

Distribution and Downward Movement of *Pasteuria penetrans* in Field Soil¹

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Abstract: Endospores of *Pasteuria penetrans* were evaluated for their vertical distribution in field soil and their downward movement through soil in the laboratory. In the field trial, the number of endospores attached to second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 varied greatly in different soil depths. There were higher percentages of J2 with endospores attached in former weed fallow plots during the first 3 years of growing peanut than in former bahiagrass and rhizomal peanut plots ($P \leq 0.05$). In weed fallow plots a higher average number of endospores per J2 were maintained in all depths, upper three depths, and upper four depths in 1999, 2000, and 2001, respectively ($P \leq 0.05$). However, in 2002, there were no differences in the percentages of J2 with endospores attached and in the average of the numbers of endospores per J2 among the treatments ($P > 0.05$). In laboratory trials, *P. penetrans* endospores were observed to move throughout the soil through the percolation of water. After one application of water, some endospores were detected 25 to 37.5 cm deep. Endospores were present at the greatest depth, 37.5 to 50 cm, after the third application of water. These results indicate that rain or water applications by irrigation are likely to move endospores to deeper levels of the soil, but the majority of endospores remain in the upper 0-to-30-cm depth.

Key words: bacterium, biological control, distribution, *Meloidogyne arenaria*, nematode, *Pasteuria penetrans*, peanut, movement, root-knot nematode, suppressive soil.

Pasteuria penetrans has been identified as one of the biological agents that cause soils to become suppressive to *Meloidogyne arenaria* (Chen and Dickson, 1998; Cho-Myoung et al., 2000; Dickson et al., 1991, 1994; Freitas et al., 2000; Timper et al., 2001). The distribution of *P. penetrans* in soil may play an important role in its efficacy and success as a biological control agent. Although endospores of *P. penetrans* can move downward in soil when water is applied (Kamra et al., 1998; Mateille et al., 1996; Oostendorp et al., 1990), little information is available on the organism's long-term persistence, distribution, and movement in soil. The percolation of *Pasteuria* endospores by rainwater or irrigation could affect their distribution and even flush them from the upper 30 cm of soil where they are likely to be most effective. This study was conducted to determine the vertical distribution of endospores in field soil and whether they were readily moved vertically through soil with the percolation of water.

MATERIALS AND METHODS

Vertical distribution: The vertical distribution of *P. penetrans* in field soil was determined at five depths (0–15, 16–30, 31–45, 46–60, and 61–75 cm) beginning in 1999 and continuing through 2002. The site chosen was a peanut field located in Williston, Levy County, Florida, that was used to investigate the persistence and suppressiveness of *Pasteuria penetrans* to *Meloidogyne arenaria* race 1 (Cetintas and Dickson, 2004). Three treatments imposed from 1991 to 1999 were two *M. arenaria* race 1

nonhost crops: bahiagrass (*Paspalum notatum* cv. Pensacola var. Tifton 9), rhizomal peanut (*Arachis glabrata* cv. Florigrade), and weed fallow. Each plot was 38 m long and 10.6 m wide with a 2.4-m-wide non-tilled border. The soil was an Arredondo fine sand (92.5% sand, 4% silt, 3.5% clay, and <1% organic matter; pH 7.2). Over the 9 years, the site received minimum inputs. During the course of the 9 years of the cropping system no fertilizer, irrigation, or pest or pathogen management tactics were applied. Peanut was planted in the bahiagrass and weed fallow plots for 4 consecutive years (1999 to 2002), and in the rhizomal peanut plots for 3 consecutive years (2000 to 2002). Production practices common for growing peanut in the area were applied (Whitty, 2002). Fungicides for peanut leafspot control were applied every 14 to 21 days during the growing season.

Twelve plots out of a total of 30 plots were selected for sampling at five depths. Four plots selected from each of the former rhizomal peanut, bahiagrass, and weed fallow plots were randomized in a complete block design with three treatments, replicated four times. Five soil cores per depth were removed via a bucket auger (10-cm-diam.) from each of five locations near the middle of each plot. The soil texture was determined for three depths: 0 to 15 cm = 92.5% sand, 4% silt, 3.5% clay, and <1% organic matter; pH 7.2; 16 to 45 cm = 91% sand, 2% silt, 7% clay, and <1% organic matter; pH 7.4; and 46 to 75 cm = 85% sand, 4% silt, 11% clay, and <1% organic matter; pH 7.4. One liter of soil at each depth within each plot was combined, mixed thoroughly, placed in polyethylene bags, and taken to the laboratory for processing. Nematodes were extracted by a centrifugal-flotation method (Jenkins, 1964), and J2 of *Meloidogyne* spp. were counted using an inverted microscope. Also, the number of *P. penetrans* endospores on the first 20 J2 observed was counted (referred to henceforth as soil extracted J2).

