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Enzyme phenotypes of *Meloidogyne* spp. populations

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Summary – Enzyme phenotypes, specifically esterases (EST), malate dehydrogenase (MDH), superoxide dismutase (SOD) and glutamate-oxaloacetate transaminase (GOT) were used to characterise different species of *Meloidogyne*, mostly from Brazil and from some American countries. Esterase activity was highly polymorphic and was the most useful in the identification of the different species. Using this enzyme it is possible to characterise and identify the four major species: *M. javanica*, *M. incognita*, *M. arenaria* and *M. hapla* from a large collection of 111 populations of *Meloidogyne* spp. Another seven less common species (*M. coffeicola*, *M. paranensis*, *M. konaensis*, *M. exigua*, *M. graminicola*, *M. oryzae*, *M. mayaguensis*), with only one or a few populations of each, were studied and exhibited species-specific EST phenotypes. The two enzymes (EST and MDH) differentiated *M. graminicola* and *M. oryzae*. It was possible to detect atypical (unidentified) phenotypes: three from Brazil, one from the USA and another from Chile. The minor bands of esterase profiles provided information to detect intraspecific variability among some populations of *M. incognita* and six populations of *M. exigua*. Profiles of MDH permitted separation of two isolates of *M. javanica* from Brazil.

Résumé – ***Phénotypes enzymatiques de populations de* Meloidogyne** – Les phénotypes enzymatiques de l'estérase (EST), la malate déshydrogènase (MDH), la superoxide dismutase (SOD) et la glutamate-oxaloacétate transaminase (GOT) ont été utilisés de manière systématique afin de caractériser plusieurs espèces de *Meloidogyne* provenant principalement du Brésil et de quelques pays des régions américaines. C'est l'activité estérasique qui a présenté le plus grand polymorphisme et s'est montrée la plus utile dans la caractérisation des espèces. A l'aide de cette enzyme il a été possible de caractériser et d'identifier les quatre espèces majeures de *Meloidogyne*: *M. javanica*, *M. incognita*, *M. arenaria*, et *M. hapla* au sein d'une collection de 111 populations de *Meloidogyne* spp. Sept autres espèces moins fréquentes (*M. coffeicola*, *M. paranensis*, *M. konaensis*, *M. exigua*, *M. graminicola*, *M. oryzae*, *M. mayaguensis*) comportant seulement quelques populations de chaque espèces ont été étudiées et ont également montré des phénotypes estérasiques spécifiques. Les deux enzymes (EST et MDH) ont permis la différenciation de *M. graminicola* et de *M. oryzae*. Il a été également possible de détecter des phénotypes atypiques non encore identifiés chez trois populations originaires du Brésil, une des USA et une du Chili. Par ailleurs, les bandes mineures des profils estérasiques ont apporté des informations sur la variabilité intra-spécifique chez quelques populations de *M. incognita* et six populations de *M. exigua*, tandis que l'observation des profils enzymatiques de la malate déshydrogènase (MDH) a permis la distinction de deux populations de *M. javanica* du Brésil.

Keywords – biochemical, electrophoresis, identification, isozymes, *Pasteuria penetrans*, root-knot nematodes, systematics.

All root-knot nematodes are currently described in the large genus *Meloidogyne* which comprises more than 80 species. Species characterisation is based primarily on morphological features of females, males and second stage juveniles (Eisenback & Hirschmann, 1979, 1980; Eisenback *et al.*, 1980). Information about host range and host specificity is also included in the original descriptions of some species. Nevertheless, precise and reliable morphological identification of *Meloidogyne* species is a formidable task even for well qualified taxonomists with expertise in the genus (Esbenshade & Triantaphyllou, 1990). For many years, extensive enzymatic studies from several countries have demonstrated that the major and some minor species of *Meloidogyne* could be differentiated by species-specific enzyme phenotypes, revealed by polyacrylamide-gel electrophoresis (Bergé & Dalmasso, 1975; Janati *et al.*, 1982; Esbenshade & Triantaphyllou, 1985; Fargette, 1987; Pais & Abrantes 1989; Carneiro *et al.*, 1996a). Unfortunately, there are enzymatic profiles for only about 26 species. Many molecular techniques have been shown to be valuable tools for species identification and isolate characterisation of root-knot nematodes (Hyman, 1996). However, these techniques remain limited in application for survey and diagnostic studies, because ob-

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Table 1. *Origin, species identification and enzyme phenotypes of* Meloidogyne *populations.*

