

Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes

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SUMMARY

A simple test was used to determine whether or not *Pasteuria penetrans* spores would attach to 17 species of nematodes. All susceptible individuals had spores attached to their cuticles after 24 h of gentle agitation in suspensions containing 10^5 spores/ml. Spores of *P. penetrans* from six populations of *Meloidogyne* only adhered to species of *Meloidogyne* and they adhered in greatest numbers to the species from which they had been originally isolated. Sonication of spores from infected females increased attachment but the effect was dependent on pH and whether the test was conducted in tap or distilled water. Invasion of tomato roots was reduced by up to 86% when, rather than using healthy juveniles, second-stage juveniles bearing 15 or more spores were added to soil at high densities (1000 or 3000/plant); at low densities (500/plant) invasion was not significantly affected. The rate of development of *M. incognita* juveniles infected with *P. penetrans* was slower than that of healthy juveniles. The numbers of second-generation of *M. incognita* were reduced by 82-93% when juveniles encumbered with 1-15 spores were added to soil instead of those bearing no spores. *Pasteuria penetrans* populations differed in their aggressiveness and when juveniles encumbered with the same number of spores from two populations were added to soil there were differences in the numbers of females that became infected. The implications of these results for the development of *P. penetrans* as a biological control agent are discussed.

INTRODUCTION

Pasteuria penetrans Sayre and Starr, is an obligate pathogen of plant-parasitic nematodes and has considerable potential as a biological control agent particularly against *Meloidogyne* spp. (Mankau, 1975; Sayre, 1980; Stirling, 1985; Brown, Kepner & Smart, 1985). Spores of *P. penetrans* attach to the cuticles of *Meloidogyne* second-stage juveniles and once spore-encumbered nematodes have entered roots and started to feed, the spores germinate. A germ tube penetrates the cuticle to form a vegetative microcolony which then proliferates throughout the body of the developing nematode. The microcolonies fragment and eventually sporulate; the mature female is filled with spores and unable to reproduce (Sayre & Wergin, 1977).

Although spores of *P. penetrans* adhere to the cuticle of many species of nematode (Sayre & Starr, 1985; Spaul, 1981; Sturhan, 1985; Williams, 1967) individual populations of the bacterium usually have a narrow host range, some being highly specific (Dutky & Sayre, 1978). The numbers of spores that attach may even differ between populations of the same nematode species (Stirling, 1985). The size of the spore differs between bacterial populations (Sayre & Starr, 1985) and it seems likely that *P. penetrans* consists of more than one species. The attachment of spores to nematodes was not affected by pH between 4.5 and 8.5 (O'Brien,

1980), or by nematicides and temperatures of 100 °C for 30 min but was increased when spores from females were sonicated and the sporangial wall disrupted (Stirling, Bird & Cakurs, 1986). Lectins have not been found to be important in the host recognition process but attachment is related to the nematode's stage of development.

Infection of nematodes is a two-stage process: attachment of spores must be followed by their germination and penetration of the nematode's cuticle. Host status is only conferred after proliferation and sporulation within the nematode. Differences in the extent of spore attachment were related to the numbers of parasitised nematodes in roots (Stirling, 1984; 1985): at least five spores per nematode were required for infection and spore burdens in excess of 40 prevented invasion (Stirling, 1984); population control was achieved in a field trial when at least 80% of *Meloidogyne* juveniles were each encumbered with 10 or more spores.

Pasteuria penetrans cannot be developed as a commercial biological control agent until it has been cultured *in vitro*. Its use may also be severely restricted unless it can be thoroughly mixed in soil to ensure contact with the nematode host at rates of application considered practical for use on arable crops (Kerry, 1987). Selection of aggressive bacterial populations which infect at low rates of spore attachment would have marked effects on the rates of inoculum required for effective control.

In this paper some factors that affect spore attachment are investigated in order to develop a standard test for screening bacterial populations, and selected populations of the bacterium were used to determine the effects of spore burden on the numbers of *M. incognita* that invade and develop on tomatoes.