In addition, a bioassay was used to determine the

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presence of *P. penetrans* endospores in soil at each soil depth (referred to henceforth as bioassay). Soil samples were air-dried and 40 g from each was placed in a 50-ml sterile polyethylene centrifuge tube. Soil water content was adjusted to 100% field capacity (saturated) to increase the rate of endospore attachment. Then, 500 1-to-3-day-old J2 of *M. arenaria* were added and the tubes left uncovered at room temperature. Three days later the J2 were extracted by the centrifugal-flotation method (Jenkins, 1964), and the number of endospores attached to 20 J2 was determined using an inverted microscope.

Movement of endospores: Polyvinylchloride plastic pipes (5-cm-id) were cut into 55-cm-long cylinders filled with soil by driving them into an undisturbed field soil located at the University of Florida Plant Science Research and Education Unit, Marion County, Citra, Florida. The soil at this site was screened for presence of *Meloidogyne* spp. and *P. penetrans*; neither was detected. The soil was an Arredondo fine sand and consisted of 93% sand, 3.5% silt, 3.5% clay, and <1% organic matter; pH = 7.4. The filled cylinders were brought to the laboratory, the top 5 cm of soil was removed to provide enough room for water to be added, and the bottom end of each cylinder was covered with Nitex screen (45- μ m opening) (TETKO, Elmsford, NY) to prevent soil from being washed out. Three representative cylinders were used to determine soil bulk density, moisture (Brady, 1998), and percentage of organic matter and texture (Bouyoucos, 1936). At the initiation of the study, bulk density and soil moisture were 1.5 g/cm³ and 18%, respectively.

Another three cylinders were used to determine water-holding capacity at the time the experiment was initiated. Tap water at room temperature was added to cylinders until it leaked from the bottom. Once water was visible at the bottom of the cylinders, the soil was considered to be at water-holding capacity. Water-holding capacity was measured as 310 ml/cylinder. The amount of water added to each experimental unit was half this amount (155 ml). The treatments consisted of 0 (control), 10,000, and 20,000 endospores/g of soil, and each treatment was replicated three times in a factorial experiment with a randomized complete block design. The factors were two densities of endospores, four water applications, and four soil depths. The cylinders were placed at room temperature, and the weight of each was recorded before and after adding water. The endospores were added at the top of each cylinder in 10 ml of water suspension. Five minutes later, 145 ml of water was added to each soil column to bring them up to approximately half their water-holding capacity. After the first water application, based on changes in weight, 155 ml of water was added to bring each cylinder back to half its water-holding capacity for the remaining three water applications. This was repeated up to four times over a period of 56 days,

i.e., one application per cylinder: added water once, two applications per cylinder: added water twice, etc. The maximum interval between water applications was 14 days.

At completion all cylinders were destructively sampled by cutting them into 12.5-cm sections, each representing a different soil depth. Presence of endospores was determined by a bioassay of the soil collected from the middle of each 12.5-cm section with a T-shaped sampling tube (2.5-cm-diam.). The bioassay was run for 3 days, after which the J2 were extracted by a centrifugal-flotation method (Jenkins, 1964) and the first 20 J2 were observed for the number of endospores attached per J2. Endospores present in the water collected from the bottom of each cylinder were concentrated to a 10 ml volume by centrifuging at 8,000g for 4 minutes, and discarding the excess water with a pipet. Then, 500 *M. arenaria* J2 were added and centrifuged in 2-ml microfuge tubes for 2 minutes at 8,000g. The J2 were recovered on a 25- μ m-pore sieve and observed for the number of endospores attached. Data were subjected to the analysis of variance, and treatment means were compared by Duncan's multiple-range test. The experiment was repeated.

***Pasteuria penetrans* and *Meloidogyne arenaria* isolates:** Endospores of a *P. penetrans* isolate (designated P-20) were collected from infected females of *M. arenaria* race 1 extracted from the roots of peanut (*Arachis hypogaea* cv. Florunner) grown in a field near Williston, Levy County, Florida. Galled roots were collected at harvest, dried, and kept in a paper bag until they were processed 5 to 6 weeks later. About 10 g of dry roots was incubated for 2 days in an enzymatic maceration solution (Charnecki, 1997), after which the females were extracted by washing the roots vigorously over stacked-sieves with 200- μ m-pore openings (top) and 30- μ m-pore openings (bottom). *P. penetrans*-infected females, conspicuous by their opaque appearance, were picked from among the females collected from the bottom sieve with a stereo-microscope (20 $\times$ magnification). Up to 1,000 endospore-filled females were collected and macerated in a glass tissue grinder in 1 ml deionized water, and the spore concentration was determined by counting in a 1-ml solution on a hemacytometer counting slide (Hausser Scientific, Horsham, PA). The *M. arenaria* race 1 isolate used was from a single egg mass culture grown in the greenhouse that was derived from peanut grown at the Williston site.

