isolates: 23 Gua, 11 Gua, 13 Gui, 14 Mar, 28C Gua, 5B Gua, 3B Gui)	513	316 (61.6%)
M. arenaria + *Meloidogyne* sp1(A2N1 + A1N1 + A2N3 + Ba2N1) (Isolates: 23 Gua, 11 Gua, 13 Gui, 14 Mar, 28C Gua, 5B Gua, 3B Gui, 19B Gua)	576	405 (70.3%)

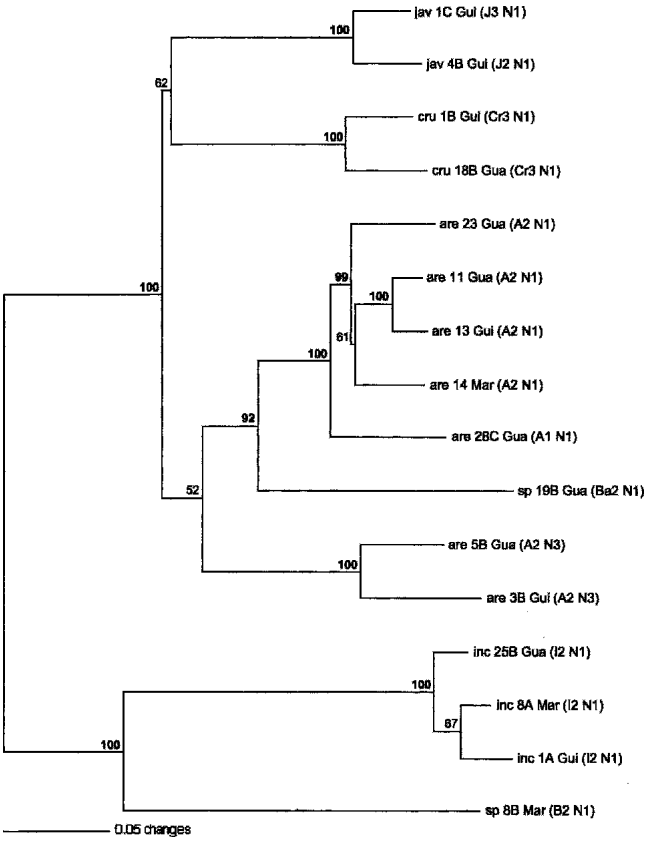

FIG. 4. Majority-rule consensus UPGMA dendrogram of relationships of 16 *Meloidogyne* spp. isolates (*M. javanica:* jav, *M. cruciani:* cru, *M. arenaria:* are, *M. incognita:* inc, *Meloidogyne* sp.: sp) based on RAPD data. (Bootstrap percentages based on 1,000 replicates are given on each node. Population codes are given in Tables 1, 2, and 3.)

phism, despite the populations having different esterase phenotypes.

The atypical population of *Meloidogyne* sp. (phenotype B2N1) from Martinique has also been found in sugar-cane (*Saccharum* spp.) in the northeast part of the island (Quénéhervé, pers. comm.). The RAPD data clustered this population with *M. incognita* isolates but with sufficient polymorphism that it could not be identified as *M. incognita.* The esterase phenotype B2N1 (labelled S1-M1) has also been reported from Nigeria, the Cote-d'Ivoire, the Philippines, Samoa (Fargette, 1987), and the United States (Esbenshade and Triantaphyllou, 1985).

During this survey, *M. javanica* was rarely found on banana but was commonly found parasitizing *Musa* in Brazil (Cofcewicz et al., 2004). Most of the *M. javanica* populations have a single esterase phenotype, J3, not found in any other *Meloidogyne* species (Esbenshade and Triantaphyllou, 1990). The phenotype J2 was first detected by Tomaszewski et al. (1994). Using morphology, isozyme profiles, karyology, host ranges, and RAPD analysis, Carneiro et al. (1998) showed that the J2 isolate did not exhibit any useful differentiating characters when compared with typical *M. javanica* isolates.

Meloidogyne cruciani, which occurred in French Guiana and Guadeloupe, also is a minor species presenting a species-specific esterase phenotype (Cr3) characterized first as M3a by Esbenshade and Triantaphyllou (1985). Based on RAPD data, *M. javanica* and *M. cruciani* were related species; the similarities in perineal patterns also suggest a close relationship between *M. cruciani* and *M. javanica* (Garcia-Martinez et al., 1982).

One population from Martinique exhibited the phenotype Hi3 similar to that found in *M. hispanica* and referred to as ''Seville populations'' (Dalmasso and Bergé, 1978; Janati et al. 1982) and later designated as S2-M1 (Esbenshade and Triantaphyllou, 1985). Two Mdh phenotypes, N1 and N3, were observed for different populations of *M. hispanica* (Esbenshade and Triantaphyllou, 1985) and for Portuguese populations (Pais and Abrantes, 1989), respectively. Our population had the same phenotype, N3, as detected for Portuguese isolates.

The lack of esterase bands (Est B0) was observed first by Esbenshade and Triantaphyllou (1985). The same phenotype was described for *M. fallax* Karssen (1996), but the Mdh is not N1 and is specific for this species.

The identification of *Meloidogyne* species parasitizing bananas needs to be more accurate. Our data indicate the value of using a combination of morphological and biochemical traits for species identification.

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