MATERIALS AND METHODS

Six populations of *P. penetrans* which had all been originally isolated from species of *Meloidogyne* (Table 1) were increased following the method of Stirling & Wachtel (1980). *Meloidogyne* second-stage juveniles were agitated in a suspension of *P. penetrans* spores until most nematodes had spores attached and were then added to soil around tomato roots cv. Rotegnom; the bacterium multiplied in the developing female nematodes within roots. For some tests, suspensions of spores were obtained from infected females dissected from the roots after 42 days. Otherwise, the root systems were air dried and suspensions of spores prepared by milling the roots and washing through a 30 µm aperture sieve to remove large root debris; the washings were collected and the spores counted in a haemocytometer. The concentration of the spore suspension was adjusted to 10⁶ spores/ml. Populations of root-knot nematodes were obtained from cultures derived from single egg masses maintained on tomato cv. Rotegnom in the glasshouse.

Table 1. Source of *Meloidogyne* species and *Pasteuria penetrans* populations

<i>Meloidogyne</i>		Source
<i>M. arenaria</i>	Race 1 No. 351	North Carolina State University, Raleigh, USA
<i>M. incognita</i>	Race 2 No. 1135	
<i>M. javanica</i>	"Tenerife"	
<i>P. penetrans</i> populations		
B7	<i>M. javanica</i>	1981 G. R. Stirling, Australia
PP1	<i>M. javanica</i>	1986 S. R. Gowen, University of Reading, UK
PP3	<i>M. javanica</i>	
PP4	<i>M. graminicola</i>	
PP2	<i>M. incognita</i>	
M1	<i>M. incognita</i>	1986 USDA, Beltsville
		R. M. Sayre, Maryland

The attachment of P. penetrans spores to M. incognita second-stage juveniles

i) *Effect of spore concentration and time of exposure.* *Pasteuria penetrans* population PP1, was obtained from mature infected females which were homogenised and suspended in tap water. The concentration of spores was adjusted to 2×10^6 spores/ml and a dilution series made down to 10^2 spores/ml. Duplicate 0.5 ml aliquots of each dilution and tap water control containing no spores were placed in staining blocks to which a further 0.5 ml of tap water containing about 200 *M. incognita* second-stage juveniles was added. The staining blocks were placed in a moist chamber and gently agitated on a rotary shaker (90 revs/min) at 25 °C for 2, 4, 6, 12 or 24 h after which 10 nematodes from each watch glass were microscopically examined ($\times 400$) and the number of spores attached to each individual was counted.

ii) *Effect of sonication and pH in distilled or tap water.* Spores of *P. penetrans* population PP1 were obtained from infected females as previously described. Spore suspensions (10^6 spores/ml) in distilled or tap water were adjusted to pH 4, 7 and 9 by the addition of either 0.5 M potassium hydroxide or hydrochloric acid made up in the appropriate water treatment. Half of each suspension was sonicated in an ice water bath for 10 min using a Kerry's ultrasound generator (Hawksley & Sons Ltd, London) fitted with a logarithmic probe. An aliquot (0.5 ml) of each spore suspension was added to 200 – 500 second-stage juveniles of *M. incognita*, suspended in the appropriate water treatment and shaken at 25 °C as before; the numbers of spores attached to 10 nematodes from each treatment were determined.

iii) *Host range of six populations of P. penetrans.* Spore suspensions containing 10^6 spores/ml were prepared from milled roots containing *Meloidogyne* females infected with each of the six *P. penetrans* populations, each bacterial population having been maintained on its original host nematode species (Table 1). Attachment of spores from each *P. penetrans* population was tested with the following nematodes: *Meloidogyne arenaria*, *M. incognita*, *M. javanica*, *Heterodera schachtii*, *H. glycines*, *Globodera rostochiensis*, *Pelodera strongyloides*, *Panagrellus redivivus*, *Pratylenchus neglectus*, *Anguina tritici*, *Caenorhabditis elegans*, *Ditylenchus dipsaci*, *Aphelenchoides* sp., *Mesodiplogaster* sp., *Diplogaster* sp., *Panagrolaimus* sp. and *Rhabditis* sp. Each test was replicated four times and done as described above.

