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Characterization of resistance breaking *Meloidogyne incognita* - like populations using lectins, monoclonal antibodies and spores of *Pasteuria penetrans*

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Summary – Six single eggmass lines of *Meloidogyne,* originating from populations collected in the Ivory Coast which showed a particular esterase phenotype (coded pVI) and an ability to overcome the resistance conferred by the *Mi* gene, were compared with *M. incognita,* using different probes. Labeling with different lectins gave similar results for the pVI lines and *M. incognita.* Strains of *Pasteuria penetrans* differentially recognized some of the populations, supporting the view that the pVI populations are different from *M. incognita* but also showing some variability among the resistance breaking group of populations. Two monoclonal antibodies, raised to *M. incognita* and specifically reacting to this species, indicated that the pVI populations were related to *M. incognita* but also demonstrated some differences.

Résumé – *Caractérisation par des lectines, des anticorps monoclonaux et des souches de* Pasteuria penetrans, *de populations de* Meloidogyne *proches de* M. incognita *et capables de se développer sur cultivars résistants* – Six clones de *Meloidogyne,* provenant de populations récoltées en Côte d'Ivoire et qui possèdent un phénotype estérasique particulier (codé pVI) ainsi que la faculté de briser la résistance conférée par le gène *Mi,* ont été comparés à *M. incognita* à l'aide de différentes sondes. Le marquage à l'aide de lectines des exsudats amphidiaux de juvéniles de second stade ne permet pas de distinguer ces lignées de *M. incognita.* Des souches de *Pasteuria penetrans* permettent de les distinguer et mettent aussi en évidence une certaine variabilité au sein de ce groupe. Deux anticorps monoclonaux, spécifiques de *M. incognita* indiquent que les clones de phénotype pVI partagent certains caractères avec *M. incognita* mais aussi qu'ils s'en distinguent.

Key-words : *Meloidogyne,* resistance breaking populations, characterization, surface components.

Meloidogyne spp. are major pests of many tropical crops. Resistant cultivars potentially provide the most effective means of controlling them. The alternatives, such as chemicals and biological control agents generally are either environmentally unacceptable or too expensive and technically demanding. Unfortunately, populations of *Meloidogyne* spp. with virulence against many sources of resistance have been identified (Riggs & Winstead, 1959; Netscher, 1976; Prot, 1984; Fargette & Braaksma, 1990). Methods for readily identifying such populations, e.g. isozyme phenotypes (Fargette & Braaksma, 1990), need to be developed. Also, it is desirable to determine from which species and how frequently such virulence has arisen. Answers of these questions would help clarify the potential sources of resistance-breaking biotypes.

Early studies (Fargette & Braaksma, 1990) focused on populations of *Meloidogyne* from the Ivory Coast that were able to overcome the resistance of a number of cultivars including tomato cv. Rossol, the resistance of which is conferred by the *Mi* gene. On the basis of morphological characters and host-range (Sasser, 1979), these populations were classified as *M. incognita.* Also their chromosome disposition during the prophase of the first division of the mitotic parthenogenetic process, as described by Triantaphyllou (1985), was characteristic of *M. incognita* (Fargette & Braaksma, 1990). In contrast, the esterase phenotype of these populations, coded pVI, was very different from that of *M. incognita* which, in previous studies, had been shown to be very consistent (Janati *et al.,* 1982; Esbenshade & Triantaphyllou, 1985; Fargette, 1987). Rather, the pVI phenotype was identical to that coded VS-S by Esbenshade and Triantaphyllou (1985) and exhibited by *M. mayaguensis* (Rammah & Hirschmann, 1988). These results distinguish the pVI lines from *M. incognita.*

The aim of the present study was to further characterize the pVI lines and their relationships with other species, especially *M. incognita.* With that objective, we used the following range of techniques and probes :

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18 were prepared in the same way. The tests were made using routine ELISA procedures (Jones *et al.,* 1988) in which the solutions containing the antibodies were allowed to react with the antigen for 1 h at 37 °C. The intensity of the reaction was recorded (absorbance) and scores grouped in four classes.

