Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 1. Stability of the esterase phenotype

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SUMMARY

The influence of different biological parameters (hatching rank or age of the larvae used to inoculate, age of the females, age and type of the host plants) on the stability of the esterase phenotype of females belonging to a single egg-mass population of *Meloidogyne incognita* has been tested. The resistant tomato cultivar Rossol was also included in the tests. None of these parameters influenced the expression of the major bands of enzymatic activity revealed by electrophoresis.

RÉSUMÉ

Utilisation des isostérases dans la systématique du genre Meloidogyne. 1. *Stabilité du phénotype estérasique*

L'influence de différents paramètres biologiques (rang d'éclosion ou âge de la larve inoculée, âge de la femelle, âge de la plante hôte et type de plante) sur la stabilité des isoestérases de femelles appartenant à un clone de *Meloidogyne incognita* a été testée. Le cultivar de tomate « Rossol », résistant aux *Meloidogyne*, a aussi été utilisé dans ces tets. Aucun de ces paramètres n'a d'incidence sur l'expression des bandes majeures révélées par électrophorèse.

The taxonomy of the genus *Meloidogyne* is generally based on morphological and biometric characters (Whitehead, 1968; Esser, Perry & Taylor, 1976; Netscher, 1978; Taylor & Sasser, 1978; Franklin, 1979; Jepson, 1983), karyological aspects (Triantaphyllou, 1962, 1963, 1966, 1970, 1971, 1979, 1981; Triantaphyllou & Hirschmann, 1964) and on host preference (Sasser, 1979; Sasser & Triantaphyllou, 1977). However, all these criteria have certain limitations. Morphological variability of perineal patterns in natural populations and even within single egg-mass lines carries ambiguous observations (Chitwood, 1949; Allen, 1952; Sasser, 1954; Whitehead, 1968; Netscher, 1978). In biometric studies, the overlapping ranges of most of the characters and the limited usefullness of others make specific identification difficult or impossible. The small chromosomes are often difficult to observe and count. Host-range studies require one and a half month delay to breed *Meloidogyne* for one generation. As mixed populations frequently occur (Netscher, 1978) and because of the difficulties of species identification mentioned above, ideally a number of single egg-mass populations should be established to analyze field populations, a time and greenhouse space consuming requirement.

Various investigators have studied several enzyme systems and have demonstrated that the esterase phenotype can be used to distinguish between species (Bergé & Dalmasso, 1975, 1976; Dalmasso & Bergé, 1975, 1977, 1978, 1979, 1983, Janati *et al.*, 1982). It has been demonstrated that the esterase phenotype of a single female unequivocably establishes its identity. Therefore analysis of sufficient females of a *Meloidogyne* population allows for an unamiguous characterization.

However it might be possible that the expression of the esterase phenotype is related to specific physiological stages of the nematode. Dickson, Huising and Sasser (1971) considered three developmental stages (the egg, the second-stage larva and the adult female) while Bergé and Dalmasso (1975) studied only two stages (the young female and the old female). Both studies revealed variations in the esterase phenotypes. When considering the host, Dickson, Huising and Sasser (1971) did not observe any difference in the esterase phenotypes of nematodes grown on different plants whereas Ishibashi (1970) and Bergé and Dalmasso (1975) noted some variations.

Because of these contradictory reports, I conducted a detailed study of the possible effects of various parameters, such as hatching rank, age of the juvenile at inoculation, age of the female and age of the plant at inoculation, on the esterase phenotype. Moreover, different host plants belonging to diverse botanical families and one *Meloidogyne* resistant cultivar of tomato were tested.

Material and methods

All experiments were made using a single egg-mass population of *M. incognita* characterized by a frequently encountered esterase phenotype (pI). Egg plant (*Solanum melongena* cv. Violette longue) was used in all tests except in experiments V and VI where particular hosts were tested.

By transfering the plants used in experiments I, II and III five days after inoculation to hydroponic cultures (Cadet & Merny, 1976), the age of the nematode could be specified within a range of five days. It also allowed detection of root-galls and facilitated the collection of females with the least possible disturbance of the plants.

