Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 2. Esterase phenotypes observed in West African populations and their characterization

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SUMMARY

Electrophoretic study of the b esterases in 57 populations of *Meloidogyne* collected in West Africa has shown that eight different phenotypes exist. Eighteen percent of the populations were composed of more than one phenotype. Phenotype pI *(M. incognita)* is the most prevalent in monospecific as well as in mixed populations. Morphobiometric and host range studies of five single egg-mass populations, characterized each by the phenotype pI, demonstrated that they all belong to *M. incognita.* The same study made on seven other single egg-mass populations, each characterized by a different esterase phenotype, agreed with the results obtained by other workers elsewhere. Electrophoretic determination is easy, accurate and more objective than any other criterion used so far.

RÉSUMÉ

Utilisation des isoestérases dans la systématique du genre Meloidogyne.
2. Les phénotypes isostérasiques présents en Afrique de l'Ouest et leur caractérisation

L'étude par électrophorèse des isoestérases de type b de 57 populations de *Meloidogyne* originaires d'Afrique de l'ouest a mis en évidence l'existence de huit phénotypes différents. Dix-huit pour-cent des populations sont composés de plus d'un phénotype. Le phénotype pI *(M. incognita)* est largement dominant dans les populations composées d'une ou plusieurs espèces. L'étude morphobiométrique ainsi que celle des gammes d'hôtes de cinq clones caractérisés chacun par le phénotype pI ont montré qu'ils appartiennent tous à l'espèce *M. incognita.* La même étude portant sur sept autres clones caractérisés chacun par un phénotype différent est en accord avec les résultats obtenus par d'autres auteurs. La détermination par électrophorèse est facile, précise et plus objective que tout autre critère utilisé jusqu'à présent.

The taxonomy of the genus *Meloidogyne* still remains ambiguous as many of the characters used to define species depend on personal judgment rather than on objective criteria (Fargette, 1987). To improve the identification of *Meloidogyne* populations, a rapid technique should be developed which is easy to apply.

The electrophoretical characterization of the b esterase system is an efficient tool to distinguish between different types (Bergé & Dalmasso, 1975; Dalmasso & Bergé, 1978, 1979, 1983; Janati *et al.*, 1982). It has been demonstrated that the b esterase system is stable and independent of the physiological state of the nematode or of the host it parasitizes (Fargette, 1987). The electrophoretical technique developed by Dalmasso and Bergé (1978) allows the study of single females of *Meloidogyne.* This avoids the time-consuming clonage of natural populations as previously required to ascertain that monospecific lines, and no mixtures of species were examined. Therefore it has become possible to use this technique as a routine method and to identify the different esterase phenotypes occurring in the world. Bergé and Dalmasso (1975, 1976) and Dalmasso and Bergé (1978) studied populations from France and neighbouring countries. Janati *et al.* (1982) and Esbenshade and Triantaphyllou (1985) studied populations collected from different parts of the world, among them, populations from West Africa.

Three species of *Meloidogyne,* i.e. *M. incognita, M. javanica, M. arenaria* are prevalent in the tropics. In the first part of the present investigation an inventory of the esterase phenotypes of populations present in West Africa is made. In the second part of the study, the esterase phenotypes present in this region are correlated to other characters currently used in the identification of *Meloidogyne* populations : morphobiometric criteria (vulval width, stylet length of males, body length of second-stage larvae, perineal patterns) and host preference. This part of the investigation has been made on twelve isolates characterized by esterase phenotypes, identified in the first part of this study.

Materials and methods

A total of 57 populations extracted from roots of different hosts collected at various locations in the Ivory Coast (41), Burkina Faso (6), Togo (9) and Senegal (1) were studied (about 1 700 females examined). An additional population from South Africa was also included. Roots of plants collected during the survey were placed in a mist-chamber and seedlings of *Hibiscus cannabinus*, *Coleus* sp. or *Solanum melongena* cv. Violette Longue, growing in the greenhouse, were inoculated with *Meloidogyne* juveniles extracted. Thirty five days after inoculation, 40 swollen white females from each population (in certain cases the inoculated plants produced fewer females) were individually squashed. Electrophoresis was carried out according to the Bergé and Dalmasso's method described by Fargette (1984). On each gel slab, protein extract of a female from an isolate with phenotype pI was included as a reference.

In the second part, twelve single egg-mass isolates selected among the 57 populations were studied. Each isolate line was multiplied on egg plants grown in the greenhouse. The eight different phenotypes observed in the first part of the study were represented each by one isolate except for the most common one (pI) of which five isolates were examined, in order to study the variability of morphological and physiological characters of isolates possessing the same esterase phenotype.

Morphobiometry

Males were extracted from roots which were placed, seven weeks after inoculation, in a mist-chamber. From each line, second-stage larvae were collected from twenty egg-sacs hatching in water for twelve hours, following a period of ten days in a 0.3 M NaCl solution (Dropkin, Martin & Johnson, 1958). Samples of males (twenty males per line) and second-stage larvae (twenty larvae per line) were fixed and mounted according to the methods described by Seinhorst (1959, 1962, 1966), Netscher and Seinhorst (1969) and Netscher (1970). The perineal patterns (twenty per line) were mounted according to the method of Taylor and Netscher (1974).

