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Evaluation of *Pasteuria penetrans* as a biocontrol agent against populations of root–knot nematodes from Crete

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Summary. The attachment of spores from different populations of *Pasteuria penetrans* was assessed using a field population of root-knot nematodes from Crete consisting of 75% *Meloidogyne incognita* and 25% *M. javanica*. Juveniles were more heavily encumbered with spores of an isolate of *P. penetrans* from South Africa and from a population consisting of a mixture of *P. penetrans* isolates than by spores from two other populations. When single egg mass lines of the component species of the Cretan root-knot nematode population were tested, it was found that *M. javanica* was the more susceptible species. A greater proportion of the nematodes encumbered with 5-8 spores eventually became infected than did juveniles that were encumbered by fewer spores.

Key words: *Meloidogyne incognita, M. javanica*, biological control, *Pasteuria penetrans*, variability.

The bacterium *Pasteuria penetrans* (Thorne, 1940; Sayre & Starr, 1985) is an obligate pathogen of certain plant parasitic nematodes. It produces spores which adhere to the cuticle of nematodes, which, after germinating and penetrating the cuticle, grow as a mycelium within the bodies of the host and complete the life-cycle (Sayre & Starr, 1985). It has the attributes of a successful biocontrol agent, particularly against root-knot nematodes (Sayre, 1980; Stirling, 1984). *P. penetrans* reduces root-knot nematode populations not only by preventing females producing eggs but also by reducing the numbers of juveniles that invade roots (Brown & Smart, 1984; Davies et al., 1988).

Meloidogyne spp. are heterogeneous with respect to susceptibility to spore attachment and *P. penetrans* is heterogeneous with respect to host specificity (Channer & Gowen, 1992). Also, juveniles that hatched from egg masses of one biotype of *M. javanica* showed a wide range of spore attachment when exposed to a suspension of spores of *P. penetrans* (Ratnasoma, 1990). A better understanding of the host parasite relationship is necessary if improvements are to be made in the efficacy of biocontrol.

We examined the attachment of ***P. penetrans*** spores to a population of *Meloidogyne* spp. from Crete, and, the invasion and subsequent infection of females using different *P. penetrans* isolates in order to find a compatible *Pasteuria - Meloidogyne* combination. The results from these investigations are presented here.

MATERIALS AND METHODS

Culturing nematodes and P. penetrans. The *Meloidogyne* population was cultured on tomato cv. Tiny Tim. Nematode inoculum was obtained by gently washing the root systems, picking off egg masses, suspending them in a small volume of tap water on a nylon sieve (100 μm dia. aperture) and collecting the hatched juveniles (J_2) after 48 h.

The *P. penetrans* isolates were grown on *Meloidogyne* spp. on tomato and spore suspensions were prepared from the roots using the method of

Stirling and Wachtel (1980) viz. attachment of *P. penetrans* spores to juveniles. A 0.1 g sample of powdered tomato roots that had contained infected females was ground for approximately 5 min in a pestle and mortar, mixed into a suspension in 100 ml tap water and passed through a 25 μm sieve to remove debris. The spores in the suspension were estimated with a haemocytometer and the suspensions adjusted to 10^4 spores/ml. *P. penetrans* populations used in the experiments were *Pp* 3 from S. Africa, *Pp* 1 from Australia, *Pp* blend containing the mixture of populations used by Channer & Gowen (1992), and *Pp* C of unknown origin. All populations except *Pp* C were in the collection at Reading and had been produced for at least one generation on the root-knot nematode population from Crete. The isolate *Pp* C had been found associated with this nematode population.

Experiment 1. Attachment of spores of different P. penetrans isolates to a mixed population of Meloidogyne incognita and M. javanica. *P. penetrans* populations were adjusted to a series of concentrations and 1 ml aliquots were placed in staining blocks to which a further 1 ml of tap water was added, containing approximately 200 J_2's of the nematode population estimated to be 25% *M. javanica* and 75% *M. incognita*. The blocks were placed in an incubator at 24° C for 24 h after which 20 nematodes from each block were examined with a binocular microscope and the number of spores attached to each nematode were counted. Five replicate blocks were used for each spore concentration.