Species	Country/States[1)]	Crops	Number of populations	Enzyme phenotypes[2)]			
				EST	MDH	GOT	SOD
M. incognita race 1	Brazil: BA, PR, PB, RN, SP	barbados cherry *Malpighia punicifolia*	12				
		banana *Musa* spp.	2				
		soybean *Glycine max*	2	I2	N1	N1	N3 (I2)[4)]
		melon *Cucumis melo*	1				
		cucumber *Cucumis sativus*	1				
M. incognita race 2	Brazil: BA, PB, RN, PE, PR	barbados cherry *Malpighia punicifolia*	7	I1	N1	N1	N3 (I2)
		pineapple *Ananas comosus*	2				
M. incognita race 3	Brazil: BA, PR, PB, RN, SP, RS	barbados cherry *Malpighia punicifolia*	5				
		banana *Musa* spp.	2				
		soybean *Glycine max*	3	I1	N1	N1	N3 (I2)
		fig *Ficus carica*	2				
		chayote *Sechium edule*	1				
		cucumber *Cucumis sativus*	2				
M. incognita race 4	Brazil: RN	barbados cherry *Malpighia punicifolia*	1	I2	N1	N1	N3 (I2)
		watermelon *Citrullus vugaris*	1				
M. incognita	USA: Hawaii	coffee *Coffea arabica*	1	I2	N1	N1	N3 (I2)
M. arenaria race 2	Brazil: SE, BH, PB, RS	barbados cherry *Malpighia punicifolia*	3				
		beet *Beta vulgaris*	2	A2, A3	N1	N1	JA2
		lettuce *Lactuca sativa*	1				
M. javanica	Brazil: BH, PB, PR, RS	barbados cherry *Malpighia punicifolia*	15				
		rosemary *Rosmarinus officinalis*	1				
		thyme *Thymus vulgaris*	1				
		lavender *Lavandula spica*	1	J3	N1	N1	JA2
		balm *Melissa officinalis*	1				
		watermelon *Citrullus vugaris*	1				
		potato *Solanum tuberosum*	1				
M. javanica	Brazil: MS	soybean *Glycine max*	2	J3	N3	N1	JA2
M. hapla	Brazil: RS, SP	rosemary *Rosmarinus officinalis*	1				
		strawberry *Fragaria hibridos*	1	H1	H1	(H2)	(H1)
		baroa potato *Arracacia xanthorrhyza*	1				
		kiwi *Actinida deliciosa*	1				
M. paranaensis	Brazil: PR	coffee *Coffea arabica*	12	P1(F1)	N1	N1	KP2(N2)
M. konaensis	USA: Hawaii	coffee *Coffea arabica*	1	K3	N1	N1	KP2
M. exigua[3)]	Brazil: SP, MG	coffee *Coffea arabica*	2	E1b (VF1)	N1	E1	N3
	Brazil: MS	rubber tree *Hevea brasiliensis*	2	E1a(VF1)	–	–	–
M. exigua	Brazil: MG	coffee *Coffea arabica*	2	E1 (VF1)	–	–	–
M. coffeicola[3)]	Brazil: SP	coffee *Coffea arabica*	1	C2	C1	C1	–
M. mayaguensis	Martinique	tomato *Lycopersicum esculentum*	1	M2(VS1-S1)	N1a	N3	N2
M. graminicola[3)]	Brazil:RS	rice *Oryza sativa*	4	G1(VS1)	N1a	N1	GO3
M. oryzae	French Guiana	rice *Oryza sativa*	1	O1(VS1)	O3	N1	GO3
M. oryzae	Surinam	rice *Oryza sativa*	1	O1(VS1)	O3	N1	GO3
Unidentified populations							
Meloidogyne sp. 1	Brazil: SP	yacon *Polymnia sonchifolia*	2	Y3	N1	N1	N2
Meloidogyne sp. 2	Brazil: RS	lavender *Lavandula spica*	1	L3	N1	N1	N2

Table 1. *(Continued).*

Species	Country/States[1)]	Crops	Number of populations	Enzyme phenotypes[2)]			
				EST	MDH	GOT	SOD
Meloidogyne sp. 3	Brazil: RS	kiwi *Actinida deliciosa*	1	Ki3	N1	N1	N2
Meloidogyne sp. 3	Chile	grape vine *Vitis vinifera*	1	Ki3	N1	N1	N2
Meloidogyne sp. 4	USA: Florida	peach *Prunus persica*	1	P3	N1	N1	N2

[1)] Brazilian States: BA — Bahia, PR — Paraná, PB — Paraíba, RN — Rio Grande do Norte, SP — São Paulo, PE — Pernambuco, SP — São Paulo, RS — Rio Grande do Sul, SE — Sergipe, MS — Mato Grosso do Sul, MG — Minas Gerais.
[2)] Phenotype designations: EST = esterase, MDH = malate dehydrogenase, GOT = glutamate-oxaloacetate transaminase, SOD = superoxide dismutase.
[3)] Tomato cv. Santa Cruz is a non-host.
[4)] ( ) phenotype designation used by Esbenshade and Triantaphyllou (1985).

taining and understanding the results is complex and time consuming. Actually, until more convenient techniques are devised, we must develop isozyme studies for a large number of *Meloidogyne* species. This paper reports the findings of an extensive survey of 104 Brazilian populations and of seven populations of *Meloidogyne* from other countries, studied with regard to four enzymes. This paper also discusses the usefulness of such information for the identification of *Meloidogyne* species.

Material and methods

One hundred and four populations originating from the Brazilian States of Rio Grande do Norte (RN), Bahia (BH), Paraíba (PB), Pernambuco (PE), Mato Grosso do Sul (MS), Minas Gerais (MG), São Paulo (SP), Paraná (PR) and Rio Grande do Sul (RS), and seven populations from other countries of the Americas (Chile, USA, French Guyana, Martinique (French West Indies) and Surinam) were examined (Table 1). Nematodes were collected from their natural host, and propagated mostly on tomato cv. Santa Cruz in a greenhouse at 25 to 30°C. Species identification was based on esterase phenotype (EST), using the technique described by Carneiro *et al.* (1996a). Other studies with the isozymes malate dehydrogenase (MDH), superoxide dismutase (SOD) and glutamate-oxaloacetate transaminase (GOT) were made using the technique described by Esbenshade and Triantaphyllou (1985). For unidentified species or populations with atypical phenotypes, complementary studies were carried out using perineal patterns and the morphology of males, females and juveniles according to previously described methods (Eisenback & Hirschmann, 1979, 1980; Eisenback *et al.*, 1980). A differential host test was made only with *M. incognita* and *M. arenaria* (Hartman & Sasser, 1985). Enzyme phenotypes were designated with letters and a number indicating the number of bands. Phenotypes with the same number of bands were differentiated by small letters as described by Esbenshade and Triantaphyllou (1985, 1990). For reliable identification of the phenotype of an unknown population, protein extract from a pure population of a known species (*e.g.*, *M. javanica* (Treub, 1885) Chitwood, 1949), was included in the same gel, where they were compared directly.

Results and discussion

The present study included numerous populations of many *Meloidogyne* species originating in Brazil and other countries that were sent to our laboratory for species identification due to their observed pathogenic activities in the field. Therefore this study provides additional and useful information on the isozyme diversity in the genus *Meloidogyne*, particularly for Brazilian populations.

Esterase phenotype (EST)

Seventeen bands for esterase (EST) activity and 18 esterase phenotypes were detected among 111 populations of *Meloidogyne* spp. A distinct EST-phenotype was associated with every population of the major and minor species (Table 1; Fig. 1). Each esterase phenotype is designated by a letter suggestive of the species it specifies or the name of the crop for unknown phenotypes.