The effect of P. penetrans on the invasion and development of M. incognita in tomato roots.

i) *Effect of spore burden on juvenile invasion.* A suspension of spores of *P. penetrans* population PP1 from air dried roots was agitated with second-stage juveniles of *M. incognita* for different periods of time (2 – 8 h) in order to obtain three groups of juvenile nematodes with 1 – 10, 11 – 20 or more than 20 spores adhering to the cuticle of each. The numbers of spores adhering to a sample of 20 individual juveniles from each group were counted and the mean and standard error determined (Table 4). A control group consisted of juveniles with no *P. penetrans* spores attached but which had otherwise been treated similarly. Approximately 500, 1000 and 3000 second-stage juveniles at each of the ranges of spore attachment were added around the roots of 6 wk old tomato plants growing in a 3:1 peat-sand mixture (Croxdon Horticultural Products Ltd, UK) at pH 6.0. Each pot was placed in a small plastic tray to reduce cross contamination on the glasshouse bench, watered daily and maintained at approximately 25 °C with a 16 h day, 8 h night photoperiod. After 14 days the roots were collected, blotted dry and weighed before being cut into 0.5 cm lengths and placed in 25% Pectinex (Novo Enzyme Products Ltd, Farnham, UK) on a rotary shaker (156 rpm). After 24 h the slurry was placed in an Atomix homogeniser (Measuring and Scientific Equipment Ltd, Crawley, UK) for 20 s before the slurry was washed through a 0.8 mm sieve and collected on a 53 μ m sieve. The residue was then resuspended in 100 ml of water. A sample of 10 ml from each treatment was placed in a Doncaster counting tray (Doncaster, 1962) and the numbers of juveniles and immature females determined. The experiment consisted of four replicate plants at each inoculum level.

ii) *Effect of spore burden on nematode multiplication in roots.* As before, second-stage juveniles of *M. incognita* were exposed to *P. penetrans* population PP1 to provide juveniles with different ranges of spores attached to each (1–5, 6–10 and 11–15 spores). A control group consisted of juveniles with no spores attached. Approximately 5000 second-stage juveniles at each spore range were added around the roots of 8 wk old tomato plants (cv. Rotegnom) growing in plastic plant pots (13 cm diameter) containing the compost mixture as previously described. After 35 days and the partial development of a second generation of nematodes the plants were harvested and the roots washed, blotted dry, weighed and the numbers of adult females and second-stage juveniles determined by the method described above. From each sample 20 adult females were removed and squashed before examination ($\times 400$) to determine how many were infected by *P. penetrans*. Each treatment consisted of four replicate plants.

iii) *Effect of two P. penetrans populations on female production.* Spores of *P. penetrans* populations PP1 and M1 differed in their ability to attach to *Meloidogyne* spp. (Table 3). Second-stage juveniles of *M. incognita* were shaken in spore suspensions of these two populations for different periods of time so that there were between one and five spores per juvenile. Approximately 0, 300, 1000, 3000 or 6000 second-stage juveniles, encumbered with spores of the two different *P. penetrans* populations, were added around the roots of 6 wk old tomato plants (cv. Rotegnom) growing in plastic pots (9 cm diameter) containing peat-sand compost, as previously described. There were four replicates of each treatment and uninfected juveniles were included as controls at each nematode density. After 36 days the plants were harvested, the roots washed, their fresh weights measured and the numbers of adult females determined. Twenty adult females infected with each *P. penetrans* population were collected from the plants grown in compost infected with 6000 second-stage juveniles.

