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Host Range and Ecology of Isolates of *Pasteuria* spp. from the Southeastern United States[1]

M. Oostendorp,[2] D. W. Dickson,[2] and D. J. Mitchell[3]

Abstract: Isolates of *Pasteuria penetrans* were evaluated for ecological characteristics that are important in determining their potential as biological control agents. Isolate P-20 survived without loss of its ability to attach to its host nematode in dry, moist, and wet soil and in soil wetted and dried repeatedly for 6 weeks. Some spores moved 6.4 cm (the maximum distance tested) downward in soil within 3 days with percolating water. The isolates varied greatly in their attachment to different nematode species and genera. Of five isolates tested in spore-infested soil, three (P-104, P-122, B-3) attached to two or more nematode species, whereas B-8 attached only to *Meloidogyne hapla* and B-1 did not attach to any of the nematodes tested. In water suspensions, spores of isolate P-20 attached readily to *M. arenaria* but only a few spores attached to other *Meloidogyne* spp. Isolate P-104 attached to all *Meloidogyne* spp. tested but not to *Pratylenchus scribneri.* Isolate B-4 attached to all species of *Meloidogyne* and *Pratylenchus* tested, but the rate of attachment was relatively low. Isolate P-100 attached in high numbers to *M. arenaria* when spores were extracted from females of this nematode; when extracted from *M. javanica* females, fewer spores attached to *M. arenaria* than to *M. javanica* or *M. incognita.*

Key words: bacterium, bioassay, biological control, host range, lesion nematode, *Meloidogyne arenaria, M. incognita, M. javanica, Pasteuria penetrans, Pasteuria* sp., *Pratylenchus brachyurus, P. scribneri,* root-knot nematode, survival.

Pasteuria, a genus of obligately parasitic endospore-forming actinomycetes, is mentioned often as a potential biological control agent of plant-parasitic nematodes (5,7,11). Several characteristics of the organisms make them promising for practical application. They are frequently encountered in many climates and environmental conditions (7). They can survive prolonged periods under dry conditions, which appears to give them an excellent shelf life (12, pers. obs.). The host range of each isolate appears to be limited, but isolates specific for many important plant-parasitic nematodes have been observed (7). Many ecological characteristics of the organism are unknown, however, and must be elucidated before development of the organism as a biological control agent can progress.

Two species of the genus *Pasteuria, P. thornei* Starr & Sayre and *P. penetrans sensu stricto* (Thorne) Starr & Sayre, were described based on host range and morphological differences (8). As the genus is likely to contain more than two species parasitic to nematodes, Starr and Sayre (8) proposed the term *Pasteuria penetrans* for all non-speciated nematode-parasitic isolates. As we have not yet performed a speciation of our isolates, we use the term *P. penetrans* in this broad sense.

Our objectives were to evaluate the host range of eight isolates of *P. penetrans* and to determine the effect of soil water on spore dispersal and survival. The reproduction of one isolate in different nematodes was determined.

Materials and Methods

Nematode populations: The nematode populations used in this study originated from greenhouse cultures maintained at the University of Florida. *Meloidogyne* spp. were reared in a greenhouse on tomato (*Lycopersicon esculentum* Miller cv. Rutgers). Eggs of *Meloidogyne* spp. were extracted from roots in 0.5% sodium hypochlorite (3) and caught on a 25-μm-pore sieve. Second-stage juveniles (J2) were hatched from these eggs on a Baermann funnel, and only J2 less

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[2] Post-doctorate and Professor, Department of Entomology and Nematology, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-0611.