The females were collected 31 to 35 days after inoculation except in experiment II, where the effect of the age of females was tested.

Females were squashed individually in a Tris-HCl buffer, pH 8, containing 17 % sucrose, 1.8 % ascorbic acid and 1.4 % cysteine hydrochlorure and centrifuged at 10 000 g for 15 minutes. The supernatant was analyzed by electrophoresis following the method of Dalmasso and Bergé (1978) using polyacrylamid gel (7 %, pH 8.4 and 0.5 mm thin slabs) (Fargette, 1984), and α or β naphthyl-acetate as staining reagents for esterases (Brewer & Singh, 1970); b and β esterases were distinguished according to Dalmasso and Bergé (1978).

The following six experiments were performed :

Experiment I : Effect of hatching rank of second-stage larvae on the esterase phenotype

Egg-sacs were collected from an egg plant inoculated five weeks earlier, placed on a small screen in a Petri-dish filled with water and allowed to hatch for eighteen days. Every three days, the hatched larvae were collected and immediatly used to inoculate to egg plant seedlings. In each of the six batches thus obtained, ten females were individually squashed and analyzed by electrophoresis.

Experiment II : Effect of the age of females on the esterase phenotype

Egg-sacs were left in a 0.3 M NaCl solution for one week and subsequently placed on a small screen in a Petri dish filled with water. Hatching was inhibited by the saline solution (Dropkin, Martin & Johnson, 1958) and took place massively after the egg-masses were placed in water. Egg plant seedlings were inoculated with freshly hatched larvae. Every five days, from the fifteenth to the 70th day after inoculation (twelve batches, $J_0 + 15$, $J_0 + 20$, ... $J_0 + 70$), third- and fourth-stage larvae and/or females were collected from the roots of the plants. Three to five nematodes were combined to form sample. Ten samples per batch were subjected to electrophoresis, although only six samples could be obtained for the first and the second batches.

Experiment III : Effect of aging of second-stage larvae on the esterase phenotype

Eggplant roots were placed in a mist-chamber for 30 days and larvae collected every three days; thus ten batches of larvae were obtained, varying from three to thirty days in age. Larvae were kept in tubes filled with water aerated by a small air pump. When the last batch of larvae was collected from the mist-chamber, each of the batches of larvae was poured on a filter paper supported by a small sieve. Subsequently the filter paper was placed in a Petri dish and water added to cover the filtered nematodes. The next day one-month-old egg plant seedlings growing in sterile soil were inoculated with living larvae collected in the Petri dish. Mortality due to starvation was high and therefore few or even no adult females could be obtained from a number of batches. Nevertheless, four, six, six, ten, ten and ten females could be subjected to individual electrophoresis in the batches $J_0 + 3$, $J_0 + 6$, $J_0 + 12$, $J_0 + 21$, $J_0 + 27$, $J_0 + 30$, respectively.

Experiment IV : Effect of age of plants on the esterase phenotype

Eleven batches of egg plants 19, 18, 16, 13, 11, 9, 7, 6, 5, 4 and 3 weeks of age were simultaneously inoculated with second-stage larvae collected from the mist-chamber. Five weeks later, ten females per batch were individually squashed and subjected to electrophoresis.

Experiment V : Effect of host type on the esterase phenotype

The following five hosts belonging to different botanical families were inoculated with second-stage larvae collected from the mist-chamber : kenaf, *Hibiscus cannabinus* and gombo, *H. esculentus* (both belonging to Malvaceae), cucumber, *Cucumis sativus* (Cucurbitaceae), *Coleus* sp. (Labiaceae), egg plant, *Solanum melongena* (Solanaceae). Five weeks later, ten females per host were individually squashed and subjected to electrophoresis.

Experiment VI : Study of the esterase phenotype of the females obtained on a resistant cultivar

A *Meloidogyne* resistant tomato cultivar, Rossol, was used in this experiment. Tomato plants were inoculated with 3 000 second-stage larvae per plant. Each of the twenty females obtained was individually squashed and subjected to electrophoresis.