The size of each female was estimated by measuring two diameters at right angles across her body (ignoring the head) and calculating the volume of a sphere based on the mean radius. The number of *P. penetrans* spores in each female was obtained by homogenising each individual in 0.1 ml of water and counting the number of fully mature spores in a subsample using a haemocytometer. The degree of development of vegetative growth stages of the bacterium could not be accurately assessed.

RESULTS

Attachment of spores

The number of spores that adhered to the cuticles of second-stage juveniles of *M. incognita* was much affected by the concentration of spores in the suspension. Few spores attached at concentrations below 10^3 /ml and there was a small residual contamination of the controls (Table 2). More spores ($P < 0.001$) attached as concentrations increased; after 24 h at 10^5 or 12 h at 10^6 /ml all juveniles had one or more spores attached. In other initial tests, extending the period of exposure from 24 h to 72 h had little effect on the numbers of spores adhering.

Sonication and pH had large effects ($P < 0.001$) on spore attachment but these were dependent on whether the tests were performed in either tap or distilled water (Fig. 1). In tap water sonication improved the number of spores that adhered irrespective of the pH; more ($P < 0.001$) sonicated and untreated spores adhered at pH 7 but the reduction in attachment at pH 4 and 9 was more marked in tests with untreated spores. Fewer spores adhered to the nematodes in distilled water than in tap water at pH 7; attachment of sonicated spores again was reduced by either increasing or decreasing the pH. However, in distilled water non-sonicated spores attached in greater numbers at pH 4 and pH 9 than at pH 7.

Table 2. Effect of concentration and time of exposure on the attachment of spores of *P. penetrans* to cuticles of *M. incognita* second-stage juveniles. (Mean no. of spores per juvenile; for replicates each of 10 worms)

Spore concentration (ml ⁻¹)	2	4	6	12	24	Mean	± S.E.D.
0 (control)	0.7	0.8	0.2	0.7	0.9	0.7	
10 ²	1.1	1.3	1.0	0.5	0.8	1.0	
10 ³	1.5	1.9	1.2	1.0	1.6	1.5	0.69
10 ⁴	2.9	2.3	3.0	2.8	6.1	3.5	***
10 ⁵	3.0	3.6	7.0	13.4	15.7	8.6	
10 ⁶	10.7	10.1	17.9	28.3	44.3	22.5	
Mean	3.3	3.4	5.1	7.8	11.6		
± S.E.D.			0.63				***

Interaction (spore concentration vs exposure time) S.E.D. = 1.55 *** differences between means were significant $P < 0.001$

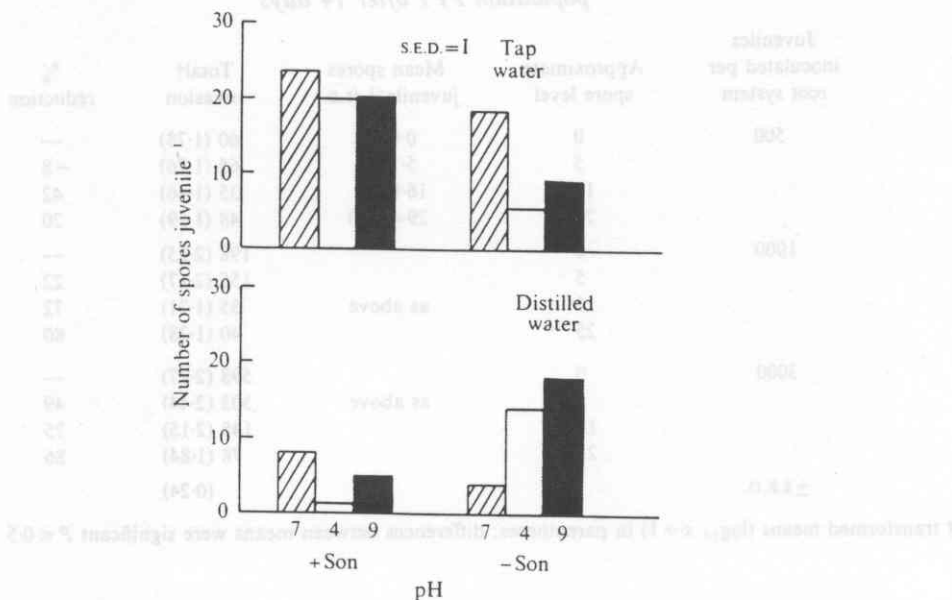


Fig. 1. Number of sonicated (+ son) and non-sonicated (- son) spores of *P. penetrans* population PP1 adhering to the cuticle of second-stage juveniles of *M. incognita* in tap and distilled water adjusted to pH's 4, 7 and 9.