Host range studies

Three host ranges were tested :

— the differential host range as defined in the International *Meloidogyne* Project (Sasser, 1979) : *Nicotiana tabacum* cv. NC 95, *Gossypium hirsutum* cv. Deltapine 16, *Capsicum annuum* cv. California Wonder, *Citrullus vulgaris* cv. Charleston Gray, *Arachis hypogaea* cv. Florrunner, *Lycopersicon esculentum* cv. Rutgers (test plant);

— a few species or cultivars known to be resistant to West African *Meloidogyne* spp. or at least to some species of *Meloidogyne* : *Ipomœa batatas* cv. CDH, *Ipomœa batatas* cv. Chinese, *Medicago sativa* cv. Interior, *Glycine max* cv. Forrest, *Panicum maximum* cv. T 58, *Lycopersicon esculentum* cv. Rossol;

— certain plants of interest to local agriculture : *Lactuca sativa* cv. Blonde de Paris, *Amaranthus viridis*, *Sesbania rostrata*, *Coffea arabica*, *Coffea canephora*.

Five plants per host type were inoculated with 500 second-stage larvae of each isolate (the number of available plants of watermelon and alfalfa allowed only two replications; because of the small size of the plants only 200 second-stage larvae were inoculated to alfalfa and 100 to *Panicum maximum*, lettuce and coffee). Five weeks after inoculation, plants were uprooted and the roots carefully washed. After examining each root system to record the rate of galling, roots were placed in the mist-chamber and the number of second-stage larvae determined. The following scale was used to score the rate of galling : — : no gall; 1 : a single gall without egg-sac; 2 : a single gall with egg-sac(s); 3 : 2-5 galls; 4 : 6-10 galls; 5 : more than 10 galls. The scale applied for the number of second-stage larvae extracted from roots was : — : 0-50 second-stage larvae; 1 : 51-200 second-stage larvae, sometimes more, but in only one of the five replications; 2 : 51-500 second-stage larvae, in each of the five replications; 3 : 501-2 500 second-stage larvae; 4 : 2 501-5 000 second-stage larvae; 5 : more than 5 000 second-stage larvae.

Results

Part 1 : Studies of natural populations

Isozymes and esterase phenotypes

Twelve isozymes of the b esterase system were observed (the isozymes are strongly stained and hydrolyse both α and β naphthyl-acetate [Dalmasso & Bergé, 1978]). Eight esterase phenotypes can be distinguished by the number, the rate of migration (Rm) and the intensity of the bands of the isozymes (Fig. 1). Comparing the results of Bergé and Dalmasso (1975), Dalmasso and Bergé (1978, 1983), Janati *et al.* (1982), Esbenshade and Triantaphyllou (1985) and those presented here, small differences in the position of the bands (Rm) can be observed. These are due to slight variations in laboratory conditions dependent of electrophoretic conditions used and beyond the control of the investigator. In certain cases band 0.71 was weak (pVI, pVII and pVIII); bands 0.56 (pVI) and 0.76 (pI) were also weak. The presence of these weak bands should similarly be attributed to slightly different laboratory conditions because these bands are present in the reference line of pI furnished by Dalmasso whereas the latter worker did not observe them in his analyses.

All phenotypes encountered during this study have already been recorded (Fig. 1).

— Phenotypes pI, pIV, and pV were described by Bergé and Dalmasso (1975), Dalmasso and Bergé (1978,

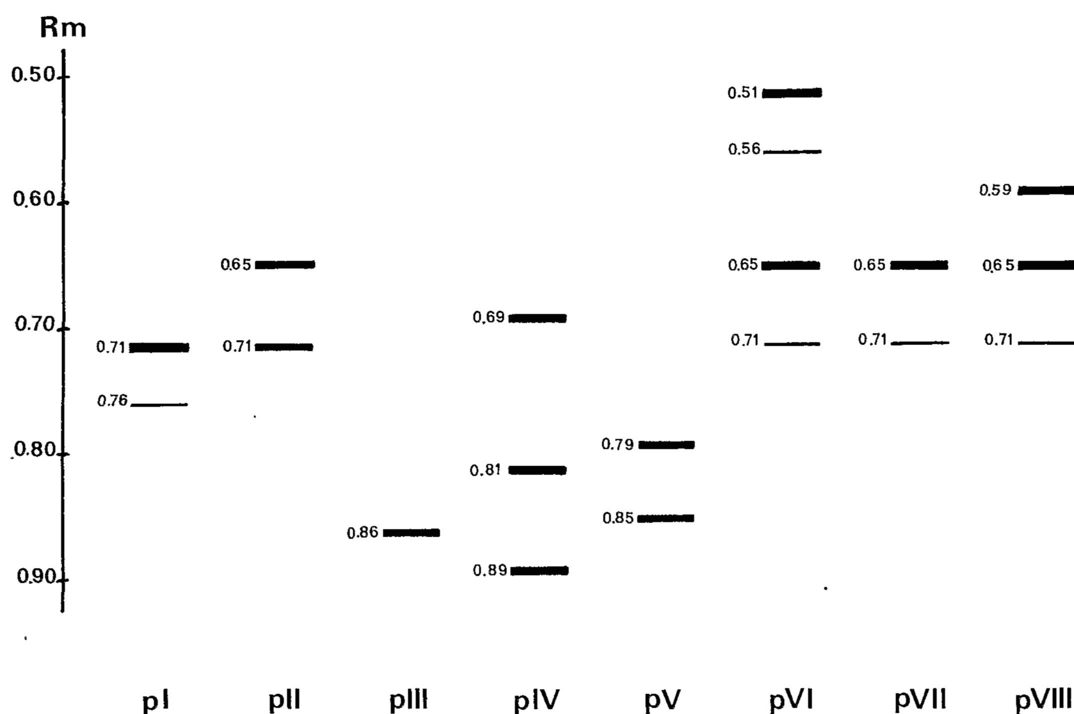

Fig. 1. The eight b esterase phenotypes found in natural populations from West Africa.