[3] Professor, Department of Plant Pathology, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611-0513.

than 2 days old were used for experimentation. *Pratylenchus scribneri* Steiner and *P. brachyurus* (Godfrey) Filipjev & Schuurmans-Stekhoven were grown in the greenhouse on mint (*Mentha* sp.) and bean (*Phaseolus vulgaris* L. cv. Harvester), respectively. Mixed-life stages of *Pratylenchus* spp. were extracted from the roots with a Baermann funnel technique. *Tylenchulus semipenetrans* Cobb and *Belonolaimus longicaudatus* Rau were cultured on citrus (*Citrus jambhiri* Lush. cv. Rough Lemon) or red clover (*Trifolium pratense* L. cv. Kenstar), respectively, and extracted by a centrifugal-flotation technique (4). *Panagrellus redivivus* (L.) Goodey, reared in petri dishes on oatmeal, and *Caenorhabditis elegans* (Maupas) Dougherty, cultivated on NG agar (1), were extracted by Baermann funnel.

Bacterial cultures: The origins of the *Pasteuria penetrans* isolates were P-20 from *M. arenaria* (Neal) Chitwood from Levy County, Florida; P-100 from *Meloidogyne* sp. from Pasco County, Florida; P-104 from *Meloidogyne* sp. from Lenoir County, North Carolina; P-122 unknown origin; B-1 from *T. semipenetrans* from Indian River County, Florida; B-3 from *Meloidogyne* sp. from Collier County, Florida; B-4 from *Pratylenchus scribneri* from Seminole County, Florida; and B-8 from *B. longicaudatus* from Alachua County, Florida. Twenty spores of isolates P-20, P-100, P-122, and B-4 were measured by light microscopy to establish that the morphology within each isolate was homogenous. Spores of isolate B-4 were smaller ($P \leq 0.05$) in diameter (3.4 ± 0.24 μm) than those of the other isolates (4.5 ± 0.4 μm).

Spores of isolate P-20 were extracted from *M. arenaria* infected peanut (*Arachis hypogaea* L. cv. Florunner) hulls grown at the original isolation site. The hulls were incubated overnight in Pectinol 59 (Genencor, South San Francisco, CA) and washed vigorously on a 600-μm-pore sieve. Females of *M. arenaria* were caught on a 150-μm-pore sieve, and spore-filled females, conspicuous by their opaque, white appearance, were collected with a stereomicroscope at a magnification of 20×; specimens were illuminated from above. The females were macerated in a glass tissue grinder in 1 ml deionized water, and the spore concentration was determined with a bacterial counting slide.

Spores of isolates P-100 and P-104 were extracted from parasitized females of *M. javanica* (Treub) Chitwood growing on tomato roots by the technique described for spores in *M. arenaria* females. *Pratylenchus scribneri* parasitized with isolate B-4 was collected from soil by centrifugal-flotation using a sucrose solution with a specific density of 1.26 g/ml (unpubl.); more spore-filled nematodes were extracted with this higher sucrose concentration than with the usual sucrose concentration. Spore-laden nematodes were cut to release their spores.

Survival of spores in soil: The persistence of *Pasteuria penetrans* spores in soil with different moisture regimes was studied in a sandy soil (95% sand, 1% silt, 4% clay; pH 6.8, < 1% organic matter). Spores of isolate P-20 were mixed into the soil to give a concentration of 3,000/g dry soil, and 40 g soil was placed into a petri dish. Experiment 1 consisted of the following treatments: no water; saturated daily with water (33.1% water); and alternately saturated with water and dried to constant weight (about 3.8% water) at room temperature one, two, four, or six times over a 6-week period. Each treatment was replicated five times.

The same soil was used in experiment 2, and the treatments were no water, saturated daily, water contents adjusted daily to 50% of the water-holding capacity (19.9% water), and alternately saturated and dried to constant weight at room temperature eight times. The dishes were left uncovered to increase drying. After 6 weeks, the soil of all treatments was adjusted to 50% of the water-holding capacity for 3 days. Preliminary experiments showed a reduced attachment of spores of *P. penetrans* isolate P-20 to *M. arenaria* race 1 in saturated soil relative to moist soil (data not shown). Therefore, all attachment assays in soil were performed at a soil moisture

content of 50% of the water-holding capacity to standardize the procedure. Four thousand J2 of *M. arenaria* in 4 ml water were inoculated per petri dish. After 3 days, the J2 were extracted by centrifugal-flotation. The number of spores attached per J2 was determined with an inverted microscope for 20 J2 per replicate at a magnification of 450×. The data were subjected to analysis of variance, and treatment means were compared by Duncan's multiple-range test.