Results

If single females are examined, the esterase phenotype consists of two bands : one strong (Rm 0.71) and the

other weaker (Rm 0.76). In experiments I, III, IV, V, VI, where females were individually squashed, no variation in the esterase phenotype could be observed. No discernable differences in esterase phenotype could be attributed to the different biological parameters tested : hatching rank and age at inoculation of the second-stage larva, age of the host at time of inoculation, host type and influence of a *Meloidogyne* resistant cultivar.

When homogenates from several females were analyzed, additional proteins, in concentrations normally too low to be detected in extracts from single females, could be observed. In experiment II, up to nine bands could be observed : Rm 0.11, 0.24, 0.45, 0.50, 0.58, 0.62, 0.71, 0.76, 0.82 (Fig. 1). Some apparently quantitative variation has been noticed depending on the age of the females (Fig. 2). The frequency is expressed as a ratio between the number of females showing a phenotype and the total number of females of each batch.

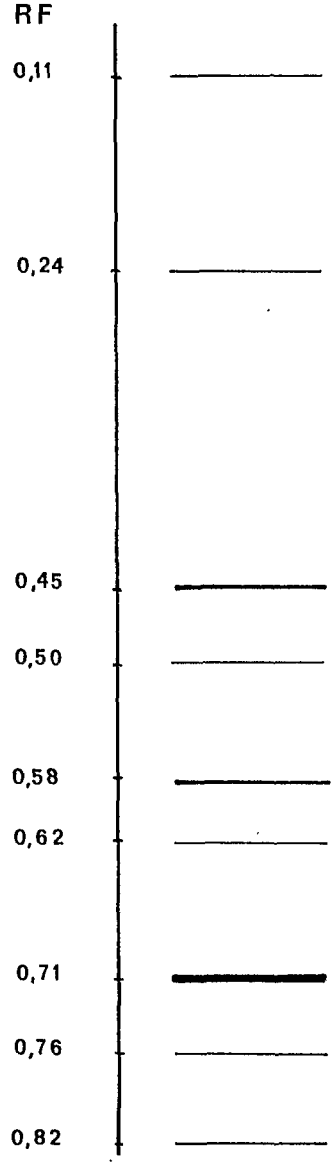

Fig. 1. Esterase isozymes, corresponding to *M. incognita*, observed in samples containing several individuals (Exp. II).

— The two upper bands (0.11, 0.24) are very weak and do not appear in each batch. There is no clear relationship between their occurrence and the age of the nematode. (They are not taken into account in Fig. 2.)

— The lowest band (0.82) seems to be associated with the young stages : it is always present on $J_0 + 15$, $J_0 + 20$, $J_0 + 25$ whereas in later stages its intensity and its occurrence decrease. It is not observed after $J_0 + 50$.

— Bands 0.45 and 0.58 are first observed at $J_0 + 20$ and remain present until the end of the experiment. From $J_0 + 20$ until $J_0 + 40$, the bands 0.50 and 0.62 are also present but disappear afterwards.

— Band 0.76 appears on $J_0 + 20$ and remains stable during the whole experiment.

— Band 0.71 is always present from the beginning to the end of the experiment. Its intensity is weak on $J_0 + 15$ but from $J_0 + 20$ until the end of the experiment it has turned stronger.

Bands 0.71 and 0.76 belong to the b esterase system as defined by Dalmasso and Bergé (1978). Bands 0.11 and 0.24 belong to the same system but they are not as useful as bands 0.71 and 0.76 because homogenates must be prepared of several females in order to detect their presence. The 0.45, 0.50, 0.58, 0.62, 0.82 bands seem to belong to the β esterase system.

Discussion

Two main kinds of esterase bands are revealed by the electrophoresis of *Meloidogyne* after staining with α and β naphthyl acetate. The major bands can be detected by electrophoresis of individual females whereas minor bands can only be demonstrated when samples composed of several females are analyzed. The major bands present in the population studied (0.71, 0.76) belong to the b esterase system which can be used for specific identification (Dalmasso & Bergé, 1978, 1983; Janati *et al.*, 1982).