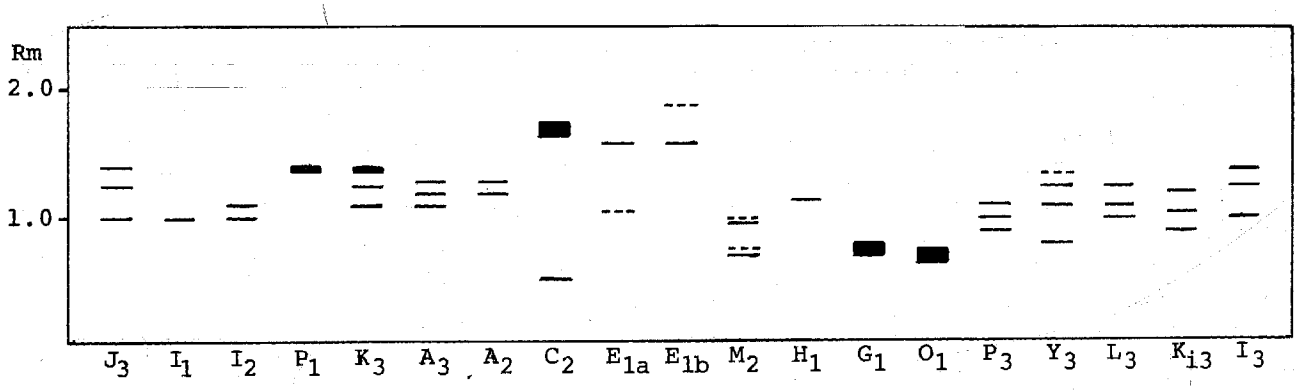

Fig. 1. *Esterase (EST) phenotypes observed in 111 populations of* Meloidogyne *spp. (For explanation of phenotype designations see Table 1).*

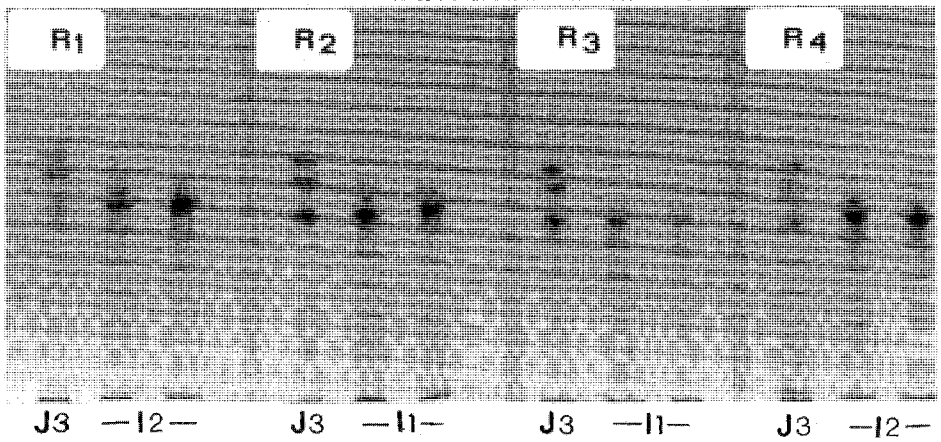

Fig. 2. *Esterase phenotypes found in four races (R1, R2, R3, R4) of* Meloidogyne incognita.

Phenotype I1 (Rm 1.0) was detected in 24 *M. incognita* (Kotoid & White, 1919) Chitwood, 1949 populations and phenotype I2 (Rm 1.0), with a minor band (Rm 1.1), was detected in 21 populations (Table 1; Figs 1, 2). *M. incognita* races 2 and 3 had the phenotype I1 and race 1 and 4, the phenotype I2. The *M. incognita* populations were isolated from different crops (Table 1). These two phenotypes, together with others, were observed by Pais and Abrantes (1989). This variation could be associated with intraspecific variability. Unfortunately, these electrophoretic profiles, together with those of the other enzymes tested (Table 1), do not provide enough information to separate the four races of *M. incognita* (Esbenshade & Triantaphyllou, 1990).

The species-specific phenotype J3 (Rm: 1.0, 1.25, 1,4) was detected in 21 *M. javanica* populations from different crops (Table 1; Fig. 1), and was used as standard control in each gel. It is interesting to note that females of *M. javanica* completely infected by the endospores of the obligate bacterial antagonist *Pasteuria penetrans* Sayer & Starr exhibited an even higher esterase concentration than non-parasitised females (Fig. 3). Consequently, it is possible to identify species of *Meloidogyne* on females parasitised by *P. penetrans*, using esterase enzymes.

Phenotypes A2 and A3 were detected in six populations of *M. arenaria* (Neal, 1889) Chitwood, 1949 isolated from Barbados cherry, beet and lettuce (Table 1). The phenotypes A3 (Rm 1.1,1.2, 1.3) and A2 (Rm: 1.2, 1.3) were species-specific for *M. arenaria* (Fig. 1) and the lack of resolution of one band in A2 was related either to the use of an individual female, the low esterase activity of this band, or the female physiological conditions (Carneiro *et al.*, 1996a). The two phenotypes (A2 and A3) had the same differential host plants (race 2).

Phenotype H1 (Rm 1.1) was detected in four populations of *M. hapla* isolated from rosemary, strawberry, parsnip and kiwi in RS and SP States (Table 1; Fig. 1).

The phenotype P1 (Rm 1.4) was detected in 12 populations of *M. paranaensis* Carneiro *et al.*, 1996 isolated from coffee in Paraná State (Table 1; Figs 1, 4A). This phenotype is the most useful characteristic to differentiate this species from *M. incognita* (I1) in coffee plantation surveys in Brazil (Carneiro *et al.*, 1996a). The phenotype K3 (Rm 1.1, 1.3, 1.4) was found in one population of *M. konaensis* Eisenbach *et al.*, 1994 isolated from coffee on the island of Hawai, USA (Table 1; Figs 1, 5). One esterase band (Rm: 1.4) was similar to the P1 (F1) phenotype detected in *M. paranaensis* populations from Brazil (Fig. 5). The esterase activity of the two bands (Rm 1.1, 1.3) was low, and only by using more than two females was it possible to see clearly the K3 Est-phenotype (Fig. 5). The original population of *M. konaensis* sent from Hawai was mixed with *M. incognita* (EST-phenotype I2). These populations were purified prior to conducting this study on isozyme characterisation. The phenotypes P1 and K3 were species specific of *M. paranaensis* and *M. konaensis*, respectively.