Movement of spores in soil: PVC plastic tubes, 8.5 cm long and 2.5 cm d, were covered at one end by a Nitex (TETKO, Elmsford, NY) screen. The lower 6.4 cm of each tube (volume = 31.5 ml) was filled with spore-free soil (described in the preceding paragraph) and compacted to give a bulk density of 1.63. The compacting prevented settling of the soil during the experiment. A 1.6-cm layer of the same soil infested with 10,000 spores of P-20/g was placed on top of the spore-free soil. Deionized water was added daily at a rate of 5.8 ml per tube which was equivalent to half the pore volume of the soil in the tube or to 12-mm rainfall. The pore volume was calculated from the weight difference between soil saturated with water and soil dried to constant weight at 105 C. Replacing half the pore volume with water each day insured that the spores would not be washed through the whole soil column by a single application of water. After 1, 3, 5, and 10 days, spore distribution was evaluated in five randomly selected tubes. The soil was removed from the tubes and divided into five 1.6-cm-thick layers. Each layer was placed in a 6-cm-d petri dish and air dried for 3 days to ca. 50% water holding capacity. Five hundred J2 of *M. arenaria* race 1 in 1 ml water were added to each section. After 3 days the nematodes were extracted and the number of spores attached to each of 20 J2 was determined. The distributions of spores at different soil depths were compared by a chi-square test at $P \leq 0.05$.

Host-range studies: To determine the nematode host-attachment range of *P. penetrans,* 40 g dry, spore-infested soil was placed in a petri dish and test nematodes were added. Nematode inoculum per dish consisted of 4,000 J2 of *Meloidogyne* spp., 4,000 mixed life stages of *Pratylenchus scribneri,* 1,000 J2 of *T. semipenetrans,* or 500 mixed-life stages of *B. longicaudatus.* The nematodes were extracted from the soil after 3 days, and the number of spores attached to each nematode was determined for 20 nematodes in each of two replicates.

When spores could be obtained from parasitized nematodes, host-attachment range was evaluated in water suspensions. One-half milliliter of the spore suspensions (2×10^4 spores/ml deionized water) was pipetted into a 2-cm-d Syracuse watch glass, and 50 nematodes in 0.5 ml water were added. After 5 hours and after 24 hours, the number of spores attached to each nematode was determined for 20 nematodes in each of five replicates.

Reproduction of Pasteuria penetrans in nematodes: One thousand J2 of *M. javanica* or of *M. incognita* race 1 (Kofoid and White) Chitwood were incubated in a culture tube containing 5×10^4 spores of isolate P-100/ml water in a total volume of 5 ml. One thousand J2 of *M. arenaria* race 1 were incubated in a spore suspension of 1.5×10^5 spores/ml water. The higher concentration for *M. arenaria* was used to obtain an attachment rate similar to that for the other test nematodes as determined in preliminary experiments.

After 20 hours, the attachment was determined on 20 J2 of each species. Each of five tomato plants was inoculated with 200 nematodes for each nematode species. After 70 days, females of each nematode species were extracted from the roots following overnight treatment with Pectinol 59, and 10 randomly selected females per replicate were evaluated for spore contents. Spore suspensions were prepared from *M. arenaria* and *M. javanica* females, and the attachment of the spores to the same three nematodes was determined on 20 J2 per replicate; a spore concentration of 2×10^4/ml was used for all test nematodes. *Meloidogyne javanica* and *M. arenaria*

TABLE 1. Attachment of spores of *Pasteuria penetrans* isolate P-20 to second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 incubated for 3 days in spore-infested soil that had been submitted to different moisture regimes at 25 C.

