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Specific Gravity of Spores of *Pasteuria penetrans* and Extraction of Spore-filled Nematodes from Soil[1]

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Abstract: The specific gravity of spores of *Pasteuria penetrans* collected from *Meloidogyne arenaria* was found to be around 1.28. Increasing the sucrose concentration used for the extraction of *Pratylenchus scribneri* from a specific gravity of 1.14 to 1.26 led to the recovery of higher numbers of specimens filled with spores of *Pasteuria* sp. ($P \leq 0.05$). The numbers of spore-filled specimens of *Hoplolaimus galeatus* recovered from field soil were not affected by the concentration of the sucrose solutions. *Belonolaimus longicaudatus* was recovered from field soil in greater numbers in sucrose solutions with specific gravities of 1.22 and 1.26 than with a specific gravity of 1.14 ($P \leq 0.05$).

Key words: *Belonolaimus longicaudatus*, biological control, centrifugal flotation, extraction, *Hoplolaimus galeatus*, nematode, *Pasteuria* spp., *Pratylenchus scribneri*, specific gravity.

Nematode recovery procedures commonly include centrifugation of soil extracts in a sucrose solution with a specific gravity of 1.14 (2,4). Because the specific gravity of most vermiform nematodes is between 1.04 and 1.09 (4), a solution with a specific gravity of 1.14 is sufficient to keep the nematodes suspended during centrifugation. Nematodes parasitized by *Pasteuria* spp. are tightly packed with endospores of the parasite in its final stages of disease development. Our objectives were to determine the density of the endospores of *Pasteuria* spp. and the suitability of published nematode extraction procedures to extract spore-filled nematodes from soil.

Materials and Methods

Specific gravity of spores: Spores of *Pasteuria penetrans* Sayre and Starr were extracted from parasitized females of *Meloidogyne arenaria* (Neal) Chitwood (3). They were suspended at a concentration of 1.2×10^6 spores/ml in water or solutions with 10, 30, 50, and 70% (w/v) sucrose. The specific gravities of these solutions (5) were found to be 1.04, 1.11, 1.18, and 1.25, respectively, using a hydrometer at 21 C. The suspensions were centrifuged for 15 minutes at 8,000 *g*, the supernatant was removed, and the pellet was resuspended in 1 ml water. The spore contents of supernatant and pellet were determined using a bacterial counting slide.

A suspension of 0.5 ml of 7×10^6 *P. penetrans* spores/ml water was pipetted onto a sucrose gradient with a specific gravity increasing from 1.17 to 1.30. After 30 minutes of centrifugation at 53,000 *g*, 10 fractions of 0.5 ml each and the pellet were collected. The sucrose was removed from the fractions by adding 1 ml water and sedimenting the spores three times. Forty second-stage juveniles (J2) of *M. arenaria* race 1 were added to each fraction to assay for spore density. The numbers of spores attaching to their cuticles were counted after incubation for 24 hours on a laboratory shaker.

Recovery of spore-filled nematodes: Pratylenchus scribneri Steiner infected with *Pasteuria* sp. was extracted from a greenhouse culture by centrifugal flotation using sucrose solutions with specific gravities of 1.14, 1.22, and 1.26. The solutions were prepared by dissolving 454, 908, or 1,362 g of sucrose in 1 liter of water. The experiment was repeated four times with two to four replicates per experiment. The data were subjected to a two-way analysis of variance, and the orthogonal contrast between specific gravities 1.14 vs. 1.26 was determined.

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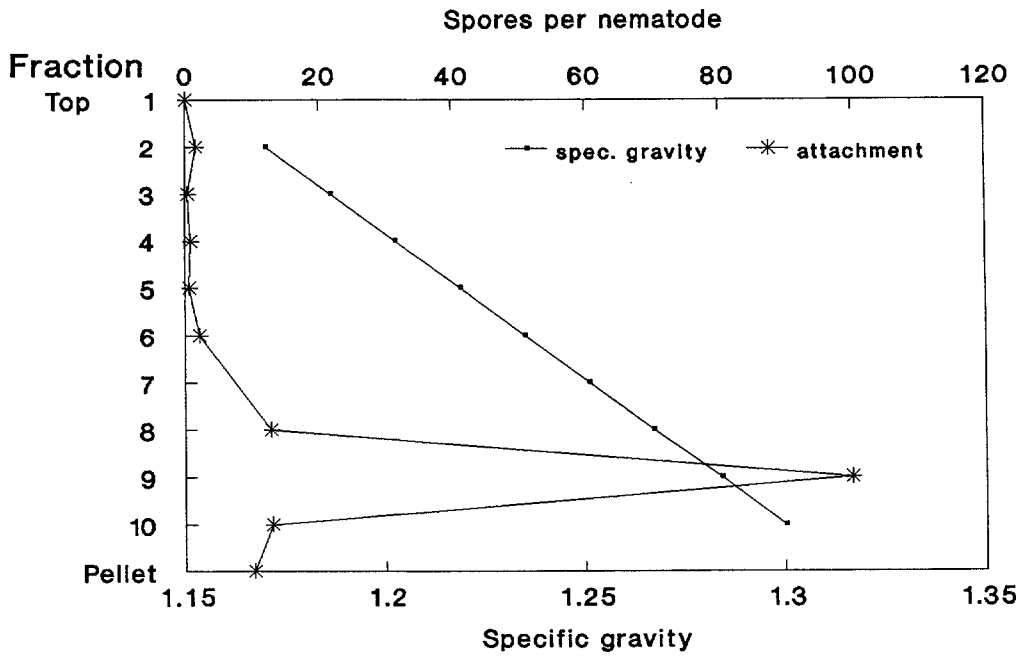

FIG. 1. Specific gravity of fractions of a sucrose gradient and number of spores of *Pasteuria penetrans* attached to the cuticle of second-stage juveniles of *Meloidogyne arenaria* race 1 incubated for 24 hours in these fractions.