Spores of the six populations of *P. penetrans* only adhered to the nematode cuticles of species of *Meloidogyne* and not to any of the other 14 species in the other genera tested. The number of spores that attached to each juvenile was variable with greatest numbers occurring on *M. javanica* followed by *M. incognita* and *M. arenaria* (Table 3). Most attachment occurred when the *P. penetrans* population was tested against the same species of nematode from which it was originally isolated. However, some populations were more aggressive than others: few spores of population (PP2) isolated from *M. incognita* attached even to their original host.

Table 3. Mean number of spores of six populations of *P. penetrans* attached to individual second-stage juveniles of three species of *Meloidogyne* after 24 h, mean of 20 juveniles

<i>Meloidogyne</i> spp.	<i>Pasteuria penetrans</i> populations						Mean (S.E.)
	B7	PP1	PP2	M1	PP3	PP4	
<i>M. incognita</i>	7.9	4.8	0.1*	3.6*	9.9	1.0	4.6 (0.3)
<i>M. javanica</i>	51.8*	31.5*	0.0	1.5	14.4*	0.1	16.5 (0.6)
<i>M. arenaria</i>	0.0	0.2	NA†	0.2	0.2	0.1	0.2 (0.1)
Mean	14.9	9.1	NA	1.3	6.1	0.3	—
(S.E.)	(0.8)	(0.5)	—	(0.1)	(0.5)	(0.1)	—

* denotes original host was the same as the juveniles tested.

† not available

Table 4. The total number of juveniles of *M. incognita* on tomato roots inoculated with second-stage juveniles to which had been attached four levels of spores of *P. penetrans* population PPI after 14 days

Juveniles inoculated per root system	Approximate spore level	Mean spores juvenile ⁻¹ (S.E.)	Total† invasion	% reduction
500	0	0.0 (0)	60 (1.78)	—
	5	5.7 (4)	65 (1.76)	-8
	15	16.8 (8)	35 (1.46)	42
	25	29.4 (13)	48 (1.59)	20
1000	0	—	198 (2.25)	—
	5	—	155 (2.17)	22
	15	as above	55 (1.71)	72
	25	—	40 (1.28)	80
3000	0	—	598 (2.77)	—
	5	as above	303 (2.44)	49
	15	—	148 (2.15)	75
	25	—	78 (1.84)	86
± S.E.D.	—	—	(0.24)	—

† transformed means ($\log_{10} x + 1$) in parentheses; differences between means were significant $P < 0.05$

Effects of *P. penetrans* on invasion and development

Invasion was reduced ($P < 0.001$) by up to 86% when 1000 or more juveniles, each bearing at least 15 spores, were added around tomato roots. However, when only 500 juveniles were added the number of spores adhering to their cuticles had less effect on invasion (Table 4). The reduction in juvenile invasion was density dependent with greatest reduction occurring at high nematode densities. The number of spores adhering to second-stage juveniles also has a small but significant effect on the nematode's development inside roots (Fig. 2). Although there was no significant effect when 500 juveniles were added per root system, at densities of 1000 and 3000 nematodes there were fewer ($P < 0.001$) second-stage juveniles and more ($P < 0.001$) third-stage juveniles and females in uninfected populations than in the presence of *P. penetrans*, which delayed development.