1983), Janati *et al.* (1982) and Esbenshade and Triantaphyllou (1985). They are commonly found. According to these studies, they correspond to *M. incognita, M. javanica, M. arenaria* respectively.

— Phenotype pIII reported by Bergé and Dalmasso (1975) was found in populations identified as *M. incognita* var. *acrita*, the statute of which still remains unclear (Hewlett & Tarjan, 1983). It was reported as phenotype F1 in one unidentified population by Esbenshade and Triantaphyllou (1985).

— Phenotype pVII is the phenotype 1 described by Janati *et al.* (1982) from populations from Senegal and Nigeria. Esbenshade and Triantaphyllou (1985) found it (their phenotype SI) associated with *M. chitwoodi, M. platani* and *M. incognita.*

— Phenotype pII from atypical *M. arenaria* populations from Nigeria, Ivory Coast, Philippines, Samoa, and one unidentified population from the USA was labeled S1-M1 by Esbenshade & Triantaphyllou (1985).

— Phenotype pVI corresponds to the VS1-S1 phenotype from *M. enterolobii* from China and from an unidentified population from Porto Rico (Esbenshade & Triantaphyllou, 1985).

— Phenotype pVIII was reported, as the S2-M1 phenotype, in atypical *M. arenaria* populations and in one unidentified population from the USA; it was also found in *M. hispanica* (Esbenshade & Triantaphyllou, 1985) and also observed in one population from the Cape Verde Islands (Merny, pers. comm.). It was recorded here only once, in the population from South Africa.

Distribution and relative frequency of the different phenotypes in field populations

The majority (82 %) of the 57 populations exhibited one of the following phenotypes : pI, pIV, pV and pVI (Tab. 1). Phenotype pI was the most prevalent in monospecific as well as in mixed populations (Tabs 1 and 2). Phenotypes pIV, pV, pVI were less common. Phenotypes pIII and pVII were found in one population each and in small numbers (Tab. 2). Phenotype pII was encountered in five mixed populations.

Occurrence of esterase phenotypes in botanical families

The populations were collected from 26 different hosts belonging to fifteen botanical families*. No pref-

* [*Saccharum officinalis, Oryza sativa, Zea mays* (Graminaceae); *Lycopersicon esculentum, Solanum melongena, S. anolanum, Capsicum* sp. (Solanaceae); *Arachis hypogaea, Phaseolus vulgaris, Canavalia ensiformis* (Leguminoseae); *Lactuva sativa* (Compositae); *Ipomœa batatas* (Convolvulaceae); *Coleus* sp. (Labiaceae); *Aglaœnema* sp. (Araceae); *Amaranthus* sp. (Amaranthaceae); *Hibiscus esculentus* (Malvaceae); *Impatiens* sp. (Balsaminaceae); *Cucumis melo, C. sativus* (Cucurbitaceae); *Allium porrum* (Liliaceae); *Musa acuminata* (Musaceae); *Beta vulgaris* (Chenopodiaceae); *Brassica oleracea* (Cruciferae)]

Table 1

Frequency of the different esterase phenotypes in 47 homogeneous field populations, expressed as the percentage of the number of females examined

Esterase phenotypes	*pI*	*pII*	*pIII*	*pIV*	*pV*	*pVI*	*pVII*
Frequency	60.5	0	0	16.3	11.6	11.6	0

Table 2

Frequency of the different phenotypes of the 10 mixed populations, expressed as the percentage of the number of females examined

Esterase phenotypes	*pI*	*pII*	*pIII*	*pIV*	*pV*	*pVI*	*pVII*
population 1	65.3			34.7			
population 2	98	2					
population 3	87.1						12.9
population 4	46.7	16.7		20		16.7	
population 5	80.9	19.1					
population 6	63.5	23.8	12.7				
population 7		2.6		94.7	2.6		
population 8	26.9			73.1			
population 9	81.6				18.4		
population 10	52.6				47.4		
Total	60.3	6.4	1.3	22.2	6.8	1.7	1.3

erence of esterase phenotypes for botanical families could be demonstrated, as a host belonging to one family can be parasitized by several phenotypes and reciprocally one phenotype can develop on plants belonging to different botanical families (Tab. 3). Probably nematodes of all phenotypes are polyphagous. This implies that the most frequently encountered phenotypes will be present in many plant species whereas the more sparcely occuring phenotypes necessarily will be found on few plant species. The fact that the rare phenotype pII was observed on five out of fifteen families is probably due to chance.