RESULTS

Vertical distribution: There were interactions and main effects of cropping systems, depths, and years on the percentages of J2 with endospores attached and the average number of endospores per J2 in the bioassay ($P \leq 0.05$) (Table 1). Of the two methods used to determine endospore densities in each of the treatments,

TABLE 1. Analysis of variance for the effects of depths (0–15, 16–30, 31–45, 46–60, 61–75 cm), cropping system (bahiagrass, rhizomal peanut, weed fallow^a), and years on the percentage of second-stage juveniles (J2) of *Meloidogyne* spp. with endospores of *Pasteuria penetrans* attached (soil extraction and bioassay), the average number of endospores attached per J2, and the number of *Meloidogyne* spp.^b Second-stage juveniles were extracted from each plot for four peanut-growing seasons (1999 to 2002).

Source of variation	df	Percentage of J2 with endospores attached ^c				Endospores/J2 ^d				No. J2 ^d	F value
		Field soil	F value	Bioassay ^e	F value	Field soil	F value	Bioassay ^e	F value		
Depth (D)	4	ns ^f	0.5	* ^g	9.7	ns	0.5	*	11.0	*	2.5
Cropping system (C)	2	*	11.7	*	49.9	*	9.7	*	65.4	*	12.1
Year (Y)	3	*	3.0	*	7.1	ns	1.3	*	3.0	*	81.6
C×D	14	*	2.2	*	10.9	*	3.8	*	15.8	ns	1.3
D×Y	19	ns	1.3	*	3.1	ns	1.0	*	2.4	*	12.4
C×Y	11	*	3.8	*	10.1	*	2.7	*	12.2	*	37.3
C×D×Y	59	ns	1.2	*	4.1	*	1.7	*	5.0	*	7.8

^a Plots with bahiagrass, rhizomal peanut, and weeds were grown from 1991 to 1999; beginning in 2000 peanut was grown 4 consecutive years in the bahiagrass and weed fallow plots, and 3 consecutive years in the rhizomal peanut plots.

^b *Meloidogyne arenaria* was the dominant species infecting peanut at this site; however, *M. javanica* also was discovered infecting peanut in this field in autumn 2001.

^{c,d} Data transformed with arcsin ($\sqrt{x}$) and $\log_{10}(x+1)$, respectively, before analysis.

^e Forty grams of air-dried soil was placed in a 50-ml-size sterile polyethylene centrifuge tube. Five hundred 1-to-3-day-old *M. arenaria* race 1 J2 were added. Three days later, J2 were extracted by a centrifugal-flotation method and the number of endospores attached per J2 was counted on the first 20 J2 observed per sample.

^f ns = Nonsignificant ($P > 0.05$).

generally the bioassay method gave a higher number (Tables 2, 3). Based on soil-extracted J2 in 1999, there were little differences among treatments or soil depths in the percentages of J2 with endospores attached or numbers of endospores per J2 (Table 2). With the bioassay in 1999 and 2000, however, weed fallow plots had

a higher percentage of J2 with endospores of *P. penetrans* attached in the top three depths than those from bahiagrass and rhizomal peanut plots ($P \leq 0.05$) (Table 3). Over the 4-year period there were increased numbers of endospores in the field soil (Table 2,3). There were greater numbers of endospores in the upper soil

TABLE 2. Percentages of second-stage juveniles (J2) of *Meloidogyne* spp.^a with *Pasteuria penetrans* endospores attached and average numbers of endospores attached per J2 (as determined by soil extraction) from soil collected at five soil depths during peanut harvest for the years 1999 to 2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow.