In the second experiment, in which < 15 spores were attached per juvenile and 5000 juveniles were added to each plant, there was also a significant reduction in the number of adult females produced in infected compared to uninfected populations (Table 5). The

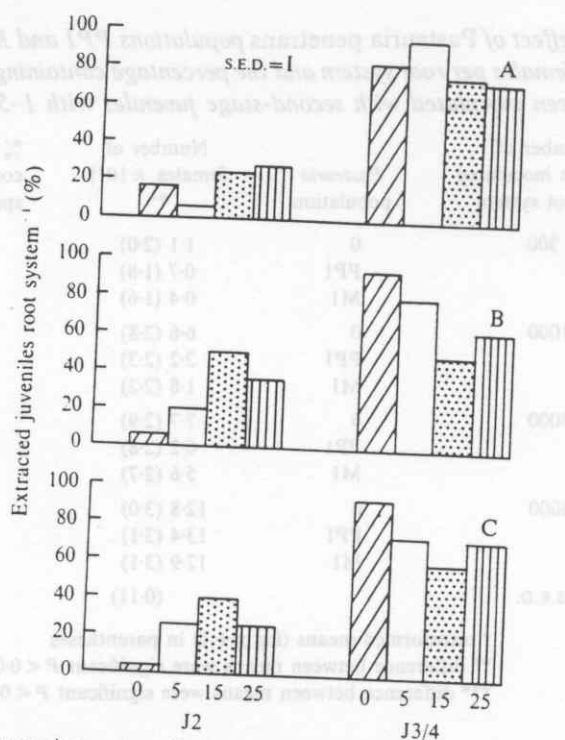


Fig. 2. Proportion of second-stage juveniles (J2) and other stages (J3/4) in a *M. incognita* population developing inside tomato roots after 500 (A), 1000 (B) or 3000 (C) second-stage juveniles encumbered with 0, 5, 15 or 25 *P. penetrans* spores were added to soil.

Table 5. The number of second generation *M. incognita* juveniles and adult females produced from an inoculation of 5000 second-stage juveniles with three levels of *P. penetrans* spore attachment together with the number of adult females containing *P. penetrans* spores

PPI spores juvenile ⁻¹	Second generation J2's†		Adult females			Total population†	
	× 10 ³ *	% reduction	× 10 ³ *	% reduction	% with <i>P. penetrans</i> *	× 10 ³ *	% reduction
0	18.9 (4.2)	—	3.4	—	0	22.3 (4.3)	—
1-5	3.4 (3.4)	82	1.6	53	70	5.0 (3.5)	77
6-10	2.7 (3.3)	86	1.9	44	58	4.6 (3.6)	79
11-15	1.4 (2.9)	93	1.2	65	92	2.6 (3.4)	88
± S.E.D.	(0.23)		1.5		16	(0.16)	

† differences between means were significant $P < 0.001$

* transformed means ($\log_{10} x + 1$) in parentheses

number of spores per juvenile (1 - 15) had no significant effect on the number of adult females. However, as the spore burden increased fewer juveniles tended to survive to become adult females, more of which were infected, so resulting in a smaller second generation. *Pasteuria penetrans* had a marked effect on the second generation which was reduced by between 82 and 93%.

There were significant differences between the numbers of females that developed from juveniles infected with only one to five spores of *P. penetrans* populations PP1 or M1 compared with uninfected controls at all nematode densities (Table 6). At densities of 3000 and 6000

Table 6. *The effect of Pasteuria penetrans populations PP1 and M1 on the number of adult M. incognita females per root system and the percentage containing P. penetrans spores that had been inoculated with second-stage juveniles with 1-5 spores attached*

Number of juveniles inoculated per root system	<i>Pasteuria</i> population	Number of females $\times 10^2$ † **	% females containing spores ***
300	0	1.1 (2.0)	5.0
	PP1	0.7 (1.8)	58.8
	M1	0.4 (1.6)	47.5
1000	0	6.6 (2.8)	5.0
	PP1	2.2 (2.3)	43.8
	M1	1.8 (2.2)	43.8
3000	0	7.7 (2.9)	2.5
	PP1	6.2 (2.8)	66.3
	M1	5.6 (2.7)	48.8
6000	0	12.8 (3.0)	0.8
	PP1	13.4 (3.1)	82.5
	M1	12.9 (3.1)	53.8
\pm S.E.D.		(0.11)	8.0