Soil treatment	Spores/J2	
	Exp. 1	Exp. 2
Dried 6 weeks	2.80 a	0.98 a
Water saturated 6 weeks	1.38 b	1.59 a
50% water capacity 6 weeks		1.20 a
Air dried 1 time†	2.21 ab	
Air dried 2 times†	1.37 b	
Air dried 4 times†	2.51 ab	
Air dried 6 times†	2.06 ab	
Air dried 8 times†		1.22 a

Mean number of spores attached per nematode, 20 J2 per replicate; number of replicates = 5. Means in each column followed by the same letter do not differ significantly ($P \leq 0.05$) according to Duncan's multiple-range test.

† Alternately saturated with water and dried to constant weight at room temperature 1–8 times by dividing the 6-week period into equal intervals.

TABLE 2. Distribution of *Pasteuria penetrans* isolate P-20 spores from an inoculated surface layer† of soil through a soil column at 25 C after daily addition of 5.8 ml water onto the soil surface as demonstrated by attachment to second-stage juveniles (J2) of *Meloidogyne arenaria* race 1.

Soil depth (cm)	Spores/J2			
	Day 1	Day 3	Day 5	Day 10
0.0–1.6	8.40	7.70	6.20	4.20
1.6–3.2	1.00	2.60	2.20	1.60
3.2–4.8	0.10	1.20	1.50	1.10
4.8–6.4	0.05	1.40	1.20	0.70
6.4–8.0	0.00	0.70	0.80	0.60

Mean number of spores attached per nematode, 20 J2 per replicate; number of replicates = 5. Spore distribution differed ($P \leq 0.05$) between day 1 and day 3 and between day 5 and day 10 according to chi-square test.

† Surface layer of soil inoculated with P-20 at the rate of 10,000 spores/g.

J2 with spores were inoculated again to tomato plants. The attachment of the next generation of spores was determined on the three test nematodes using the same procedures. The data were subjected to analysis of variance, and treatment means were compared by Duncan's multiple-range test.

RESULTS

Survival of spores in soil: In experiment 1, the number of spores per J2 was higher ($P \leq 0.05$) when spores were in dry soil for 6 weeks than when spores were in continuously wet soil or in soil that was wetted and dried two times (Table 1). Attachment of spores in soil that had been alternately wetted and dried 1–6 times showed no clear trend. In experiment 2, none of the treatments resulted in differences in attachment.

Movement of spores in soil: Some spores of isolate P-20 moved downward in soil more than 3.2 cm after a single application of 5.8 ml water (Table 2). After day 3, spores were detected consistently at the greatest distance from the surface. The distribution of spores differed significantly ($P \leq 0.05$) at day 3 vs. day 1 and at day 10 vs. day 5. No spores were observed in the water collected from the bottom of the tubes. Most of the spores stayed in the top layer of the column.

Host-range studies: In a soil bioassay, the attachment of spores to the test nematodes differed greatly among the isolates of *P. penetrans.* Isolates B-3 and P-122 attached to all *Meloidogyne* spp. tested, whereas isolate B-1 failed to attach to any of the test nematodes (Table 3).

Isolates for which sufficient quantities of spores were available to prepare spore suspensions were tested in an in vitro assay. Attached spores were counted after 5 hours and again after 24 hours. In one experiment, attachment of isolate B-4 to some nematodes was observed after 5 hours but not after 24 hours. In all other cases, at-

TABLE 3. Attachment of spores of five *Pasteuria penetrans* isolates to nematodes after 3 days incubation at 25 C in spore-infested soil.