Experiment 2. Attachment of spores of different P. penetrans isolates to single egg mass populations of Meloidogyne incognita and M. javanica. Juveniles of single egg mass lines of *M. incognita* and *M. javanica* were exposed to populations of *P. penetrans* under conditions as described previously. The spore concentrations per 1 ml were as follows: *Pp* 3 - 3 x 10^4, *Pp* blend - 1.7 x 10^4, *Pp* C - 3 x 10^4 and *Pp* 1 - 10 x 10^4. The mixed *Meloidogyne* population was also used with the *Pp* 3 population. Replication was fivefold and 20 juveniles from each replicate were assessed after 24 h.

Experiment 3. Influence of nematode host on which P. penetrans was cultured on attachment of spores on Meloidogyne spp. Groups of 200 J_2's of the *Meloidogyne* population were placed in suspensions of *Pp* 3 and *Pp* blend which had been cultured on *Meloidogyne* spp. from Papua New Guinea and on the Cretan *Meloidogyne* spp. population. Spore concentrations were 3 x 10^4 spores per ml, replication was threefold and spore attachment was recorded on 20 nematodes from each replicate after 24 h at 28° C.

Experiment 4. The effect of number of P. penetrans spores attached to infective root-knot nematode juveniles and the incidence of infected adult females after 47 days. A bulk samples of J_2 hatched from the field population of *Meloidogyne* spp. was placed in a suspension of *Pp* 3 at 6 x 10^4 spores/ml. At four periods up to 24 h groups of juveniles were removed from the suspension and the spore attachment on 20 individuals was recorded. A control group was kept in water. One thousand J_2 from each group with 2.86, 3.73, 5.26 and 8.25 spores/J_2 respectively were inoculated on 42 day old tomato plants growing in 15 cm pots and kept in a glasshouse for 47 days. After washing out the roots a random sample of 12 females was taken from each root system, and these were crushed under a cover-slip and examined at x 400 for the presence of *P. penetrans* spores. The remainder of the root systems was dried, milled and spore concentrations estimated from 100 mg samples.

RESULTS

Experiment 1: Attachment of the spores of the different *P. penetrans* populations to juveniles of the *Meloidogyne* spp. population varied greatly. Generally, there was greater attachment with increased spore concentration (Fig. 1, Table 1 A-D). There were many nematode juveniles which were unencumbered with spores in the *Pp* 1 and *Pp* C suspension, whereas in *Pp* 3 and *Pp* blend relatively few nematodes were observed without spores attached.

Experiment 2: The spores of *Pp* 3 attached in far greater numbers to the single egg mass line of *M. javanica* (28.13 spores/J_2) than to *M. incognita* (4.06 spores/J_2 (Table 2), whereas with *Pp* blend attachment was similar, and there were mean attachment levels of < 1 with *Pp* 1 and *Pp* C on *M. incognita*. Attachment was always greatest on *M. javanica*.

Table 1. Variation in ranges of spore attachment of four *Pasteuria penetrans* isolates (A - D) on *M. incognita*/*M. javanica* juveniles at different spore concentration.

	Approx. spore concentration (per ml)	Numbers of spores per J2* min	max	% nematodes with no spores
	Pp 3			
	12×10^4	7	75	0
	6×10^4	3	60	0
(A)	3×10^4	1	36	0
	1.5×10^4	0	26	10
	0.75×10^4	0	12	8
	Pp 1			
	10×10^4	0	9	13
	5×10^4	0	15	11
(B)	2.5×10^4	0	6	24
	1.25×10^4	0	6	27
	0.625×10^4	0	4	33
	Pp blend			
	10×10^4	12	75	0
	5×10^4	2	57	0
(C)	2.5×10^4	3	46	0
	1.25×10^4	1	37	0
	0.625×10^4	0	16	3
	Pp C			
	3×10^4	0	43	23
(D)	1.5×10^4	0	20	21
	0.75×10^4	0	11	39
	0.374×10^4	0	7	35

* mean from 5 replicates each with 20 nematodes.

