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SPORE ATTACHMENT OF *PASTEURIA PENETRANS* ON JUVENILES OF *MELOIDOGYNE INCOGNITA* AS AFFECTED BY PH AND ORGANIC MATTER

by

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Summary. The attachment of spores of a population of *Pasteuria penetrans* on juveniles of *Meloidogyne incognita* was significantly less at pH 3 than at greater pH. Similarly, attachment in acidic spore suspensions prepared directly from infested root systems and containing plant debris was less than in such suspensions that had been diluted even though the spore concentration was lower.

The use of *Pasteuria penetrans* (Thorne) Sayre and Starr for the control of *Meloidogyne* spp. has been investigated in many laboratories in recent years. The biological evaluation of this parasite is still a priority as there remain several factors relating to spore attachment to nematodes which are poorly understood. For mass production of spores, the method devised by Stirling and Wachtel (1980) has not been improved upon. Spore suspensions derived from this technique contain debris of the root systems of the host plant. For laboratory studies the concentration of root debris in the spore suspensions might influence the level of spore attachment on juveniles.

Materials and methods

Juveniles of *Meloidogyne incognita* (Kofoid *et* White) Chitw. were hatched from egg masses collected from cultures maintained on tomato plants in the glasshouse. A population of *Pasteuria penetrans* (Pp3) was maintained on *Meloidogyne* spp. having originally been isolated from *M. javanica* in South Africa.

The root system of a tomato plant, *Lycopersicon esculentum* Mill. cv. Tiny Tim, infested with *M. incognita* which were infested with *P. penetrans* (population Pp3) was divided in two parts. One part was cut in 2-2.5 cm pieces which were left in a beaker of tap water for four days at room temperature (15-21 °C) until they had become soft. The roots were then comminuted for 30-50 sec at low speed in a kitchen blender. The suspension was washed over a 500 µm sieve and female nematodes that passed through were collected on a 250 µm sieve. The females were separated from the slurry that accumulated on the 250 µm sieve by washing into a beaker and stirring in a 10% sugar solution until the females floated to the surface. These were collected with a Pasteur pipette washed to remove sugar and remaining debris and then crushed in a small volume of tap water to release spores of *P. penetrans* contained in the bodies of the females.

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The spore suspension was passed through a 21 µm sieve to separate spores from the female body cuticles. Spore concentration of this relatively clean suspesion was estimated with a haemocytometer. The pH was 8.0.

The other part of the root system was dried and ground with a pestle and mortar. A spore suspenison was prepared in tap water following the technique of Stirling and Wachtel (1980).

This suspension was left for four days before passing through a 21 µm sieve to remove the larger particles of root and nematode debris and then calibrated with a haemocytometer. The pH was 3.8. Adjustments were made to the volumes so that the spore concentrations of each suspension were similar at $1.3x10^5$ spores/ml.

A range of six spore concentrations was then prepared by serial dilutions of the original preparations.

Solitary, freshly hatched juveniles of a single egg-mass line were placed in glass cavity blocks containing 1 ml of each dilution of each spore suspension and placed randomly in a 18 °C incubator for two days. Spore attachment was recorded on each individual at x 400 magnification. There were ten replicates for each treatment.

A suspension of *P. penetrans* spores ($0.8x10^5$ spores/ml) was prepared in tap water directly from infected females. The suspension was pH 8. A range of pH levels was established by adding either 0.1N KOH or 0.1N HCl to aliquots of the original suspension. Solitary *M. incognita* juveniles were added to 1 ml aliquots of the amended suspensions and these were placed randomly in an incubator at 28 °C for two days when spore attachment was recorded as described above. Replication was five-fold.

Results and discussion

Juveniles which were placed in an undiluted spore suspension produced directly from nematodes had twice the number of spores attached to them than those which were placed in a similar undiluted suspension made from powdered roots (Table I). However, when these suspensions were diluted, the differences in levels of attachment between the suspensions were minor. It is assumed that all of the spores were released when the suspension was prepared from powdered roots and that the numbers trapped on plant debris were minimal.

In the second experiment, the fewest spores attached in the suspension at pH 3.0 than in suspension with pH 4-10 (Table II). Greatest attachment was at pH 5.

TABLE I - *Attachment of spores of* Pasteuria penetrans *on* Meloidogyne incognita *juveniles exposed to different concentrations of spores in suspensions prepared from powdered roots or directly from infected nematodes.*

Approximate spore concentration spores/ml (x 10^5)	Spores/nematode	
	Root-powder suspension	Pure suspension
1.30	39.9 (6.30)	72.5 (8.51)
0.65	47.7 (6.89)	54.8 (7.41)
0.32	32.2 (5.67)	33.1 (5.75)
0.16	25.6 (5.04)	15.7 (3.95)
0.08	18.2 (4.25)	9.2 (3.01)
0.04	9.9 (3.13)	7.6 (2.72)
0.02	6.2 (2.46)	3.1 (1.72)
SED	(0.19)	(0.21)

Square root transformation of means given in parentheses.

TABLE II - *Attachment of* P. penetrans *spores on* M. incognita *juveniles at different pH levels.*

Spores/nematode	Spore attachments/juvenile	
3	3.40	(1.74)
4	27.83	(5.09)
5	40.40	(6.11)
6	28.80	(5.24)
7	23.00	(4.70)
8	19.60	(4.42)
9	22.20	(4.67)
10	19.00	(4.32)
SED		(0.72)

Square root transformation of means given in parentheses.

Undiluted spore suspensions derived from powdered roots following the technique of Stirling and Wachtel (1980) may be too acidic for optimum spore attachment. Other studies have shown that optimum pH levels for attachment are between pH 4.5 and 5.5 (O'Brien, 1980). This may be a problem when evaluating *P. penetrans* in laboratory and glasshouse studies if greater quantities of powdered root have to be used to produce suspensions of sufficient concentration to obtain desired attachment levels.

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