Nematodes were extracted from field soil containing *Belonolaimus longicaudatus* Rau and *Hoplolaimus galeatus* (Cobb) Filipjev and Schuurmans Stekhoven parasitized by *Pasteuria* spp. and unidentified fungi using the three sucrose solutions described in the preceding paragraph. Counts were made of the number of nematodes with spores of *Pasteuria* spp. attached to their cuticle, of spore-filled bodies, and of bodies filled with parasitic fungi.

RESULTS

Specific gravity of spores: Spore counts of the supernatant and the pellet showed that more than 98% of the spores were in the pellet after centrifugation, even in the highest concentrated sucrose solution (Table 1). The specific gravity of the spores was, therefore, greater than 1.25.

The attachment of spores to the J2 of *M. arenaria,* which were used to assay for the spore density in the fractions of a sucrose gradient, was highest in fraction nine. This fraction had a specific gravity of 1.28 and corresponds to the specific gravity of the spores (Fig. 1).

Recovery of spore-filled nematodes: The number of *P. scribneri* extracted with spores attached to their cuticles did not change with increasing sucrose concentrations, but the percentage of nematodes filled with spores increased with increasing specific gravities ($P \leq 0.05$, Table 2). The per-

TABLE 1. *Pasteuria penetrans* spore concentration in supernatant and resuspended pellet after centrifugation for 15 minutes at 8,000 *g* in water or sucrose solutions of increasing specific gravities.

	P. penetrans spores/ml at specific gravity				
	0	1.04	1.11	1.18	1.25
Supernatant	4.0×10^4	0	4.0×10^4	0	1.5×10^4
Pellet	3.2×10^6	4.6×10^6	3.7×10^6	2.8×10^6	1.6×10^6

TABLE 2. Percentage of specimens of *Pratylenchus scribneri* filled with spores of *Pasteuria* sp. after extraction from soil in four different tests with three different sucrose concentrations.

Specific gravity	Percentage of *P. scribneri* with *Pasteuria* sp.			
	Test 1	Test 2	Test 3	Test 4
1.14	3	14	1.3	46
1.22	17	24	3.7	53
1.26	22	30	4.9	59
	n = 3	n = 2	n = 3	n = 4

The effects of test and specific gravity were significant in a two-way analysis of variance. The orthogonal contrast between specific gravities 1.14 and 1.26 was significant across all tests at $P = 0.009$. n = number of replicates.

centage of spore-filled nematodes was consistently higher ($P \leq 0.05$) in extractions made in a sucrose solution with a specific gravity of 1.26.

The number of spore-filled *H. galeatus* recovered from field plots infested with *Pasteuria* spp. was very low, and no spore-filled *B. longicaudatus* was recovered (Table 3). At other sampling dates, however, spore-filled *B. longicaudatus* were recovered (pers. obs.). The three sucrose concentrations did not affect the recovery of *H. galeatus,* nor was there an effect on the number with spores attached or filled with spores. The percentage of *H. galeatus* bodies recovered that were filled with an unidentified fungus ranged from 2.4 to 7.8%. *Belonolaimus longicaudatus* was extracted from the same plots with higher efficiency with specific gravities of 1.22 and 1.26 than with a specific gravity of 1.14 ($P \leq 0.05$). A low percentage (0.3–1.2%) of the *B. longicaudatus* were filled with an unidentified fungus.

DISCUSSION

The specific gravity of spores of *P. penetrans* extracted from *M. arenaria* females was found to be much higher than the specific gravity of the sucrose solutions commonly used for nematode extraction (4). The centrifugation–flotation technique normally is not used during the extraction of root-knot nematode females, but it was concluded that the extraction of spore-filled vermiform nematodes may be improved by using more concentrated sucrose solutions.

Subsequent experiments showed that higher sucrose concentrations were indeed effective in extracting a higher percentage of *P. scribneri* filled with spores of *Pasteuria* sp. The specific gravity of spores extracted from *Pratylenchus scribneri* was not determined, but it may be similar to that reported for *M. arenaria,* because the spores of both origins are morphologically similar. The high sucrose concentration technique was also used in attempts to extract spore-filled J2 of *Meloidogyne* spp., but none were found in three attempts. Spore-filled J2 of *Meloidogyne* spp. have been reported from a turfgrass site in south Florida (1).

The extraction efficiency for *H. galeatus* filled with spores of *Pasteuria* sp. was not increased by using higher sucrose concentrations, but the results of this experiment may have been compromised by the low number of spore-filled specimens in the soil at the time of our experiment. No spore-filled *B. longicaudatus* were recovered

TABLE 3. Percentage of specimens of *Hoplolaimus galeatus* and *Belonolaimus longicaudatus* with spores of *Pasteuria* spp. attached to their cuticle, filled with spores, or infected with fungi after extraction from soil with three different sucrose concentrations.

Specific gravity	*H. galeatus*/250 cm³ soil				*B. longicaudatus*/250 cm³ soil	
	Total no. recovered	Spores attached	Spore-filled	Fungi-filled	Total no. recovered	Fungi-filled
1.14	45.8	27.7	0.1	2.4	37.4	0.3
1.22	52.6	22.3	0.1	7.8	72.4	1.2
1.26	44.3	21.4	0.4	5.5	74.6	1.2

Total number of *B. longicaudatus* recovered at specific gravities 1.22 and 1.26 was higher ($P \leq 0.05$) than at 1.14 according to the orthogonal contrast of specific gravities 1.22 and 1.26 vs. 1.14. Number of replicates was 18.

during the period of this experiment; however, they have been recovered during other studies.

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