None of the variables tested in this study had influence on the presence and position of the major esterase bands.

The presence of the minor bands varies with the age of the nematode up to $J_0 + 50$. Probably the physiologic state of the nematodes influences their appearance. After $J_0 + 50$, the minor bands remain stable. On the contrary, starting from $J_0 + 20$ the major bands which characterize the pI phenotype are always present. There-

fore the phenotype can be determined using individual females, independently of their age. In most cases females are squashed about five weeks after inoculation. At that time they are white and swollen and the major bands are sufficiently distinct to be clearly detected in single females.

It is difficult to judge whether the frequency of the minor bands depends on the age of the females or on the quantity of material analyzed. In practice it is almost impossible to distinguish between these two factors which moreover are closely correlated.

In these experiments we did not observe any effect of the host on the esterase phenotype. These results are in line with those of Dickson, Huisingh and Sasser (1971). Ishibashi (1970), however, found that the nature of the host had an influence on the esterase phenotype. According to Dickson, Huisingh and Sasser (1971), Ishibashi's results could be explained by considering the extraction method of females from roots by subsequent freezing and thawing. Host cells were damaged and released proteins which contaminated the homogenates of the nematodes. Apart from a single observation by Bergé and Dalmasso (1975), who observed that the esterase phenotype of a population of *M. hapla* parasitizing marrow differed considerably from the esterase phenotype obtained when this population was cultivated on tomato or cowpea, the esterase phenotype of *Meloidogyne* populations have been stable. The stability of the esterase phenotype has been confirmed by other studies. Thus, Fargette (1987) studying 57 populations belonging to three *Meloidogyne* species found that the nature of the host did not influence the esterase phenotype of the nematodes.

The results of this article demonstrate that various conditions did not influence the esterase phenotype of the population studied. Consequently the esterase phenotype may be considered as a reliable and usefull tool in the taxonomy of the genus *Meloidogyne.*

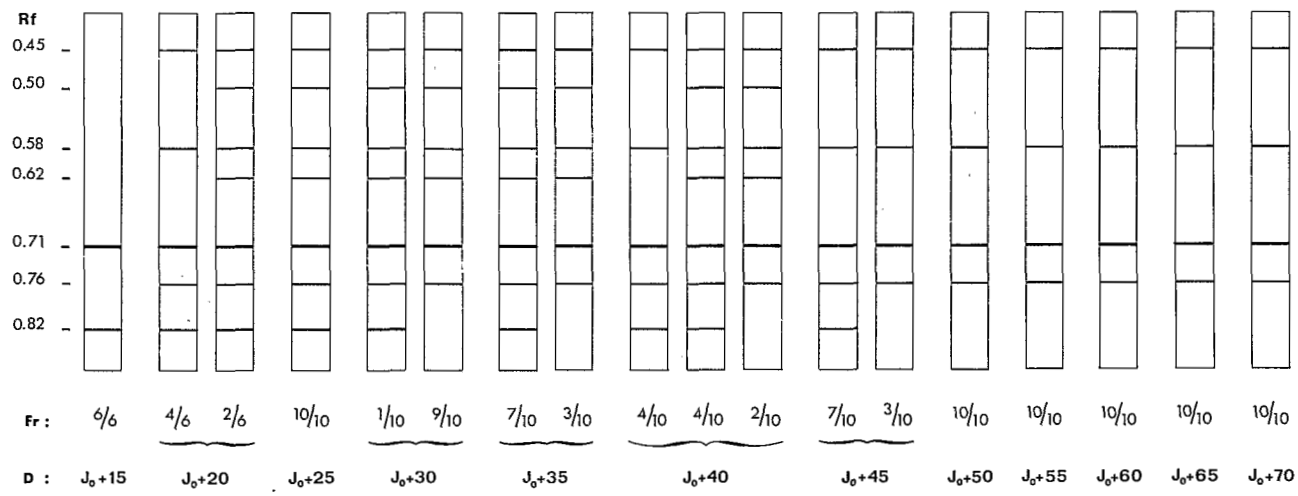

Fig. 2. Esterase phenotypes of aging females (Exp. II). For each date (D) of squashing, the different phenotypes are given with their frequency of occurrence (Fr). The slow bands 0.11 and 0.24 of Fig. 1 are not taken into account here because they were much irregularly visible.