Table 2. Attachment of different populations of *P. penetrans* to single egg mass lines of *Meloidogyne incognita* and *M. javanica* from Crete.

P. penetrans	*M. incognita*	*M. javanica* Spores/J2*	*Meloidogyne* Cretan mixed field population
Pp 1	0.45	1.34	—
Pp 3	4.06	28.13	9.36
Pp blend	3.41	6.61	—
Pp C	0.17	14.30	—
Water control			0.047

* mean count from 100 J2's.

Table 3. The mean percentage of infested females recovered from tomato roots, spore production and the galling index after being inoculated with second stage *Meloidogyne* juveniles encumbered with different numbers of spores.

№ of spores per J2	Infested females %	Gall index (0-10)	Spore production per 100 mg ($\times 10^4$)
0	0	5.2	0
2.86 (0-12)	11.11	3.28	100
3.73 (1-7)	30.55	2.62	0
5.26 (0-12)	64.02	2.71	400
8.25 (1-12)	47.47	2.71	246
SE		0.44	

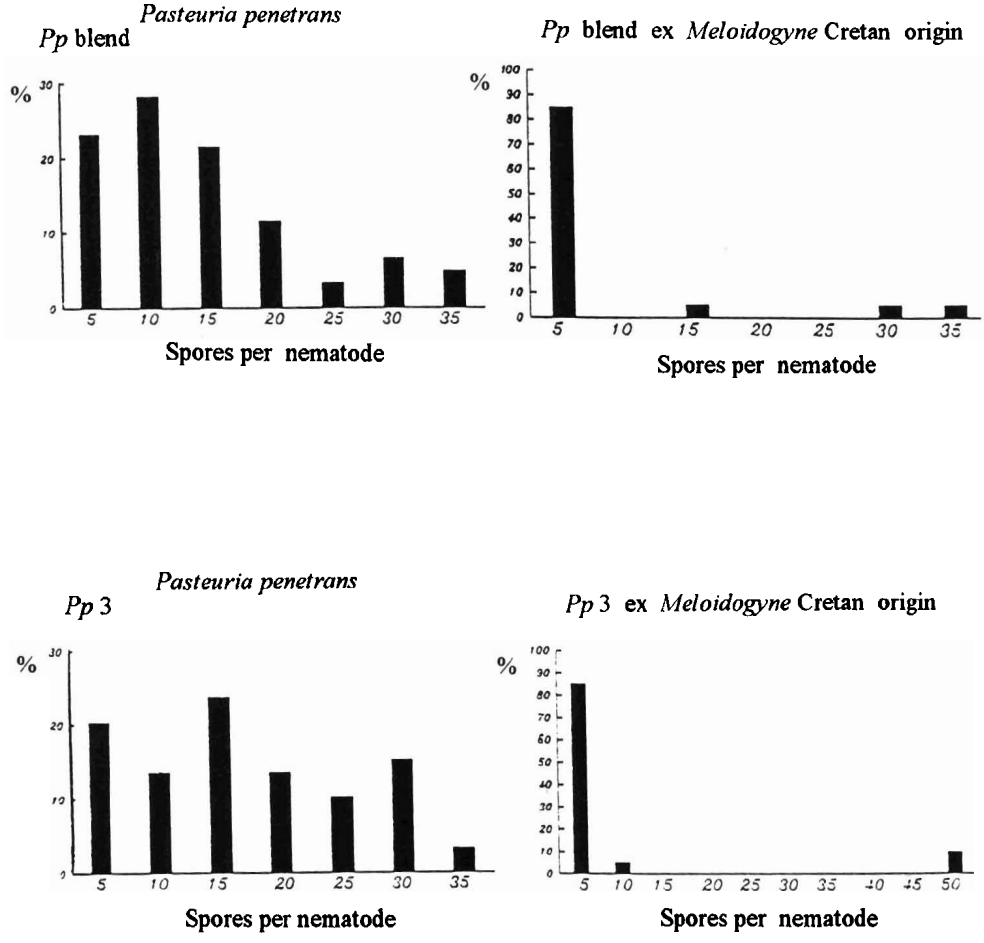

Fig. 1. Spore attachment on juveniles of a Cretan population of *Meloidogyne*, containing 25% *M. javanica* and 75% *M. incognita*, incubated in suspensions of different *Pasteuria penetrans* at different spore concentrations.