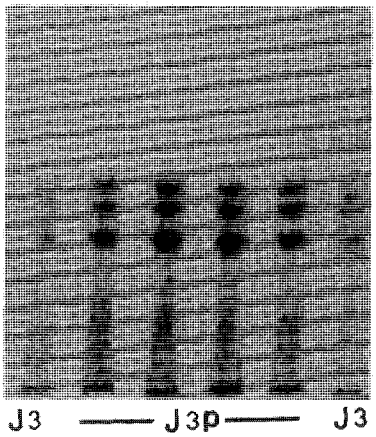

Fig. 3. *Esterase phenotypes of* Meloidogyne javanica. *J3: normal females; J3p: females containing endospores of* Pasteuria penetrans.

The phenotypes E1 (Rm 1.6) were detected on six populations of *M. exigua* Göldi, 1892 isolated from coffee and rubber trees from SP and MS States (Table 1; Figs 1, 4B, C), using a large number (more than ten) of macerated females (Carneiro *et al.*, 1996a). It is possible to differentiate *M. exigua* in two phenotypes (Figs 1, 4B, C) by the two minor bands (Rm 1.1 and 1.9) and by the ability of populations to reproduce or not on tomato. The phenotype C2 (Rm 0.5, 1.7) with high enzymatic activity was detected in one population of *M. coffeicola* Lordello & Zamith, 1960 isolated from coffee from SP State (Table 1; Figs 1, 4D) which did not reproduce on tomato.

The phenotype M2 (Table 1; Figs 1, 4E) with two major bands (Rm 0.7 and 0.9) was isolated from one population of *M. mayaguensis* Rammah & Hirschmann, 1988 from tomatoes (Martinique). Occasionally, one of these bands resolved into two minor bands (Rm 0.75, 0.95). Other morphological and morphometric characters currently used in the identification of *M. mayaguensis* were used to compare with the species description (Rammah & Hirschmann, 1988).

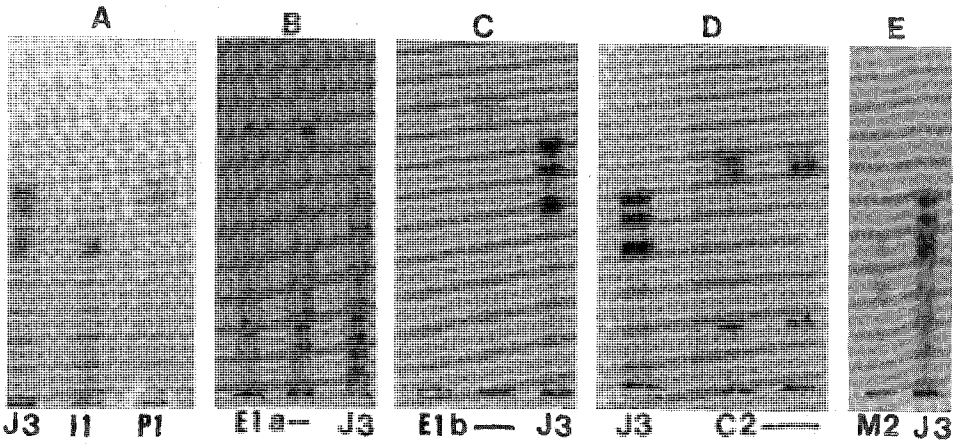

Fig. 4. *Esterase phenotypes of Brazilian populations from coffee. A-D:* Meloidogyne incognita *race 2 (I1);* M. paranaensis *(P1);* M. exigua *(E1a, E1b);* M. coffeicola *(C2); E:* M. mayaguensis *(M2).*

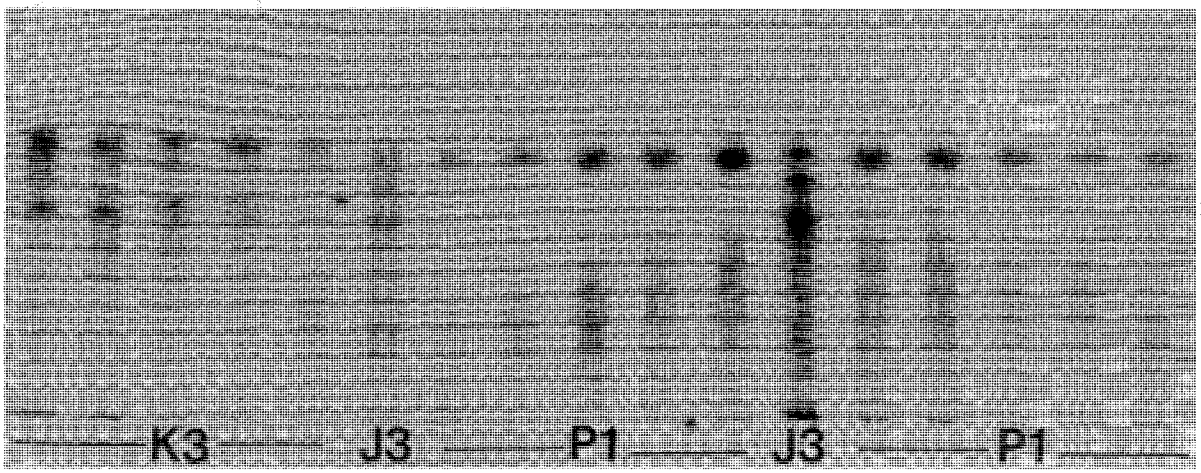

Fig. 5. *Esterase phenotypes of* Meloidogyne konaensis *(K3) and two populations of* M. paranaensis *(P1). The characterisation of these two species was made using different numbers of macerated females (one to five females corresponding to low — strong esterase activity, respectively).*

The phenotype G1 with a very slow and widely drawn-out band (Rm: 0.75) of high enzymatic activity was observed in four populations of *M. graminicola* (RS State). This esterase phenotype G1 was very close to the phenotype O1 of two populations of *M. oryzae* Maas *et al.*, 1978 (Rm 0.7) from Surinam and French Guyana (Table 1; Figs 1, 6A, B). The EST-band of *M. oryzae* was reported as being the same as *M. graminicola* with a slightly slower band (Esbenshade & Triantaphyllou, 1985). The esterase phenotype is correlated to other characters used in the identification of *M. oryzae* populations (Maas *et al.*, 1978).