Table 3

Number of botanical families on which each phenotype was found (a total of 15 botanical families were examined* : one population only was studied in each of the family where the pI phenotype was not found)

Esterase phenotypes	*pI*	*pII*	*pIII*	*pIV*	*pV*	*pVI*	*pVII*
Number of botanical families	12	5	1	5	7	6	1

Geographical distribution of esterase phenotypes

The climate of Ivory Coast is characterized by a gradual decrease of the yearly precipitation from South to the North (2 000 mm in the forest area of southern Ivory Coast; 800 mm in the much more arid northern region of Bobo-Dioulasso, Burkina Faso). In the southern region, the dry season lasts from December to March, whereas in the region of Bobo-Dioulasso the dry season is much longer (from November to May). Roughly, the region studied was divided in two areas : one South of Bouaké and one North of Bouaké. The pI phenotype is found everywhere and is prevalent. All seven esterase phenotypes were present in the South whereas only the pI and pIV phenotypes were present in the North (Tab. 4).

In general the climate in Togo is rather dry. In this region the center has the most humid climate, the North and the South being more arid. Four phenotypes were observed. Compared to the Ivory Coast, pI phenotype occurs less frequently in this country (20 % of the cases *vs* 79 % in the Ivory Coast and Burkina Faso), whereas pIV occurred in 50 % of the nematodes examined compared to 13.5 % in Ivory Coast.

Table 4

Frequency of esterase phenotypes in different geographical locations (*, **, *** : 25, 22, and 9 populations were examined respectively)

Esterase phenotypes	*pI*	*pII*	*pIII*	*pIV*	*pV*	*pVI*	*pVII*
Frequency in the South*	73.8	2.4	0.3	10.8	5.8	3.7	3.2
Frequency in the North**	83.7	0	0	16.3	0	0	0
Togo***	20	0	0	47.5	15.8	16.7	0

PART 2 : STUDIES ON SINGLE EGG-MASS ISOLATES

Morphology

To evaluate variability of the perineal pattern, six classes were defined : three classes were reserved for the patterns which closely matched the original descriptions of the species *incognita* (i), *javanica* (j), *arenaria* (a), and three groups to classify nematodes which showed characters intermediate between two of the latter species : *incognita-javanica* (i-j), *javanica-arenaria* (j-a) and *arenaria-incognita* (a-i). For each of the twelve isolates studied, the distribution of the patterns according to

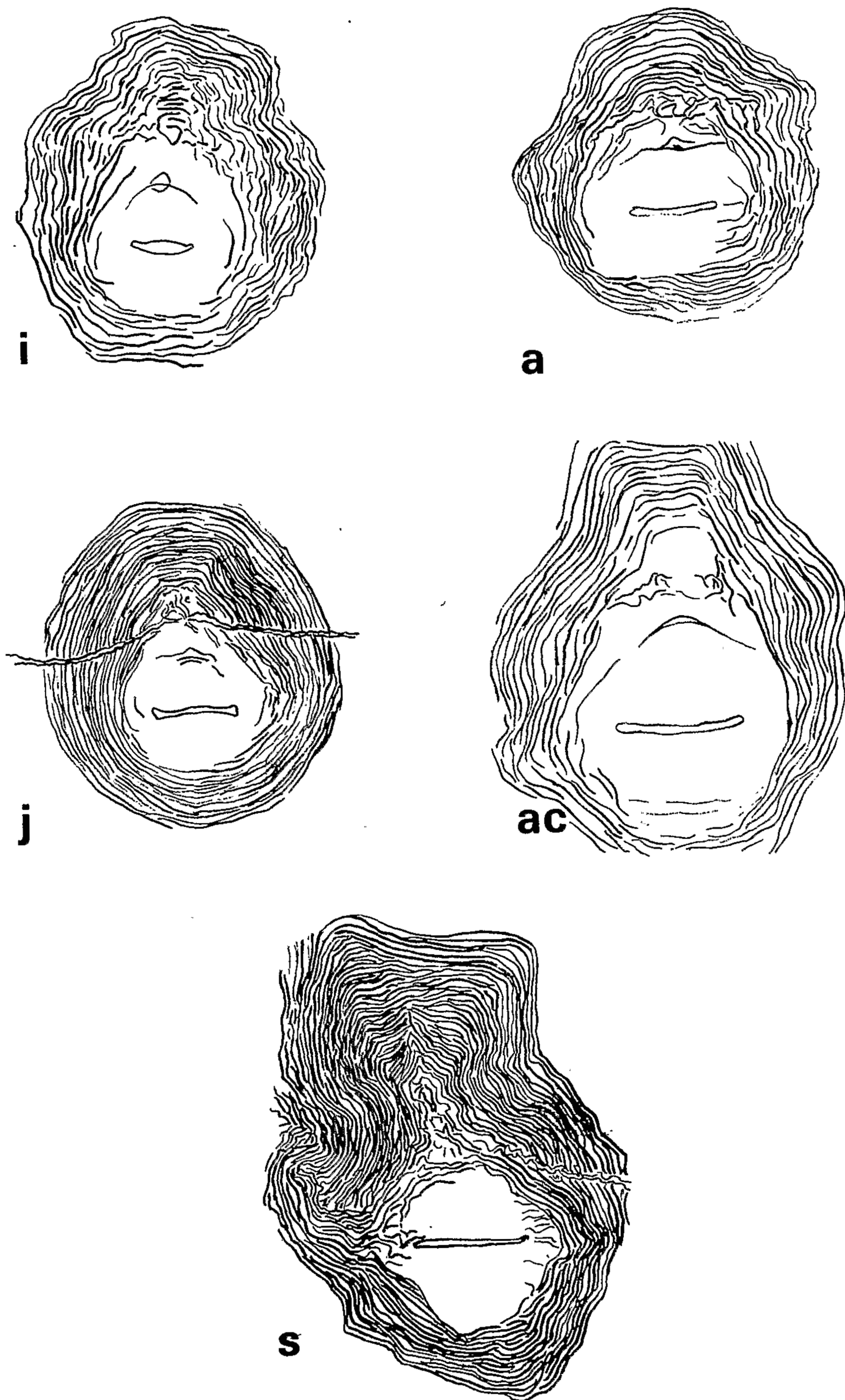

Fig. 2. The five typical forms of the perineal patterns observed.