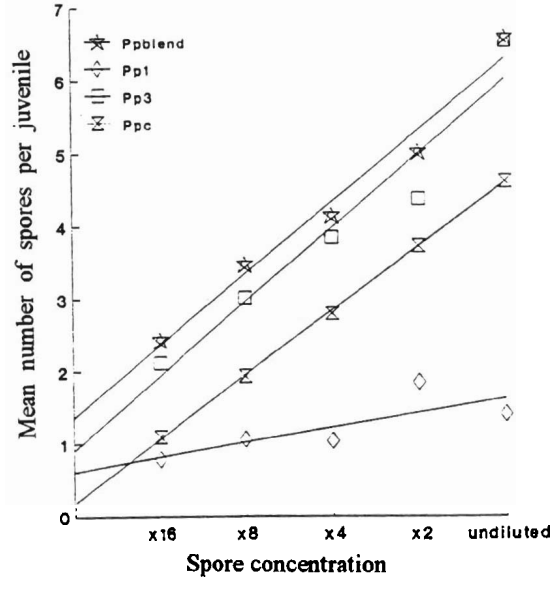

Fig. 2. Frequency distribution of spore attachments of two *Pasteuria penetrans* isolates (*Pp* 3, from South Africa and *Pp* blend, containing a mixture of populations used by Channer & Gowen (1992)) produced on different nematode hosts on juveniles of a Cretan population of *Meloidogyne*, containing 25% *M. javanica* and 75% *M. incognita*.

Experiment 3: *Pp* 3 and *Pp* blend cultured on *Meloidogyne* spp. from Papua New Guinea gave similar levels of attachment (Fig. 2). However, when these populations had been cultured on *Meloidogyne* spp. from Crete the pattern of attachment was very different with more than 80% of the J_2's being encumbered with less than 5 spores. These differences in attachment were not random according to the χ^2 test ($P = 0.001$).

Experiment 4: There was significantly greater galling on tomato roots inoculated with unencumbered juveniles than on those inoculated with J_2's with *P. penetrans* spores attached (Table 3). The incidence of infection of females was highest with J_2 inoculum, encumbered with 5 spores and lower with the other spore attachment levels. The spore production in the root systems was not consistent with spore concentrations of up to 400×10^4 spores per 100 mg dried root in one treatment.

DISCUSSION

The attachment tests showed how a nematode population was differentially encumbered by spores of populations of *P. penetrans* of different origin. Stirling (1985) and Davies et al. (1988) reported the differential susceptibility to spore attachment was evident even between populations within *Meloidogyne* species. Recently, molecular techniques have demonstrated how differences in attachment may be due to heterogeneity of the nematode cuticle and also heterogeneity within the *Pasteuria* population (Davies et al., 1994). The biochemical mechanisms that account for binding of spores to cuticles is still not well understood but could be related to acetylglucosamine (Davies & Danks, 1993).

Differences in attachment were also apparent when the species components of the Cretan root-knot nematode population was probed with different *P. penetrans* isolates. Spore attachment was greater on *M. javanica* than on *M. incognita* which probably explains why attachment on the field (mixed) population was lower, as *M. incognita* was the predominant species.

Generally, where mixtures of *P. penetrans* populations have been used to probe a nematode population, spore attachment is more consistent. This suggests that the mixture of *P. penetrans* populations being used in such tests contain spores with wider adaptability and thus are potentially more useful in a field situation (Channer & Gowen, 1992; Tzortzakakis & Gowen, 1994).

A feature of the spore attachment studies reported here is the relatively small numbers of spores that became attached when nematode juveniles were placed in suspensions of > 100,000 spores for 24 hours. The reasons for this are uncertain but could relate to poor viability, dormancy or to the heterogeneity that has recently been described by Davies et al. (1994). When considering *P. penetrans* as a potential biocontrol agent in the field, or in protected cropping, the relationships between spore concentrations in soil and the incidence of infection will be of primary importance.