Using the esterases, four atypical phenotypes of *Meloidogyne* spp. (Table 1; Figs 1, 7) remained to be identified precisely. The EST-phenotype Y3 with three major bands (Rm: 0.8, 1.1, 1.25) and one minor band (Rm 1.35) was detected in two populations from yacon (*Polymnia sonchifolia* Poep & Endl), a plant recently introduced from Japan in SP State (Capão Bonito). The perineal pattern of this population is similar to that of *M. incognita* (Mendes *et al.*, 1997). The EST-phenotype Ki3 (Rm 0.9, 1.05, 1.20) was detected in one population taken from kiwi introduced from Chile in RS State (Lagoa Vermelha) and the same phenotype was detected in another population from Casa Blanca in Chile. This nematode is a very important pest on grapevines in Chile where it was identified as *M. hapla* Chitwood, 1949 (Magunacelaya, pers. comm.). Therefore, the perineal pattern and other morphological characteristics are not typical of *M. hapla* and this population has been misidentified. It is probable that two atypical populations (Y3 and Ki3) have recently been intro-

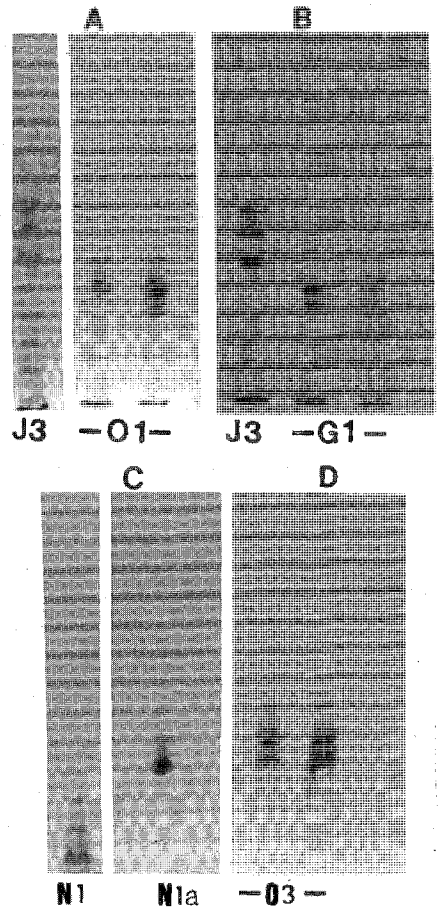

Fig. 6. *Esterase (A, B) and malate dehydrogenase (C, D) phenotypes observed in* Meloidogyne oryzae *(O1, O3) and* M. graminicola *(G1, N1a).*

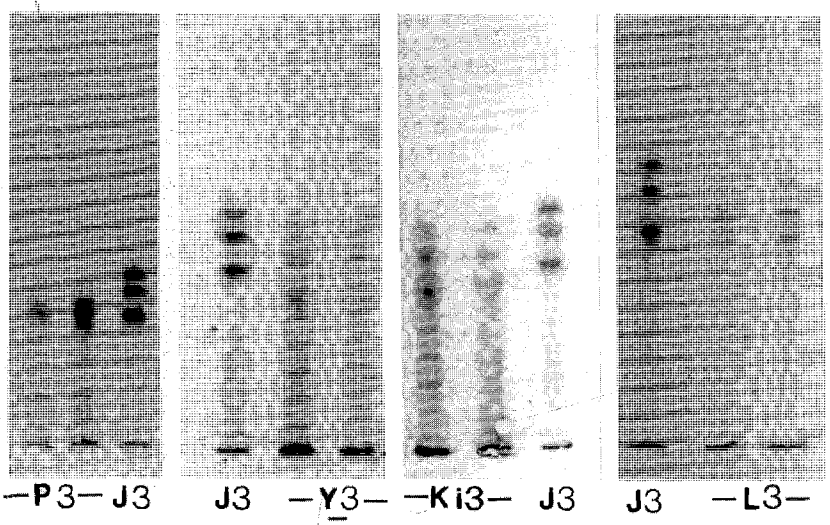

Fig. 7. *Esterase phenotypes of four unidentified populations of* Meloidogyne *spp. P3: peach, FL, USA; Y3: yacon, São Paulo, Brazil; Ki3: kiwi, Rio Grande do Sul, Brazil and grape vine, Chile; L3: lavender, Rio Grande do Sul, Brazil.*

duced in Brazil and belong to unidentified species. The population from lavender, EST-phenotype L3 (Rm: 1.0, 1.1, 1.25), is probably a natural population from RS State. In field conditions, this population is mixed with *M. javanica*, with the latter being prevalent. The population taken from peaches in Florida, USA, P3 (Rm: 0.9, 1.0, 1.1) is a still unidentified species. More accurate studies in morphology must be undertaken to identify these atypical nematodes to provide new data to help establish whether or not they have been introduced from different countries.

With the present electrophoretic procedure, esterase phenotypes of as many as 20 to 25 individual females can be compared in the same gel. Egg-masses can be saved and used latter as inoculum to obtain progeny of the individual females. These features render this biochemical approach a valuable tool in *Meloidogyne* research and confirm that esterase phenotype can be used as a rapid and efficient method to: *i*) carry out extensive field surveys to determine the frequency and relative distribution of *Meloidogyne* spp.; *ii*) characterise *Meloidogyne* species and detect atypical phenotypes; and *iii*) detect mixed species populations for purification prior to conducting studies on taxonomy, plant resistance or host specificity (Esbenshade & Triantaphyllou, 1990; Carneiro *et al.*, 1996a).

