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ORSTOM, Laboratoire de Nématologie, B.P. 1386, Dakar, Sénégal[1]
University Gadja Mada, Department of Agronomy, Yogyakarta, Indonesia[2]

EFFECT OF THE RHIZOSPHERE MICROFLORA ON *PASTEURIA PENETRANS* PARASITIZING *MELOIDOGYNE GRAMINICOLA*

by
R. DUPONNOIS[1], C. NETSCHER (†)[2] and T. MATEILLE[1]

Summary. The effect of the rhizosphere microflora associated with spores of *Pastèuria penetrans* on the attachment of the spores on juveniles of *Meloidogyne graminicola* has been studied. The microflora stimulated the attachment of the spores on the juveniles and consequently reduced the invasion of the roots of tomato plants by the nematodes. The role of a helper rhizosphere microflora for biocontrol of root-knot nematodes by *P. penetrans* is suggested.

Pasteuria penetrans is an obligate endoparasite of nematodes which has potential as a biological control agent (Oostendorp *et al.*, 1991; Zaki and Maqbool, 1992). The relationship between this actinomycete and root-knot nematodes is well documented (Davies *et al.*, 1992; Davies and Danks, 1992). However, the ways in which soil factors influence the *P. penetrans* – nematode interaction are virtually unknown, particularly that between the nematodes, *P. penetrans* and the microflora. The aim of this paper is to examine the influence of the rhizosphere microflora on the parasitic relationship between *P. penetrans* Sayre *et* Starr and *Meloidogyne graminicola* Golden *et* Birchfield.

Materials and methods

A population of *Meloidogyne graminicola* was cultured for two months on tomato (*Lycopersicon esculentum* Mill.) cv. Roma. Then the tomato roots were harvested, cut into short lengths and placed in a mist chamber for one week to allow second stage juveniles (J2) to hatch from the eggs (Seinhorst, 1950).

Seeds of tomato cv. Roma were planted in 0;3 dm^3 pots previously filled with a sandy soil (sand 84.7%; silt 4.1%; clay 11.2%; pH (H_2O) 7.6) sampled in a vegetable field from the experimental station of the École Nationale Supérieure d'Agriculture (ENSA, Thiès, Sénégal) heavily infested with juveniles of *M. javanica* parasitized by *P. penetrans* (20,000 J2 per dm^3, 80% of infected juveniles). Two months after sowing, the plants were uprooted, the root systems were gently washed, cut into short lengths and blended in 100 ml sterile water using an Ultraturax blender. The suspension was sieved through a 45 µm sieve and the filtrate was divided in two parts, each of which were centrifuged (3000 g, 20 min); the supernatants were discarded. The pellet of the first sample was resuspended in alcohol (97%) for 12 h at 25 °C to obtain *P. penetrans* spores free of contaminant microflora, and centrifuged (3000g, 20 min) three times to replace the alcohol with sterile sodium-phosphate buffer (50ml, pH 7).

The pellet of the second sample was directly resuspended in the same sterile buffer without sterilization by alcohol. The two suspensions of spores disinfected or not were incubated for two weeks in the dark at 25 °C.

Each week, attachment tests were performed in glass tubes (18x2 cm) with the spores of *P. penetrans* disinfected or not, adding 5 µl of the suspensions (10^4 spores) to one ml of distilled water containing 100 juveniles of *M. graminicola*. There were five replicates per condition. The suspensions were incubated for 12 hours at 25 °C. The number of juveniles encoumbered with *P. penetrans* spores and the number of spores per juvenile were determined with the aid of a dissecting microscope (magnification x 400). All the data were statistically analysed according to the Mann Whitney U test ($P < 0.05$). At the same time, the populations of bacteria were determined in the two suspensions of *P. penetrans* spores disinfected or not by spread-plate counts on 0.3% tryptic soy agar (Martin, 1975).