	P-104	P-122	B-1	B-3	B-8
Meloidogyne arenaria	−	+	−	+	−
M. javanica	+	+	−	+	−
M. hapla				+	+
M. incognita race 1	−		−	+	−
M. incognita race 2	+		−		
M. incognita race 3	−	+			
Pratylenchus scribneri		−	−	−	−
Belonolaimus longicaudatus				−	−
Tylenchulus semipenetrans			−		

+ = attachment; − = no attachment. Number of replicates = 2.

TABLE 4. Attachment of spores of four *Pasteuria penetrans* isolates from a spore–water suspension to nematodes after 24 hours incubation at 25 C.

	P-20			P-100		P-104	B-4	
	Exp. 1	Exp. 2	Exp. 3	Exp. 1	Exp. 2	Exp. 1	Exp. 1	Exp. 2
Meloidogyne arenaria race 1	2.40	4.90	0.32	0.02	0.57	0.92	0	0.12
M. javanica	0.03	0.09		0.92	3.24	2.10†		0.07
M. hapla	0	0		0.08				0.20
M. incognita race 1	0	0.09			2.54	9.90	0.48	0.18
M. incognita race 2	0.11	0.06				7.80		
M. incognita race 3			0.11	0	2.42	10.20	0.05	
Pratylenchus scribneri			0	0	0	0	1.00	0.43
P. brachyurus							0.71	
Belonolaimus longicaudatus			0.02					
Panagrellus redivivus	0	0						
Caenorhabditis elegans				0				

Mean number of spores attached per nematode, 20 J2 per replicate; number of replicates = 5.
† Counted after 5 hours, all other observations after 24 hours.

tachment after 5 hours and 24 hours was qualitatively the same, but attachment was greater after 24 hours. Isolate P-20 showed high rates of attachment to *M. arenaria* race 1 but low or no attachment to the other nematodes tested (Table 4). Isolate P-104 attached to all *Meloidogyne* spp. populations tested, but attachment to *M. arenaria* race 1 was markedly lower than attachment to the other *Meloidogyne* spp. Isolate P-100 attached almost exclusively to *M. javanica* in the first experiment, but it showed a broader host range in a subsequent experiment. Isolate B-4 showed a broad spectrum of attachment. Spores attached to *Pratylenchus brachyurus, P. scribneri,* and to the *Meloidogyne* spp. tested. The attachment varied greatly among experiments, even for the same nematode–spore combination.

Reproduction of Pasteuria penetrans in test nematodes: After incubating *M. incognita* race 1, *M. javanica,* and *M. arenaria* race 1 in suspensions of isolate P-100 overnight, the attachment was > 20, 1.5, and 2.5 spores/nematode, respectively. Seventy days after the inoculation of tomato plants with these spore infested nematodes, *P. penetrans* spores were observed in females of all three nematodes species. In the case of *M. incognita,* only four females were detected on the five root systems evaluated. One of these four females was spore filled. Seventy-three percent of the *M. javanica* females and 57% of the *M. arenaria* females were filled with spores. In the latter two cases, spore suspensions were prepared and the attachment of spores was evaluated. Spores from *M. javanica* attached equally well to *M. javanica* and *M. incognita,* but fewer ($P \leq 0.05$) spores attached to *M. arenaria* (Table 5, Exp. 1). Spores from *M. arenaria* showed greater ($P \leq 0.05$) attachment to *M. arenaria* than to *M. javanica* or *M. incognita. Meloidogyne javanica* and *M. arenaria* J2 were exposed again to the spores and inoculated onto tomato, and the next generation of spores was evaluated for attachment to the three test nematodes; no differences ($P \leq 0.05$) in attachment were found (Table 5, Exp. 2).

TABLE 5. Attachment of spores of *Pasteuria penetrans* isolate P-100 grown on two different host nematodes to *Meloidogyne javanica, M. incognita* race 1, and *M. arenaria* race 1.