Results

LECTIN BINDING

Where lectin binding occurred, it was mainly to the amphids (Table 2). ConA and UEA also sparingly labelled the cuticle of some lines.

Peanut agglutinin (PNA) which binds to galactose or galactosamine (Goldstein & Poretz, 1986) did not bind to any of the lines of *Meloidogyne* tested. Soybean agglutinin (SBA) bound specifically but weakly to the amphid apertures of *M. arenaria* only. The competitive sugar (N-acetyl, D-galactosamine; Goldstein & Poretz, 1986) blocked binding, indicating the presence of N-acetyl-galactosamine in the amphidial exudate of *M. arenaria* but its absence from the *M. incognita* and the pVI lines.

Concanavalin agglutinin (ConA) and *Ulex europaeus* agglutinin (UEA) bound to all lines, although binding by ConA on the amphids was stronger in *M. arenaria* than in *M. incognita* and the pVI lines. The binding was specific as it was blocked by the specific sugars (respectively methyl-mannopyranose and fucose) on *M. arenaria* and *M. incognita.* On the pVI lines, binding of ConA was totally specific (except on 18, where some non-specific binding persisted with the competitive sugar on 20 to 60 % of the juveniles observed; however, this non-specific fluorescence was diffuse and confined to the head region; quantitatively it was less important than the binding observed with the lectin only, showing that the lectin binding is at least partly specific with this line). With UEA, binding was specific on the pVI lines except for 15 where some 40 % of the juveniles observed showed some non-specific binding. On the cuticle, some specific but weak binding of ConA was observed on 11, 12, 13 and *M. incognita,* while UEA sparingly, but still specifically, bound to some juveniles (without any clear pattern). Hence, glucose/mannose and fucose are present in amphidial exudates of all lines and variably present on their cuticle.

Table 2. *Labelling of the amphidial exudates of the different lines of* Meloidogyne *by the four rhodamine conjugated lectins; the occurence or absence of fluorescence is given for each lectin in absence (L) or presence (L + S) of the corresponding competitive sugar.*
Scale : – = no recognition; + = recognition; ++ = strong recognition.

	PNA	SBA		ConA		UEA	
	L	L	L + S	L	L + S	L	L + S
M. incognita	–	–		+	–	++	–
pVI lines	–	–		+	– *	+/++	– **
M. arenaria	–	+	–	++	–	++	–

* : except for 18 (see text)
** : except for 15 (see text)

PASTEURIA PENETRANS ATTACHMENT

Attachment rates were similar within each line/species (standard error usually < five spores per nematode) except with *M. arenaria* (standard error > 30 spores per nematode with PP1 and PNG). The variability observed in the numbers of spores attached to *M. arenaria* must be intrinsic to this line/species (Table 3). There were consistent differences in spore attachment between the races/populations of *M. incognita;* races 1 and 2 were heavily infested (> 50 spores/J2) by all three *P. penetrans* populations whereas races 3 and 4 generally carried fewer spores. Few spores of PP1 and PNG attached to the *M. incognita* line from the Ivory Coast; however more spores of PCal attached. Few or no spores of all three *Pasteuria* populations attached to the pVI lines 12, 13, 17 and 18 and to *M. mayaguensis.* However, spores of *P. penetrans* PP1 adhered to the pVI lines 11 and 15 in similar numbers to those observed with *M. incognita* races 3 and 4 and *M. arenaria* whereas attachment of PCal and PNG were lower.

Table 3. *Attachment of three populations of* Pasteuria penetrans *to the different lines and species of* Meloidogyne *tested.*
Scale : 0 = 0 spore; A = 1-5 spores; 2 = 6-10 spores; 3 = 11-50 spores; 4 = > 50 spores.

	Pasteuria penetrans		
	PNG	*PCal*	*PP1*
M. incognita			
race 1	4	4	4
race 2	4	4	4
race 3	1	2	3
race 4	1	2	3
Ivory Coast	1	3	1
pVI lines			
1	0	0	3
2	0	1	1
3	0	0	0
5	1	0	3
7	0	1	0
8	1	1	1
M. mayaguensis	1	1	1
M. arenaria	3	1	3

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