Two week-old seedlings of tomato cv. Roma were transplanted in 1 dm^3 pots filled with the same sandy soil that was used for the production of spores of *P. penetrans* but which had been previously autoclaved (140 °C, 40 min). Two weeks after transplanting, the plants were inoculated either with 100 juveniles of *M. graminicola*, or with $5.9x10^6$ spores of *P. penetrans* disinfected or not with alcohol following the technique described above, or with 100 juveniles of *M. graminicola* and with $5.9x10^6$ spores of *P. penetrans* disinfected or not, together. There were ten replicates for each inoculum condition placed in a randomised block design in a glasshouse (25 °C day, 20 °C night). One month later, each root system was gently washed free of soil and the galls with egg masses were counted. The roots were then cut into short lengths and placed in a mist chamber for two weeks to collect the juveniles as described above. The oven dried weights of shoot and root (1 week at 65 °C) were measured and compared to non inoculated plants (control). Then the root systems were ground to a powder and resuspended in distilled water. The spores of *P. penetrans* were recovered as described before and counted using a Mallassez counting cell. The data were analysed with the Mann Whitney U test ($P < 0.05$).

Results

In the attachment tests 100% of the juveniles of *M. graminicola* were parasitized by *P. penetrans* whatever the time of incubation of the spores in the buffer and there were no differences between the disinfected and the non disinfected spores. Only the number of spores per juvenile differed (Fig. 1). Twenty to twenty-four spores attached to the juveniles in the first test with no difference between the disinfected and the non-disinfected spores. However, after one and two weeks of incubation, the number of spores attached per juvenile decreased, more with the spores previously disinfected with alcohol than with the non-disinfected spores. In the suspension containing the non-disinfected spores of *P. penetrans*, the population of bacteria remained constant during the two weeks of incubation in the sodium-phosphate buffer. No bacteria were detected in the suspension containing the spores previously disinfected with alcohol.

One month after the inoculation of tomato plants with juveniles of *M. graminicola*, 87.8% of the juveniles were able to invade the roots and to mature in gravid females (Table I). When the nematode inoculum was supplemented with the spores of *P. penetrans*, the development rate of the juveniles was strongly reduced, significantly more when the spores were not disinfected with alcohol than when they were. Comparing with the non-inoculated plants (control), the juveniles of *M. graminicola* alone reduced the development of the roots without any effect on the shoots. When the spores of *P. penetrans* were added to the juveniles, the root growth

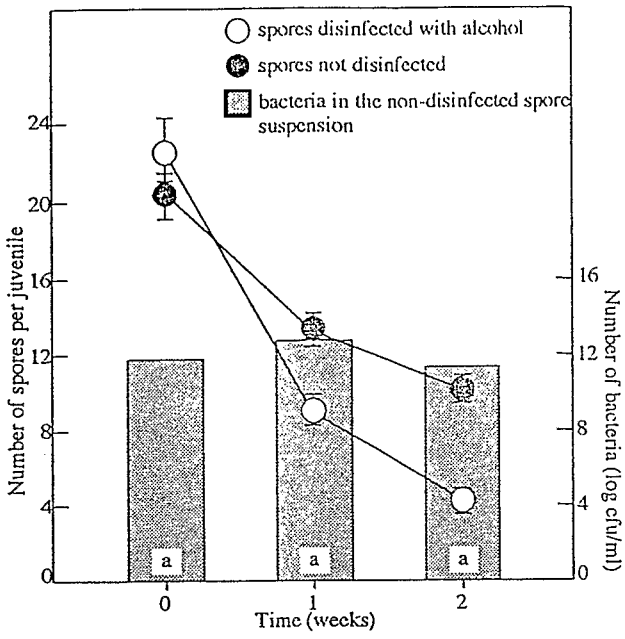

Fig. 1 - Evolution of the number of spores of *Pasteuria penetrans* disinfected or not with 97% alcohol on the juveniles of *Meloidogyne graminicola* (bars represent standard errors) and evolution of the total population of bacteria in the suspension of non disinfected spores (columns with the same letter are not significantly different: $P \geq 0.05$).

was restored. Plant growth was greater when the spores, especially not disinfected, were inoculated alone. Most of the next generation of juveniles which were extracted from the roots in the mist chamber were encumbered with spores of *P. penetrans* (66 to 74% of the juveniles), but without any significant effect of the alcohol disinfection previous to the inoculation of the plants. After the roots were dried and ground to powder, the number of spores of *P. penetrans* extracted was the same whether the parent spores were disinfected or not.