Table 5

Distribution of the perineal patterns of the twelve isolates according to the classes defined as follows :
i = *incognita;* j = *javanica;* a = *arenaria;*
i-j : *incognita-javanica;* j-a : *javanica-arenaria;*
a-i = *arenaria-incognita*

Isolates	*i*	*j*	*a*	*i-j*	*j-a*	*a-i*
pI-1	18			2		
pI-2	11	3		6		
pI-3	16	2		2		
pI-4	19			1		
pI-5	17			2		1
pII			17		2	1
pIII	20*					
pIV		18			2	
pV			9		10	1
pVI	16	1		2		1
pVII	18	1		1		
pVIII	18**					2

* *M. acrita* form; ** Seville form.

these classes is given in Table 5. Generally, most of the patterns belong to one type although some heterogeneity exists. Nevertheless, in the pI-2 and pV lines a much greater variability was observed.

Five typical types of perineal pattern were observed (Fig. 2) :

— the *incognita* type, observed in the five pI, in pVI and pVII lines. The five pI and pVII patterns are almost similar while the pVI pattern is quite distinct from the others by the presence of lateral wings;

— the *javanica* type, observed in the pIV line;

— the *arenaria* type, observed in the pII and pV lines, though to a lesser extent in the latter;

— the *acrita* type, observed in the pIII line, occupied more space and had a high and regular arch;

— the « Seville » type, observed in the pVIII line was characterized by sinuous lines.

For these two latter types, the morphology of the twenty perineal patterns was very homogenous.

Biometry

For each of the twelve lines studied, the mean and confidence interval of the width of the vulva, the length of the stylet of the males and the total body length of the second-stage larvae were calculated of twenty individuals (Tab. 6). For each of the characters studied, differences between certain lines were highly significant (Fig. 3).

Host-range studies

The reaction of the twelve lines to the differential host plants defined by the International *Meloidogyne* Project are given in Table 7. Table 8 summarizes the reactions of the lines to the other plants tested in this study. Using Sasser's (1979) criteria, they should be classified as follows :

— the five pI lines and the pVII line : *M. incognita*, race 1;

— the pVI line : *M. incognita*, race 4;

— the pIV and pII lines : either *M. javanica* or *M. arenaria*, race 2 (indistinguishable according to Sasser host-ranges);

— the pV line : *M. incognita*, race 2 (there is some contradictory evidence, see discussion).

The pIII and pVIII lines have host-ranges which have never been described and therefore cannot be classified in this system. It is of interest to note that although watermelon reacted to the penetration of juveniles of pVIII by the formation of numerous small galls, no juveniles could be recovered from this plant.

The reaction of the isolates to the other plants studied (Tab. 8) demonstrates the great physiological variability among them :

— the pVI line vigorously develops on *Meloidogyne* resistant soya (cv. Forrest), resistant tomato (cv. Rossol) and sweet potato (cv. CDH which is resistant to all the other lines and cv. Chinese on which only the pI lines develop otherwise). On the contrary, it cannot develop on *Amaranthus*, a plant susceptible to the other eleven lines;

— the pV line does not parasitize lettuce, and parasitizes only weakly *Sesbania;*

— the pVIII line develops, but weakly, on most of the tested plants;

Table 6

Mean (µm) and standard error of vulva width (A), stylet length of males (B) and body length of second stage larvae (C)

Lines	*A*	*B*	*C*
pI-1	21.9 (0.75)*	22.7 (0.39)	348.6 (5.81)
pI-2	16.5 (0.89)	19.9 (0.71)	346.2 (5.17)
pI-3	19.4 (0.78)	22.2 (0.47)	361.8 (10.72)
pI-4	20.3 (0.85)	19.8 (0.43)	346.6 (6.63)
pI-5	18.5 (0.62)	20.3 (0.85)	366.6 (3.27)
pII	21.0 (0.66)	20.9 (0.45)	366.2 (4.55)
pIII	23.6 (1.78)	21.5 (0.34)	440.4 (5.93)
pIV	19.0 (0.63)	19.5 (0.54)	407.4 (5.07)
pV	23.4 (0.81)	20.0 (0.58)	406.2 (6.16)
pVI	24.4 (1.15)	18.6 (0.44)	415.0 (6.3)
pVII	23.4 (0.81)	22.6 (0.35)	360.9 (8.44)
pVIII	23.0 (1.07)	19.3 (0.70)	376.8 (5.05)

* Mean and standard error calculated from twenty individuals.