References

Allen, M. W. (1952). Observations on the genus *Meloidogyne* Gœldi, 1887. *Proc. helminth. Soc. Wash.,* 19 : 44-51.

Bergé, J.-B. & Dalmasso, A. (1975). Caractéristiques biochimiques de quelques populations de *Meloidogyne hapla* et *M.* spp. *Cah. ORSTOM Sér. Biol.,* 10 : 263-271.

Bergé, J.-B. & Dalmasso, A. (1976). Variations génétiques associées à un double mode de reproduction parthénogénétique et amphimictique chez le nématode *Meloidogyne hapla. C. r. hebd. Séanc. Acad. Sci., Paris,* 282, D : 2087-2090.

Brewer, G. J. & Singh, C. F. (1970). *An introduction to ensozymes techniques.* London & New York, Academic Press, 186 p.

Cadet, P. & Merny, G. (1976). Méthode d'étude de la pénétration et du développement des deux sexes chez les *Heterodera. Cah. ORSTOM, Sér. Biol.,* 10 : 151-155.

Chitwood, B. G. (1949). " Root-knot nematodes " Part 1; a revision of the genus *Meloidogyne* Goeldi, 1887. *Proc. helminth. Soc. Wash.,* 16 : 90-104.

Dalmasso, A. & Bergé, J.-B. (1975). Variabilité génétique chez les *Meloidogyne* et plus particulièrement chez *M. hapla. Cah. ORSTOM, Sér. Biol.,* 10 : 233-238.

DALMASSO, A. & BERGÉ, J.-B. (1977). Variabilité liée aux phénomènes de reproduction chez les *Meloidogyne. Ann. Zool. Ecol. anim.*, 9 : 568-569.

DALMASSO, A. & BERGÉ, J.-B. (1978). Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp. : application to the taxonomy of *Meloidogyne. J. Nematol.*, 10 : 323-332.

DALMASSO, A. & BERGÉ, J.-B. (1979). Genetic approach to the taxonomy of *Meloidogyne* species. In : Lamberti, F. & Taylor, C. E. (Eds). *Root-knot nematodes* (Meloidogyne species). *Systematics, biology and control.* New York & London, Academic Press : 111-113.

DALMASSO, A. & BERGÉ, J.-B. (1983). Enzyme polymorphism and the concept of parthenogenetic species, exemplified by *Meloidogyne.* In : Stone, A. R., Platt, H. M. & Khalil, L. F. (Eds). *Concepts in nematode systematics.* Systematics Assoc. Special Vol., nº 22, London & New York, Academic Press : 187-196.

DICKSON, D. W., HUISINGH, D. & SASSER, J. N. (1971). Dehydrogenases, acid and alkaline phosphatases and esterases for chemotaxonomy of selected *Meloidogyne, Ditylenchus, Heterodera, Aphelenchus* spp. *J. Nematol.*, 3 : 1-16.

DROPKIN, V. H., MARTIN, G. C. & JOHNSON, R. W. (1958). Effect of osmotic concentration on hatching of some plant parasitic nematodes. *Nematologica,* 3 : 115-126.

ESSER, R. P., PERRY, V. G. & TAYLOR, A. L. (1976). A diagnostic compendium of the genus *Meloidogyne* (Nematoda : Heteroderidae). *Proc. helminth. Soc. Wash.,* 43 : 138-150.

FARGETTE, M. (1984). *Utilisation de l'électrophorèse dans l'étude de deux organismes d'intérêt agricole :* Trichogramma *supersp.* evanescens *(Hymenoptera, Chalcidoidea) et* Meloidogyne *spp. (Nematoda, Tylenchida).* Thèse Dr-Ingr, École nat. sup. agron., Montpellier, 189 p.