Discussion

The disinfection of the spores of *P. penetrans* with alcohol eliminated the associated microflora as no bacteria were detected later in the suspensions, but it did not affect the attachment of the spores to the juveniles of *M. graminicola.*

The spores of *P. penetrans* which were incubated in the sodium-phosphate buffer became less aggressive to the juveniles of *M. graminicola* over one and two weeks. The attachment is between two organisms whose external surfaces are electronegatively charged and hydrophobic (Himmelhoch *et al.*, 1979; Afolabi *et al.*, 1995; Davies *et al.*, 1996). Thus, the decrease of agressiveness could be due to a change of hydrophobic characteristics or to a progressive saturation of the negative sites of the spore surface by the $Na^{2}+$ cations of the buffer at neutral pH. For example, these characteristics of the $Na^{2}+$ cations are commonly used in soil particule dispersion (Bartoli *et al.*, 1990; Feller *et al.*, 199&). Nevertheless, the spores of *P. penetrans* which were incubated together with the microflora over two weeks, were more aggressive against the nematodes than the spores whose associated microflora was eliminated with alcohol. Even if the microflora did not develop in the buffer, bacterical metabolites would have been excreted in the suspension. They could act enzymatically on the external tissues of the spores thereby exposing the parasporal fibres and enabling their attachment to the cuticle of the nematodes (Davies and Danks, 1993). They might also modify the chemical environment of the spores and facilitate their attachment by producing or excreting directly (Rozycki, 1987) simple sugars and oligosaccharides which could involve lectin-carbohydrate interaction (Bird *et al.*, 1989; Ibrahim, 1991).

The same helper potential of the microflora may act efficiently in the soil. Despite sterilization of the soil, the microflora associated with the spores of *P. penetrans* induces the best attachment of the spores to the cuticle of the juveniles, unless compounds toxic to nematodes (Hoffmann-Hergarten and Sikora, 1995) are produced in addition. Consequently, the motility of the juveniles (Stirling *et al.*, 1990) and the invasion of the plant roots (Brown and Smart, 1985; Davies *et al.*, 1988) are reduced. The subsequent production of spores detected

TABLE I - *Effect of the disinfection of the spores of* Pasteuria penetrans *on the development of Meloidogyne graminicola on tomato plants and consecutive plant growth.*

Inoculum	No of galls per plant	Shoot biomass (mg dry weight)	Root biomass (mg dry weight)	No of spores per plant
Control		1880 b	818 b	
M. graminicola	87.8 a	1700 b	372 d	–
P. penetrans disinfected	–	2070 ab	1117 a	–
P. penetrans not disinfected	–	2400 a	1112 a	–
M. graminicola + *P. penetrans* disinfected	43.9 b	2200 ab	540 bc	4.6×10^6 a
M. graminicola + *P. penetrans* not disinfected	20.7 c	1900 b	424 c	3.7×10^5 a

In each column, data followed by the same letter are not significantly different according to the Mann Whitney U test ($P < 0.05$)

in the powdered roots are not different, certainly because most of the spores were spread during nematode extraction in the water mist.

Thus, the rhizosphere microflora can enhance the biocontrol fo the root-knot nematodes by *P. penetrans* by increasing the attachment of the spores to the nematodes. Further research is required to study the bacteria which are concerned and the helper mechanisms they induce, and to study and manage the abiotic factors which involve such favorable biocenotic relationships.

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