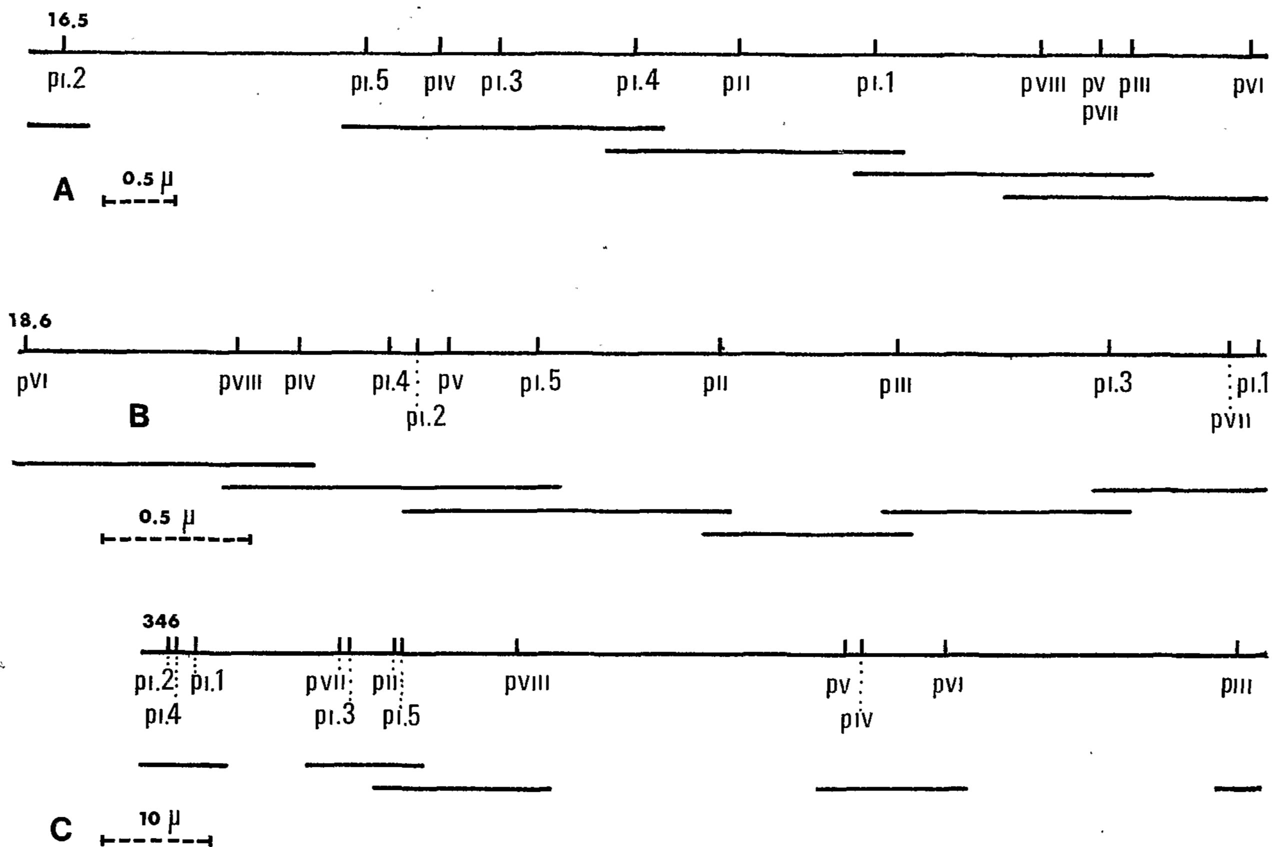

Fig. 3 : grouping by the multiple range test (Duncan test) of the mean values of each of the twelve lines for the characters. A : vulva width (µm); B : stylet length of males (µm); C : body length of second stage larvae (µm).

— the pVII line does not develop on sweet potato cv. Chinese, whereas the pI lines do. Therefore, this host allows the distinction between the pVII line and the five pI lines which all belong to *M. incognita*, race 1 according to Sasser host-ranges.

Discussion

Netscher (1978) found that, in West Africa, 25 % of the natural populations were type-mixed; Sivapalan (1981) in Sri-Lanka, Saeed *et al.* (1981) in Pakistan, Razak (1981) in Malaysia, Hadisoeganda (1981) in Indonesia, Sasser (1982) who summarized the information of the International *Meloidogyne* Project, and Dalmasso (pers. comm.) in the French West Indies, observed that *M. incognita* is the most widely spread species. Our observations indicating that 18 % of the populations studied were mixed and that the majority of the females examined belonged to *M. incognita*, are in agreement with the findings of the authors cited above.

The distribution of the various esterase phenotypes seems to be related to climatic factors. In the wet areas a maximum number of different phenotypes was recorded while in the drier and hotter zones only pI and pIV were found. In Togo, the predominance of the pIV phenotype might be related to the dry conditions prevailing in some areas, as was suggested by Abu-Garbieh (1982) discussing *Meloidogyne* populations studied in Jordan.

All the perineal patterns of each of the five pI lines studied belonged to *M. incognita*. For each of the other phenotypes studied, identification based on the study of perineal patterns allowed to classify them into recognized species. Identifications based on this characteristic are in agreement with those given by Bergé and Dalmasso (1975, 1976), Dalmasso and Bergé (1977, 1978, 1979, 1983), Janati *et al.* (1982) and Eșbenshade and Triantaphyllou (1985). Nevertheless, the variability already reported in the morphology of the perineal pattern (Chitwood, 1949; Allen, 1952; Sasser, 1954; Whitehead,

Table 7

Reaction of the twelve lines to the differential host plants defined by the international *Meloidogyne Project.*
(For each host : first line = root gall index; second line = rate of reproduction. For scales see materials and methods.)