† transformed means ($\log x + 1$) in parentheses

** difference between means were significant $P < 0.01$

*** difference between means were significant $P < 0.001$

Table 7. *The mean number of mature Pasteuria penetrans spores per M. incognita female and mean volume of M. incognita females extracted from a root system that were infected by P. penetrans populations M1 and PP1*

<i>Pasteuria</i> population	Volume of female* $\text{mm}^3 \times 10$	No. of mature* <i>P. penetrans</i> spores per adult female $\times 10^5$
Control	1.61	0.0
PP1	1.47	8.5
M1	1.37	8.9
\pm S.E.D.	0.22	0.6

* mean of 21 individual females

nematodes per root system infection with PP1 resulted in a greater ($P < 0.001$) proportion of infected females than did infection with M1. The effect of *P. penetrans* infection on the mean volume of females was not significant (Table 7), but the number of *P. penetrans* spores per female was proportional to the size of the female and this relationship was similar in females infected with populations PP1 or M1 of the parasite (Fig. 3).

DISCUSSION

P. penetrans spores attached readily to the cuticles of second-stage juveniles of *Meloidogyne* spp. when both were agitated in water for 24 h. In this study reproducible results were achieved using only small volumes (0.5 ml) of spore and nematode suspensions; rates of attachment were similar to those achieved by Stirling & Wachtel (1980). This simple test could be used to screen large numbers of bacterial populations for their ability to adhere to different nematodes (Stirling, 1985) provided that the pH and quality of water are controlled.

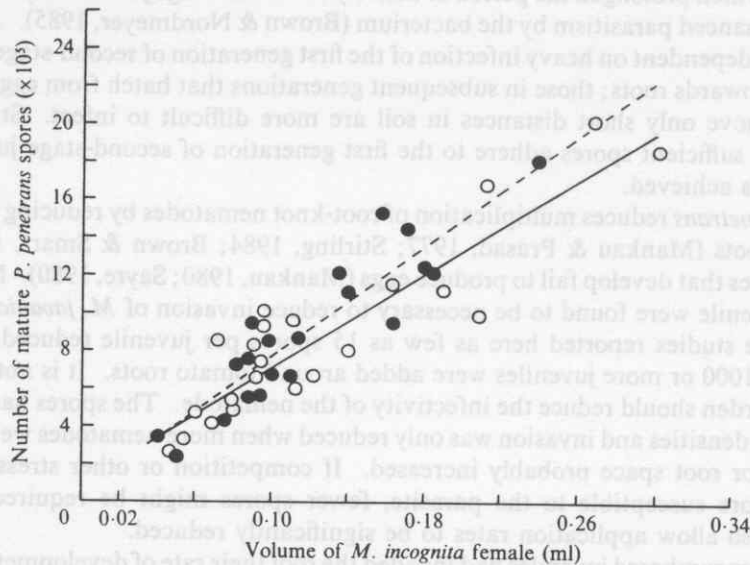


Fig. 3. Relationship between the volume of 36 day old *M. incognita* females and the number of mature *P. penetrans* spores from populations PPI (—●) and M1 (—○).

Spores have no net charge at neutral pH (Mankau, 1980) when they generally adhered in greatest numbers. However, changes in pH and the use of tap water which was rich in salts compared with distilled water both affected attachment. Thus, the presence of charged groups on spore and nematode surfaces may be important in this process. Sonication increased attachment by removing the sporangial wall and exposing the parasporal fibres that attach the spore to the cuticle of the nematode (Stirling *et al.*, 1986). Spores obtained from infected female nematodes frequently are still within their sporangia which should be disrupted by sonication before attachment studies are begun. The sporangial wall was sloughed off spores that had been collected from air-dried roots and macerated in water with a pestle and mortar.