Host nematode	Test nematode	Attachment	
		Exp. 1	Exp. 2
M. javanica	*M. javanica*	10.1 d	0.7 a
	M. incognita	9.5 d	2.9 a
	M. arenaria	1.9 b	0.3 a
M. arenaria	*M. javanica*	0.1 a	0 a
	M. incognita	0.1 a	0 a
	M. arenaria	3.7 c	1.6 a

Mean number of spores attached per nematode, 20 J2 per replicate examined; number of replicates = 5. Means followed by the same letter do not differ ($P \leq 0.05$) according to Duncan's multiple-range test.
† Spores used had been grown on the host nematode for at least two generations.

Discussion

To study the influence of environmental factors on spores of *P. penetrans* in soil, a method for quantifying spores in soil was required. Because the extraction of spores from soil proved to be difficult in initial experiments, a bioassay was developed. Bioassays for spores in soil have been used previously (13). Obviously, temperature (9), soil moisture, soil texture, and other factors that influence nematode movement in soil are likely to influence spore attachment to nematodes.

The levels of attachment differed greatly among different experiments. As these differences were observed in soil as well as in suspensions, we believe that they indicate variations in the quality of spore suspensions rather than variable soil factors. Great variations in the quality of spore preparations made from dry, ground up plant roots have been reported (12). These variations may have been caused by differences in development of nematodes or in numbers of the nematodes used to produce the spores.

The attachment of isolate P-20 in soil submitted to different moisture regimes demonstrates the suitability of the organism for practical applications. Although attachment of spores to the nematode's cuticle does not necessarily indicate that the spores are still able to parasitize the nematodes, attachment is an important factor in reducing the penetration of nematodes into roots (11). In unsterilized soil, it is possible that numerous organisms may feed on spores especially if the spore concentrations in soil are high.

Under practical conditions, it may be difficult to mix spore inoculum into the soil deeper than 15–20 cm. Significant numbers of nematodes may exist at greater depths, and it is important to know whether *P. penetrans* spores move downward with percolating water, at least in sandy soils. Soils with small pores may restrict movement. Furthermore, spores may adhere to clay particles or organic matter. Spore movement in other soil types, therefore, needs further study. In our experiment, no spores were observed in the water collected from the bottom of the tubes, but clay particles in the leachate may have obscured any spores present.

Two nematode-parasitic species of *Pasteuria* have been reported. *Pasteuria penetrans sensu stricto* parasitizes *M. incognita,* whereas *P. thornei* parasitizes *Pratylenchus brachyurus* but not *P. scribneri* (8). An isolate of *Pasteuria penetrans* that showed a different pattern by attaching to the four major *Meloidogyne* spp. and to *Pratylenchus scribneri* but not to *P. brachyurus* has been reported (6). Isolate B-4 is unique insofar as it attached to *Meloidogyne* spp. and to both *Pratylenchus* species. The spores of isolate B-4 were homogenous in size and appearance, but this does not rule out the possibility of a mixed culture of different types of *Pasteuria penetrans.* Furthermore, the specificity of *P. penetrans* isolates may be a response to nematode populations rather than to nematode species (10).

It has been observed that *P. penetrans* spores showed greater attachment to the nematode species or population they were originally grown in than to other nematodes (2). In the present study, significant levels of isolate P-100 attached to *M. arenaria* only after propagation on this nematode, indicating that *P. penetrans* may have the capability to adapt to host nematodes. Whether this adaptation involves genotypic selection or phenotypic adaptation (without genotypic changes) is not known. Spores of this isolate extracted from *M. javanica* and *M. arenaria* did not differ morphologically.

When isolate P-100 was produced in *M. javanica,* the attachment to *M. incognita* and *M. javanica* was similar. This indicates a similar mechanism of recognition or attachment with both nematodes. Isolate P-100 reproduced in all nematode populations that it attached to, although a high spore concentration was needed for spore attachment to *M. arenaria.* This indicates that attachment, not penetration, may be the step limiting the host range of *P. penetrans* isolates.

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