HOSTS	LINES											
	pI 1	*pI 2*	*pI 3*	*pI 4*	*pI 5*	*pII*	*pIII*	*pIV*	*pV*	*pVI*	*pVII*	*pVIII*
Nicotiana tabaccum	—	—	—	—	—	4	5	5	5	5	—	2
cv. NC 95	—	1	1	—	—	3	5	5	5	5	—	2
Gossypium hirsutum	—	—	—	—	—	—	—	—	—	—	—	—
cv. Deltapine	—	—	—	—	—	—	—	—	—	3	—	—
Capsicum annuum	5	4	4	4	5	—	—	—	3	5	5	4
cv. California Wonder	5	4	5	5	5	—	—	—	3	5	5	3
Citrullus vulgaris	3	4	3	4	4	3	—	4	3	3	4	4*
cv. Charleston Gray	—	—	1	1	2	1	—	1	1	5	1	—
Arachis hypogaea	—	—	—	—	—	—	—	—	—	—	—	—
cv. Florrunner	—	—	—	—	—	1	—	—	—	—	—	1
Lycopersicon esculentum	5	5	5	5	5	5	5	5	5	5	5	5
cv. Rutgers	5	5	5	5	5	5	5	5	5	5	5	5

* Watermelon reated to the penetration of the juveniles by the formation of numerous small galls, but no juveniles could be recovered..

1968; Netscher, 1978) has also been observed here. The individual variability of morphological characteristics within single egg mass lines (espacially the perineal patterns) complicates the identification of *Meloidogyne* populations, therefore the use of the esterase phenotype which eliminates this kind of variation is preferable. Apart from the pI lines, all other esterase phenotypes were studied on one population each, therefore more populations of these phenotypes should be studied to verify whether the relation between esterase phenotype and morphology, as presented here, is valid in general.

The width of the vulva and the length of stylet of the males enables one to create classes which however, do not fit into the groups established by the esterase study. In addition, no group of classes can be clearly defined though Jepson (1983) could distinguish *M. incognita* from *M. javanica* and *M. arenaria* on the basis of the male stylet. In the present study, the length of the stylet of males of *M. incognita* varied between 20 and 23 µm; these values perfectly correspond to those given by Netscher (1983). On the other hand Eisenback (1985) found that the length of stylets of *M. incognita* was between 23 and 25 µm. Thus, it seems doubtful whether this characteristic should be used for the identification of *Meloidogyne* populations.

The length of the second-stage larva is more or less related to the different phenotypes : the five pI lines are grouped in an interval equel to 20 % of the total variation, which is not likely to be a random distribution (the probability of this distribution would be $(1/5)^5$ i.e. 0.032 %).

Actually there is a large heterogeneity in the biometrical characters : the data given by Esser, Perry and Taylor (1976), Franklin (1979) and Eisenback *et al.* (1981) significantly overlap the range observed in our study. Although this may reflect the various origins of the populations or various physiological stresses and possibly, differences in methodology, it seriously limits their use in taxonomy.

The identification of *Meloidogyne* through differential hosts has also serious limitations :

— both pII and pIV lines may refer either to *M. javanica* or *M. arenaria* race 2, which have the same host-range. This is also the case for the pI and pVII lines which all correspond to *M. incognita,* race 1; but here the use of an additional host, sweet potato cv. Chinese, allowed the distinction between these two esterase types;

— some results can be misleading : the pV line was named *M. incognita,* race 2 through Sasser discrimination test but shows the perineal pattern of *M. arenaria.* Netscher (pers. comm.) noticed that in West Africa *M. arenaria* populations have a different host-range from those described by Sasser as they develop on sweet pepper cv. California Wonder but not on groundnut cv. Florrunner. The pV esterase phenotype corresponds to *M. arenaria* according to Janati *et al.* (1982). This suggests that we are dealing with a new race of *M. aren-*

Table 8

Reaction of the twelve lines to the other plants studied. (For each host : first line = root gall index; second line = rate of reproduction; for scales : see " Materials and methods ".)

HOSTS	*LINES*											
	pI 1	*pI 2*	*pI 3*	*pI 4*	*pI 5*	*pII*	*pIII*	*pIV*	*pV*	*pVI*	*pVII*	*pVIII*
Ipomœa batatas	—	—	—	—	—	—	—	—	—	5	—	—
cv. CDH	—	—	—	1	—	—	—	—	—	5	—	—
Ipomœa batatas	3	3	3	4	3	—	—	—	—	5	—	—
cv. Chinese	3	3	4	3	3	1	—	—	—	5	—	—
Medicago sativa	—	—	—	—	—	—	—	—	—	—	—	—
cv. Interior	—	—	—	—	—	—	—	—	—	1	—	—
Glycine max	2	2	2	—	—	—	—	2	—	5	—	—
cv. Forrest	3	2	3	2	1	—	3	2	3	5	—	1
Panicum maximum	—	—	—	—	—	—	—	—	—	—	—	—
cv. T 58	—	—	—	—	—	—	—	—	—	—	—	—
Lycopersicon esculentum	—	—	2	—	—	—	1	—	3	5	—	2
cv. Rossol	—	—	3	1	—	1	3	—	1	5	1	1
Coffea canephora	3	3	3	3	—	—	2	—	—	—	3	—
	1	2	3	1	—	—	2	—	—	—	1	—
Coffea arabica	—	—	—	—	—	—	—	—	—	—	—	—
	—	—	1	1	—	—	1	—	—	—	—	—
Amaranthus viridis	5	5	5	5	5	5	5	5	4	—	5	5
	5	3	5	5	5	5	5	5	4	1	5	3
Sesbania rostrata	5	5	5	5	5	5	5	5	3	5	5	5
	4	5	5	5	5	5	5	5	4	5	5	5
Lactuca sativa	4	4	5	5	5	3	4	4	—	5	5	3
	4	3	4	3	4	3	3	4	—	3	2	2