Pasteuria penetrans only adhered to *Meloidogyne* juveniles and there were significant differences in the numbers of spores adhering to the different species tested. The results confirm that individual populations of the parasite are highly specific. *M. arenaria* was probably a poor host for all six *P. penetrans* populations; all spores do not germinate and if few attach the nematode may not be parasitised (Stirling, 1984). Also, there was clear evidence that the greatest attachment of spores occurred when they were exposed to the species of *Meloidogyne* from which they were originally isolated; this suggested that spores that were specific to a particular host were being selected. However, Stirling (1985) reported that *P. penetrans* retained a wide host range despite having been cultured for 15 yr on a population of *M. javanica* derived from a single egg mass. Although lectins are considered important in the recognition of hosts of some nematophagous fungi (Nordbring-Hertz & Mattiasson, 1979; Jansson, Jeyaprakash & Zuckerman, 1985; Nordbring-Hertz, 1984; Zuckerman & Jansson, 1984) they have not yet been implicated in the attachment of *P. penetrans* spores (O'Brien, 1980; Stirling *et al.*, 1986).

Biological control using *P. penetrans* will depend upon the number and distribution of spores and the length of time the target nematode is active in the soil. *P. penetrans* spores are resistant to the nematicides, aldicarb (as Temik) and carbofuran (as Furadan) amongst others (Mankau & Prasad, 1972), and their simultaneous application has been suggested by Stirling (1984).

Nematicides which prolonged the period of activity of second-stage juveniles of *M. javanica* in soil greatly enhanced parasitism by the bacterium (Brown & Nordmeyer, 1985). Also, control of root-knot is dependent on heavy infection of the first generation of second-stage juveniles as they migrate towards roots; those in subsequent generations that hatch from eggs on the root surface and move only short distances in soil are more difficult to infect. Stirling (1984) reports that if sufficient spores adhere to the first generation of second-stage juveniles then good control is achieved.

Pasteuria penetrans reduces multiplication of root-knot nematodes by reducing the numbers that invade roots (Mankau & Prasad, 1977; Stirling, 1984; Brown & Smart, 1985). Also, infected females that develop fail to produce eggs (Mankau, 1980; Sayre, 1980). More than 40 spores per juvenile were found to be necessary to reduce invasion of *M. javanica* by Stirling (1984). In the studies reported here as few as 15 spores per juvenile reduced invasion by > 70% when 1000 or more juveniles were added around tomato roots. It is not clear why a large spore burden should reduce the infectivity of the nematode. The spores had no effect at low nematode densities and invasion was only reduced when more nematodes were added and competition for root space probably increased. If competition or other stresses make the nematodes more susceptible to the parasite, fewer spores might be required to prevent invasion and so allow application rates to be significantly reduced.

Once spore encumbered juveniles had invaded the root their rate of development was slower than that of healthy individuals. Bird (1986) was unable to demonstrate any effect of the parasite on the early stages of nematode development but the results presented here again indicate that this effect was density dependent. Sayre & Wergin (1977) estimated that only 20% of spores germinated whereas Stirling (1984) indicated that the proportion was nearer 30%. This suggests that on average between three and five spores on each juvenile are required to ensure infection. About 70% of females that developed from juveniles bearing one to five spores became infected in our tests; as many juveniles had fewer than three spores adhering about 30% must have germinated. At this level of infection the second generation was reduced by 82%. Each mature infected female contained about 2×10^6 spores which was similar to the number observed by Mankau (1975) but half that of Stirling & Wachtel (1980). However, the spore content was dependent on the size and, presumably, age of the female. The factors that affect spore germination are unknown but attachment may occur in conditions that do not favour further development of the parasite. Some populations of *P. penetrans* were more aggressive than others and even at the same spore burdens more females became infected. The selection of aggressive strains that would be effective at lower densities in soil is important in the development of *P. penetrans* as a biological control agent.

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