MALATE DEHYDROGENASE (MDH)

Eight bands of MDH activity were detected in 111 populations of *Meloidogyne* spp. and six phenotypes were recognized on the basis of particular bands or band combination (Figs 8, 9).

The phenotypes N1 (Rm 1.0) and N3 (Rm 1.0, 1.6, 1.8) detected in 23 populations of *M. javanica* (Table 1; Figs 8, 9) differentiated between two groups of populations of *M. javanica* from Brazil: N1 from 21 populations and N3 from two populations in MS State. These two groups of populations were reported to be variable with regard to morphological characters and to cytogenetic and DNA analysis (Carneiro *et al.*, 1998). The N3 population induced four to five times more galls and eggs on soybean cultivar UFV4 than other populations (Tihohod & Ferraz, 1986). Consequently, the MDH activity permitted detection of intraspecific variability between two *M. javanica* isolates. The phenotypes N1a (Rm 1.4) and O3 (Rm 1.4, 1.6, 1.8) (Table1; Figs 6, 8) contrary to previous findings by Esbenshade and Triantaphyllou (1985) who reported similar MDH phenotype for these *Meloidogyne* species. The phenotype C1 (Rm: 2.0) was species-specific to *M. coffeicola* which had the highest rate of migration of all bands observed. The phenotype N1a (Rm 1.4) detected in one population of *M. mayaguensis* was reported not to be species-specific (Esbenshade & Triantaphyllou, 1985). These authors detected a different MDH phenotype (N3c) for this species. The variability among populations of *M. mayaguensis* was detected first using sequences from the ribosomal DNA (Blok *et al.*, 1997). The phenotype H1 (Rm 1.9) of *M. hapla* is species-specific with one major band (Table 1; Figs 8, 9). The remaining eight species had

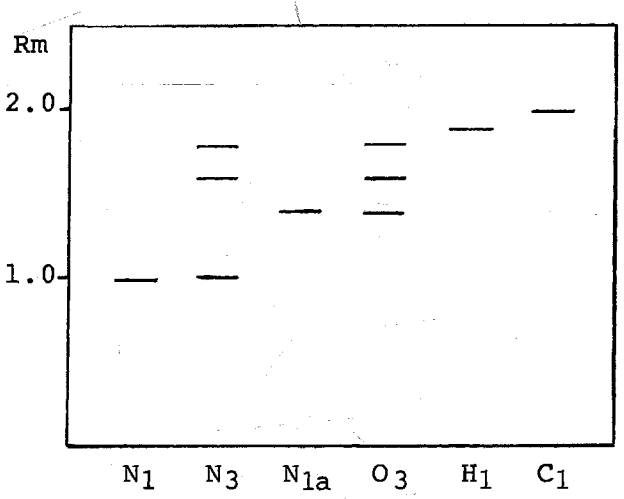

Fig. 8. *Phenotypes of malate dehydrogenase (MDH) observed in 111 populations of* Meloidogyne *spp. (For explanation of phenotypes designation see Table 1).*

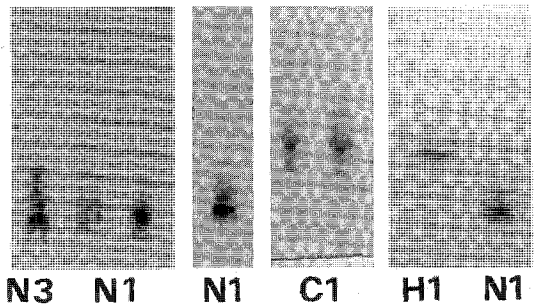

Fig. 9. *Phenotypes of malate dehydrogenase of* Meloidogyne javanica *from Mato Grosso do Sul State (N3) and* Meloidogyne javanica *from Paraná State (N1),* M. coffeicola *(C1) and* M. hapla *(H1).*

non-species-specific phenotypes and were designated by the letter N1, standing for non-specific-phenotypes (Table 1; Figs 8, 9).

Superoxide dismutase (SOD)

Five phenotypes of SOD were identified in the 111 populations of *Meloidogyne* spp. Additional minor bands were also detectable in many populations in several electrophoretic runs (Table 1; Figs 10, 11). Due to the negative staining procedure and to the low enzymatic response (Esbenshade & Triantaphyllou, 1985), we also used five females to produce individual bands of activity and overall phenotypes. A total of seven major bands was identified. Some phenotypes were not characteristic of only one major nematode species. For this reason, such phenotypes were designated with two letters suggesting their

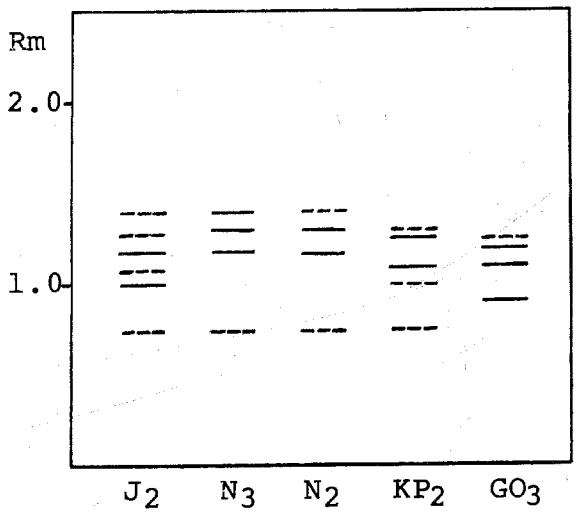

Fig. 10. *Phenotypes of superoxide dismutase (SOD) observed in 111 populations of* Meloidogyne *spp. (For explanation of phenotype designations see Table 1).*