aria, race 3, which might be typical of West Africa. It is interesting that this line does not develop on lettuce cv. Blonde de Paris;

— among the five pI lines, some variation does exist regarding the response to some hosts such as soybean cv. Forrest and *Coffea canephora* (coffee). These differences are too small to allow distinction between these lines but probably refer to physiological heterogeneity (Netscher, 1977);

— two lines, pII and pIV, morphologically corresponding respectively to *M. arenaria* and *M. javanica*, have similar host-ranges.

If we consider the most useful characters, i.e. esterase phenotype, perineal pattern and host-range, we can identify the various isolates as follows :

— pI : esterase phenotype characteristic of *M. incognita* (Janati *et al.*, 1982; Esbenshade & Triantaphyllou, 1985), perineal pattern of the *M. incognita* type and host-range of race 1 of *M. incognita* (Sasser, 1979). Thus, the five pI lines can be named *M. incognita* (race 1);

— pII : perineal pattern of the *M. arenaria* type and host-range corresponding to both *M. javanica* and *M. arenaria* (race 2). This phenotype was refered to *M. arenaria*, as atypical populations, by Esbenshade and Triantaphyllou (1985). We propose to name it *M. arenaria* (race 2);

— pIII : esterase phenotype observed in one population of *M. incognita acrita* (Bergé & Dalmasso, 1975) and in unidentified populations (Esbenshade & Triantaphyllou, 1985). This line has a peculiar perineal pattern of the *M. incognita acrita* type. According to Sasser (1979), the host-range has not been studied so far. It is proposed to name this line *M. incognita acrita.* First mentioned by Chitwood (1949), *M. incognita* variety *acrita* has been considered respectively as a variety, a subspecies and even as a species, sometimes synonymized with *M. incognita;* its precise position in taxonomy remains in question (Hewlett & Tarjan, 1983). However

in our study, the esterase phenotype, the perineal pattern, the body length of the second-stage larva and the host-range make this line very different from the others and further studies need to be made to compare this phenotype to other *M. incognita acrita* populations;

— pIV : esterase phenotype characteristic of *M. javanica* (Janati *et al.*, 1982; Esbenshade & Triantaphyllou, 1985). This line shows a perineal pattern of the *M. javanica* type and a host-range which corresponds either to *M. javanica* or to *M. arenaria* (race 2) according to Sasser (1979). Therefore, it should be considered as *M. javanica;*

— pV : this line shows the esterase phenotype of *M. arenaria* (Janati *et al.*, 1982; Esbenshade & Triantaphyllou, 1985) and has a perineal pattern of the *M. arenaria* type. The host-range does not fit the one proposed by Sasser (1979) for *M. arenaria* (races 1 and 2); our observations on the host-range, in agreement with that of Netscher (pers. comm.) justify the creation of a new race : *M. arenaria* (race 3);

— pVI : this phenotype often encountered during this study was recorded only twice before, once in an unidentified population and once in a population of *M. enterolobii* (Esbenshade & Triantaphyllou, 1985). The perineal pattern is of the *M. incognita* type and its host-range fits that of race 4 of *M. incognita.* Acosta and Negron (1982) found that this race was able to develop on *Meloidogyne* resistant soybean cv. Forrest. This is in agreement with our observations and suggests that this line corresponds to the race 4 of *M. incognita.* More detailed studies on numerous lines should be made in order to confirm this hypothesis;

— pVII : this phenotype was observed in unidentified populations from Nigeria and Senegal (Janati *et al.*, 1982) and in *M. chitwoodi, M. platani* and *M. incognita* populations (Esbenshade & Triantaphyllou, 1985). Our line shows a perineal pattern of the *M. incognita* type and the host-range of *M. incognita,* race 1. It can probably be considered as *M. incognita* race 1, but it should be noted that it does not develop on sweet potato cv. Chinese in opposition to former (pI) lines;

— pVIII : this phenotype has been found in one population from South Africa (this study), in unidentified populations from Seville, Porto-Rico, Australia, Portugal (Janati *et al.*, 1982), from Cape Verdian Islands (Merny, pers. comm.), from USA (Esbenshade & Triantaphyllou, 1985), in an atypical population of *M. arenaria* and in *M. hispanica* (Esbenshade & Triantaphyllou, 1985). It has a particular perineal pattern and its host-range has not been described. The population from Seville is also able to develop on a resistant cultivar of hot pepper resistant to *Meloidogyne :* INRA P.M. 687 (Hendy, 1984). These observations justify its identification as *M. hispanica* (*in* Esbenshade & Triantaphyllou, 1985).

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