FARGETTE, M. (1987). Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne.* 2. The esterase phenotypes observed in West African populations and their characterization. *Revue Nématol.,* 10 : 45-56.

FRANKLIN, M. T. (1979). Taxonomy of the genus *Meloidogyne.* In : Lamberti, F. & Taylor, C. E. (Eds). *Root-knot nematodes* (Meloidogyne *species*). *Systematics, biology and control.* New York & London, Academic Press : 37-54.

ISHIBASHI, N. (1970). Variations of the electrophoretic protein patterns of Heteroderidae (Nematoda : Tylenchida) depending on the developmental stages of the nematode and on the growing conditions of the host plants. *Appl. Entomol. Zool.,* 5 : 23-32.

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

Accepté pour publication le 26 août 1986.

JEPSON, S. B. (1983). Identification of *Meloidogyne :* a general assessment and a comparison of male morphology using light microscopy. *Revue Nématol.,* 6 : 291-309.

NETSCHER, C. (1978). Morphological and physiological variability of species of *Meloidogyne* in West Africa and implications for their control. *Meded. Landb.hogesch., Wageningen,* 78 : 1-46.

SASSER, J. N. (1954). Identification and host-parasite relationships of certain root-knot nematodes (*Meloidogyne* spp.). *Univ. Maryland, Bull. A 77, :* 1-30.

SASSER, J. N. (1979). Pathogenicity, host-ranges and variability in *Meloidogyne* species. In : Lamberti, F. & Taylor, C. E. (Eds). *Root-knot nematodes* (Meloidogyne *species*). *Systematics, biology and control.* New York & London, Academic Press : 257-268.

SASSER, J. N. & TRIANTAPHYLLOU, A. C. (1977). Identification of *Meloidogyne* species and races. *J. Nematol.,* 9 : 283.

TAYLOR, A. L. & SASSER, J. N. (1978). *Biology, identification and control of root-knot nematodes.* Raleigh; N. C. State Univ., 111 p.

TRIANTAPHYLLOU, A. C. (1962). Oogenesis in the root-knot nematode *Meloidogyne javanica. Nematologica,* 7 : 105-113.

TRIANTAPHYLLOU, A. C. (1963). Polyploidy and parthenogenesis in the root-knot nematode *Meloidogyne arenaria. J. Morphol.,* 113 : 489-499.

TRIANTAPHYLLOU, A. C. (1966). Polyploidy and reproductive patterns in the root-knot nematode *Meloidogyne hapla. J. Morphol.,* 118 : 403-413.

TRIANTAPHYLLOU, A. C. (1970). Cytogenetic aspects of evolution of the family Heteroderidae. *J. Nematol.,* 2 : 26-32.

TRIANTAPHYLLOU, A. C. (1971). Genetics and cytology. In : Zuckerman, B. M., Mai, W. F. & Rhode, R. A. (Eds). *Plant parasitic nematodes, Vol. 2.* New York & London, Academic Press : 85-109.

TRIANTAPHYLLOU, A. C. (1979). Cytogenetics of root-knot nematodes. In : Lamberti, F. & Taylor, C. E. (Eds). *Root-knot nematodes* (Meloidogyne *species*). *Systematics, biology and control.* New York & London, Academic Press : 85-109.

TRIANTAPHYLLOU, A. C. (1981). Oogenesis and chromosomes of the parthenogenetic root-knot nematode *Meloidogyne incognita. J. Nematol.,* 13 : 95-104.

TRIANTAPHYLLOU, A. C. & HIRSCHMANN, H. (1964). Reproduction in plant and soil nematodes. *Ann. Rev. Phytopathol.,* 2 : 57-80.

WHITEHEAD, A. G. (1968). Taxonomy of *Meloidogyne* with descriptions of four new species. *Trans. Zool. Soc. Lond.,* 31 : 263-401.