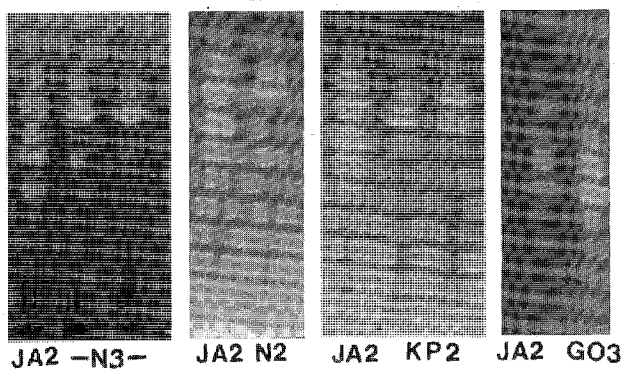

Fig. 11. *Phenotypes of superoxide dismutase (SOD) of* Meloidogyne *spp. JA2:* M. javanica *and* M. arenaria*; N3:* M. incognita*; N2:* M. mayaguensis*; KP2:* M. konaensis *and* M. paranaensis*; GO3:* M. graminicola *and* M. oryzae.

species specificity for two species. The phenotypes JA2 (Rm 1.0, 1.2) are specific for *M. javanica* and *M. arenaria* and the KP2 (Rm 1.1, 1.2) was specific for *M. konaensis* and *M. paranaensis*. The phenotype GO3 (Rm 1.0, 1.1, 1.2) was specifc for *M. graminicola* and *M. oryzae*. The remaining N3 (Rm 1.25, 1.30, 1.40) and N2 (Rm 1.25, 1.30) were not specific and are very similar. The only difference is band Rm 1.4, detectable as strong in N3 and as a minor band in N2 (Table 1; Figs 10, 11). Using five females, we had more major and minor bands and more differences among the phenotypes. Consequently, our results differ slightly from those obtained by Esbenshade and Triantaphyllou (1985).

GLUTAMATE-OXALOACETATE TRANSAMINASE (GOT)

A total of five major bands of GOT activity were detected and recognized in the study of 111 populations of *Meloidogyne* spp. (Table 1; Figs 12, 13). Additional very slow minor bands were also detectable in many populations in different electrophoretic runs. These bands were not considered in the designation of the phenotype because their expression was inconsistent and therefore their presence or absence seemed to have no taxonomic significance. The most prevalent phenotype of GOT was designated as N1 (Rm 1.0), for non-specific phenotype with one band. Phenotype N1 was detected in populations of *M. incognita*, *M. javanica*, *M. arenaria*, *M. graminicola*, *M. oryzae*, *M. paranaensis* and *Meloidogyne* sp. 4, *Meloidogyne* sp. 1, 2 and 3.

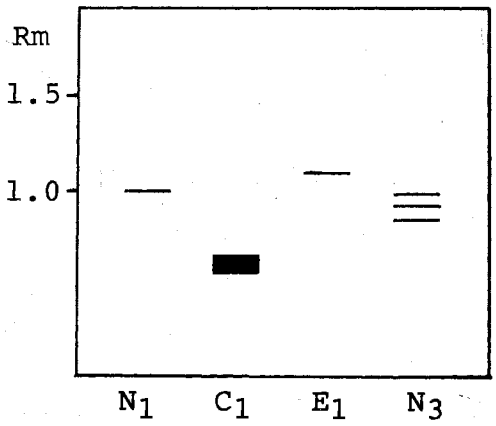

Fig. 12. *Phenotypes of glutamate-oxaloacetate transaminase (GOT) observed in 111 populations of* Meloidogyne *spp. (For explanation of phenotype designations see Table 1).*

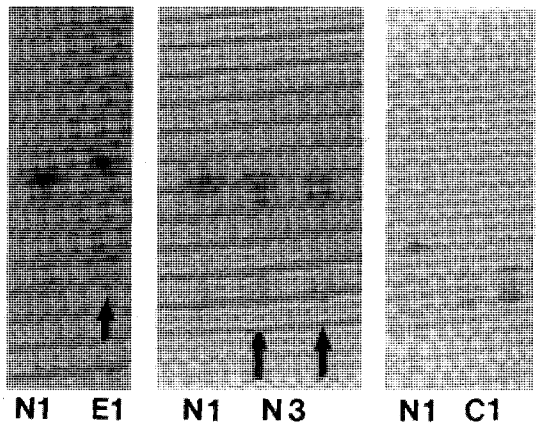

Fig. 13. *Phenotypes of glutamate-oxaloacetate transaminase (GOT) of* Meloidogyne *spp. N1:* M. javanica*; E1:* M. exigua*; N3:* M. mayaguensis*; C1:* M. coffeicola. *(Arrows indicated inconsistent bands that were observed in some gels).*

M. coffeicola, *M. exigua* and *M. mayaguensis* had species-specific GOT enzymatic activity, phenotypes C1 (Rm 0.6), E1 (Rm 1.1) and N3 (Rm 1.0, 0.95, 0.9), respectively (Table 1; Figs 12, 13). This N3 phenotype was reported not to be species-specific by Esbenshade and Triantaphyllou (1985).

EVALUATION OF MULTI-ENZYME PHENOTYPES

A single multi-enzyme phenotype was observed on *M. coffeicola* (EST-C1, MDH-C1, GOT-C1), *M. mayaguensis* (EST-M2, GOT-N3), *M. exigua* (EST-E1, GOT-E1), *M. paranaensis* (EST-P1, SOD-KP2), *M. konaensis* (EST-K3, SOD-KP2) and *M. hapla* (EST-H1, MDH-H1, GOT-H2, SOD-H1).

After esterase, the SOD phenotypes showed the highest specificity in their association with certain species. Furthermore, detecting SOD bands and determining SOD phenotype proved harder than determining esterase phenotypes. These results agree with the observation made by Esbenshade and Triantaphyllou (1985).

Multi-enzyme phenotypes, formed by phenotypes of two or more enzymes, offered a biochemical profile or a variety of profiles for each species. These profiles often characterised *Meloidogyne* species more definitively than did the constituent single enzymes considered individually. For example, EST-G1 and EST-O1 and MDH-N1a and MDH-O3 permitted better differentiation between *M. graminicola* and *M. oryzae* (Fig. 6). The intraspecifc variability among *M. javanica* populations can be shown by phenotypes EST-J3 and EST-J2 (Carneiro *et al.*, 1996a, 1998) and MDH-N1 and MDH-N3 (Figs 8, 9).