The critical number of spores attached to free-living, infective juveniles to ensure that the ensuing females are infected has been estimated to be five (Stirling, 1984). When the spore burden is greater the number of nematodes invading roots declines. It is possible that this would account for the greater percentage infection of the females encumbered with five spores than those with eight, on the assumption that fewer of the heavily encumbered nematodes succeeded in locating and/or penetrating the roots. Also, this has important implications for field epidemiology of this host-pathogen interaction as it suggests that populations of *P. penetrans* that attach readily may not perpetuate if the encumbered juveniles are unable to invade and provide the means for reproduction of the bacterium.

The concentration of spores in the roots was not as great as had been anticipated. The conditions under which the plants were grown were similar to those recommended by Stirling (1981) in which the appropriate number of day degrees had been achieved. However, under glasshouse conditions the temperatures did fluctuate and it is possible that the periods of lower temperature adversely affected the development of the *P. penetrans*.

REFERENCES

Brown, S. M. & Smart, G. C. 1984. Attachment of *Bacillus penetrans* to *Meloidogyne incognita*. *Nematropica* 14: 171-172.

Channer, A. G. de R. & Gowen, S. R. 1992. Selection for increased host resistance and increased pathogen specificity in the

Meloidogyne-Pasteuria penetrans interaction. *Fundamental and Applied Nematology* 15: 331-339.

Davies, K. G. & Danks, C. 1993. Carbohydrate/protein interactions between the cuticle of infective juveniles of *Meloidogyne incognita* ands spores of the obligate hyperparasite *Pasteuria penetrans*. *Nematologica* 39: 53-64.

Davies, K. G., Kerry, B. R. & Flynn, C. A. 1988. Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. *Annals of Applied Biology* 112: 491-501.

Davies, K. G., Redden, M. & Pearson, T. K. 1994. Endospore heterogeneity in *Pasteuria penetrans* related to adhesion to plant-parasitic nematodes. *Letters in Applied Microbiology* 19: 370-373.

Ratnasoma, H. A. 1990. Studies on the use of *Pasteuria penetrans* for the control of root-knot nematodes and its evaluation on the perennial crops in Sri Lanka. Ph.D. thesis, Department of Agriculture, University of Reading.

Sayre, R. M. 1980. Biocontrol: *Bacillus penetrans* and related parasites of nematodes. *Journal of Nematology* 12: 260-270.

Sayre, R. M. & Starr, M. P. 1985. *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., a mycelial endospore forming bacterium parasitic in plant-parasitic nematodes. *Proceedings of the Helminthological Society of Washington* 52: 149-165.

Stirling, G. R. 1981. Effect of temperature on infection of *Melodogyne javanica* by *Bacillus penetrans*. *Nematologica* 27: 458-462.

Stirling, G. R. 1984. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. *Phytopathology* 74: 55-60.

Stirling, G. R. 1985. Host specificity of *Pasteuria penetrans* within the genus *Meloidogyne*. *Nematologica* 31: 203-209.

Stirling, G. R. & Wachtel, M. F. 1980. Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. *Nematologica* 26: 308-312.

Tzortzakakis, E. A. & Gowen, S. R. 1994. Resistance of a population of *Meloidogyne* spp. to parasitism by the obligate parasite *Pasteuria penetrans*. *Nematologica* 40: 258-266.

Vouyoukalou E., Gowen S. Оценка *Pasteuria penetrans* как биологического агента в борьбе с галловыми нематодами на Крите.

Резюме. Оценивали прилипаемость спор различных популяций *Pasteuria penetrans* к полевой популяции галловых нематод из Крита, состоящей из 75% *Meloidogyne incognita* и 25% *M. javanica*. К личинкам нематод в большей степени прилипали споры изолята *P. penetrans* из Южной Африки и изолята, являющегося смесью популяций, чем споры изолята из Австралии и изолята неизвестного происхождения. Использование в опытах линий галловых нематод, выделенных из одного яйцевого мешка, показало, что *M. javanica* является более восприимчивым к этим бактериям видом. Больше самок было обычно поражено бактериями в том случае, если к личинкам прикреплялось 5-8 спор.