Since data about many 'minor' *Meloidogyne* species (*M. coffeicola*, *M. mayaguensis*, *M. oryzae* and *M. exigua*) are based on the study of only one or a few populations, the enzyme phenotype survey must be extended to many more populations to establish the stability and variability of enzyme phenotypes within each species. As more laboratories deploy this diagnostic tool, more data will become available for use. Still, a reliable diagnosis of a species through conventional taxonomic criteria, possibly including scanning electron microscopy and other common techniques, will be necessary before enzyme phenotype data are permanently assigned to the majority of species.

For routine identification of the phenotypes described in this paper, one should not rely heavily on the numerical expression of the migration rates of the various

bands. The rate of migration (Rm) of proteins in a gel is highly dependent upon the conditions of protein extraction, sample storage and electrophoretic separation conditions (Rollinson, 1980). Such factors or conditions may vary among laboratories and from one electrophoretic run to another in the same laboratory.

The methodology employed and the evaluation of results were kept to the simplest level possible in an attempt to assess the feasibility of this biochemical approach for routine work in laboratories interested in reliable identification of root-knot nematodes but not equipped with sophisticated facilities for electrophoretic work.

References

BERGÉ, J.B. & DALMASSO, A. (1975). Caractéristiques biochimiques de quelques populations de *Meloidogyne hapla* et *Meloidogyne* spp. *Cahiers de l'Office de la Recherche Scientifique et Technique Outre-Mer, Série Biologie* 10, 263-271.

BLOK, V.C., PHILLIPS, M.S. & FARGETTE, M. (1997). Comparison of sequences from the ribosomal DNA intergenic region of *Meloidogyne mayaguensis* and other major tropical root nematodes. *Journal of Nematology* 29, 16-22.

CARNEIRO, R.M.D., ALMEIDA, A.R.A. & CARNEIRO, R.G. (1996a). Enzyme phenotypes of Brazilian isolates of *Meloidogyne* spp. *Fundamental and Applied Nematology* 19, 555-560.

CARNEIRO, R.M.D.G., CARNEIRO, R.G., ABRANTES, I.M.O., SANTOS, M.S.N. & ALMEIDA, M.R.A. (1996b). *Meloidogyne paranaensis* n.sp. (Nemata: Meloidogynidae) a root-knot nematode parasitising coffee from Brazil. *Journal of Nematology* 28, 177-189.

CARNEIRO, R.M.D.G., CASTAGNONE–SERENO, P. & DICKSON, D.W. (1998). Variability among four populations of *Meloidogyne javanica* from Brazil. *Fundamental and Applied Nematology* 21, 319-326.

EISENBACK, J.D. & HIRSCHMANN, H. (1979). Morphological comparison of second-stage juveniles of six isolates of *Meloidogyne hapla* by SEM. *Journal of Nematology* 11, 5-16.

EISENBACK, J.D. & HIRSCHMANN, H. (1980). Morphological comparison of *Meloidogyne* males by scanning electron microscope. *Journal of Nematology* 12, 23-32.

EISENBACK, J.D., HIRSCHMANN, H. & TRIANTAPHYLLOU, A.C. (1980). Morphological comparison of *Meloidogyne* female head structures, perineal patterns, and stylets. *Journal of Nematology* 12, 300-313.

ESBENSHADE, P.R. & TRIANTAPHYLLOU, A.C. (1985). Use of enzyme phenotypes for identification of *Meloidogyne* species (Nematoda: Tylenchida). *Journal of Nematology* 17, 6-20.

ESBENSHADE, P.R. & TRIANTAPHYLLOU, A.C. (1990). Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology* 22, 10-15.

FARGETTE, M. (1987). Use of esterase phenotype in the taxonomy of the genus *Meloidogyne*. 2. Esterase phenotypes observed in West Africa populations and their characterization. *Revue de Nématologie* 10, 45-56.

HARTMAN, R.M. & SASSER, J.N. (1985). Identification of *Meloidogyne* species on the basis of differential host test and perineal pattern morphology. In: Barker, K.R., Carter, C.C. & Sasser, J.N. (Eds). *An advanced treatise on* Meloidogyne. *Volume 2. Methodology*. Raleigh, NC, USA, North Carolina State University Graphics, pp. 69-77.

HYMAN, B.C. (1996). Molecular systematics and population biology of phytonematodes: some unifying principles. *Fundamental and Applied Nematology* 19, 309-313.

JANATI, A.A., BERGÉ, A., TRIANTAPHYLLOU, A.C.& DALMASSO, A. (1982). Nouvelles données sur l'utilisation des isoestérases pour l'identification des *Meloidogyne*. *Revue de Nématologie* 5, 147-154.

MAAS, P.W.T., SANDERS, H. & DEDE, J. (1978). *Meloidogyne oryzae* n.sp. (Nematoda, Meloidogynidae, infesting irrigated rice in Surinam (South America). *Nematologica* 24, 305-361.

MENDES, M.L., SILVA, M.F.A., VILHENA, S.M.C., CARNEIRO, R.M.D.G. & ALMEIDA, M.R.A. (1997). Patogenicidade dos nematóides de galhas ao yacon, *Polymnia sonchifolia*. *Nematologia Brasileira* 21, 23. [Abstr.]

PAIS, C.S. & ABRANTES, I.M. DE (1989). Esterase and Malate dehydrogenase phenotypes in Portuguese populations of *Meloidogyne* species. *Journal of Nematology* 21, 342-346.

RAMMAH, A. & HIRSCHMANN, H. (1988). *Meloidogyne mayaguensis* n.sp. (Meloidogynidae), a root-knot nematode from Puerto Rico. *Journal of Nematology* 20, 58-69.

ROLLINSON, D. (1980). Enzymes as a taxonomic toll: a zoologist's view. In: Bisby, F.A., Vaughan, J.C. & Wright, C.A. (Eds). *Chemosystematics: principles and practice*. New York, NY, USA, New York Academic Press, pp. 123-146.

TIHOHOD, D. & FERRAZ, S. (1986). Variabilidade de três populações de *Meloidogyne javanica* em plantas de soja. *Nematologia Brasileira* 10, 162-171.