Cropping system ^b	Soil depth (cm)					Soil depth (cm)				
	0–15	16–30	31–45	46–60	61–75	0–15	16–30	31–45	46–60	61–75
	Percentage of J2 with endospores attached ^c					Endospores/J2 ^d				
1999										
Bahiagrass	0 a	10 a	5 a	15 a	5 a	0.0 a	0.3 a	0.2 a	0.4 a	0.1 a
Rhizomal peanut	0 a	5 a	5 a	0 b	5 a	0.0 a	0.1 a	0.1 a	0.0 a	0.2 a
Weed fallow	5 a	10 a	10 a	15 a	10 a	0.1 a	0.4 a	0.4 a	0.4 a	0.1 a
2000										
Bahiagrass	5 b	5 b	9 a	3 b	6 a	0.3 a	0.2 a	0.5 a	0.1 a	0.1 a
Rhizomal peanut	5 b	6 b	3 a	6 ab	4 a	0.2 a	0.2 a	0.1 a	0.2 a	0.3 a
Weed fallow	15 a	15 a	3 a	10 a	8 a	0.5 a	0.4 a	0.1 a	0.4 a	0.1 a
2001										
Bahiagrass	56 ab	25 b	25 b	15 b	11 a	1.0 b	0.2 b	0.2 b	0.2 b	0.2 a
Rhizomal peanut	18 b	16 b	18 b	18 ab	11 a	0.3 b	0.2 b	0.1 b	0.3 b	0.1 a
Weed fallow	75 a	83 a	66 a	46 a	35 a	3.2 a	5.0 a	5.1 a	1.8 a	0.1 a
2002										
Bahiagrass	38 b	13 b	4 b	5 a	5 a	0.8 b	0.1 a	0.1 a	0.1 a	0.1 a
Rhizomal peanut	16 b	10 b	14 b	10 a	5 a	0.5 b	0.1 a	0.2 a	0.2 a	0.1 a
Weed fallow	82 a	59 a	48 a	34 a	15 a	4.7 a	4.5 a	3.5 a	3.5 a	0.3 a

^a *Meloidogyne arenaria* is the dominant species infecting peanut at this site; however, *M. javanica* also was discovered infecting peanut in this field in autumn 2001.

^b Main treatments were established in 1991 and were left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were removed for planting peanut; however, the rhizomal peanut could not be removed because of heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

^{c,d} Data were transformed with arcsin ($\sqrt{x}$) and $\log_{10}(x+1)$, respectively, before analysis, but untransformed arithmetic means are presented.

Data are means of four replications with 20 J2 per replication. Means followed by the same letter within a column are not different according to Duncan's multiple-range test ($P \leq 0.05$).

TABLE 3. Percentages of second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 with *Pasteuria penetrans* endospore attached and average numbers of endospores attached per J2 (as determined by soil bioassay^a) from soil collected at five soil depths taken at harvest for each peanut season 1999–2002 following 9 years of bahiagrass, rhizomal peanut, and weed fallow.

Cropping system ^b	Soil depth (cm)					Soil depth (cm)				
	0–15	16–30	31–45	46–60	61–75	0–15	16–30	31–45	46–60	61–75
	Percentage of J2 with endospores attached ^c					Endospores/J2 ^d				
1999										
Bahiagrass	30 b	20 b	15 b	10 a	5 b	0.8 b	0.5 b	1.8 a	0.8 b	0.5 b
Rhizomal peanut	10 b	13 b	15 b	10 a	32 a	0.5 b	1.0 b	0.8 b	0.8 b	0.5 b
Weed fallow	75 a	57 a	63 a	32 a	35 a	2.8 a	2.8 a	2.0 a	1.5 a	3.0 a
2000										
Bahiagrass	26 b	5 b	14 b	13 a	7 a	1.2 b	1.2 b	0.3 b	0.7 a	0.2 a
Rhizomal peanut	9 b	6 b	9 b	5 a	11 a	0.2 b	0.2 b	0.2 b	0.1 a	0.2 a
Weed fallow	66 a	43 a	45 a	30 a	14 a	5.6 a	5.6 a	1.9 a	0.3 a	0.3 a
2001										
Bahiagrass	25 ab	25 b	13 b	13 b	19 a	3.8 ab	1.4 b	0.7 b	0.4 b	0.3 a
Rhizomal peanut	25 b	27 b	13 b	33 ab	31 a	1.8 b	0.5 b	0.8 b	0.5 b	0.4 a
Weed fallow	76 a	75 a	46 a	50 a	38 a	35.5 a	18.5 a	7.0 a	2.8 a	1.0 a
2002										
Bahiagrass	66 a	44 a	14 a	30 a	11 a	15.1 a	2.1 b	1.6 a	1.4 a	0.3 a
Rhizomal peanut	69 a	49 a	16 a	16 a	3 a	6.3 a	2.9 ab	0.4 a	0.5 a	0.2 a
Weed fallow	80 a	83 a	44 a	45 a	19 a	32.3 a	16.6 a	3.8 a	5.4 a	1.1 a

^a Forty grams of air-dried soil from each plot was placed in a 50-ml sterile polyethylene centrifuge tube. Five hundred 1-to-3-day-old J2 *M. arenaria* were added. Three days later, J2 were extracted by a centrifugal-flotation method and the number of endospores attached per J2 was counted on the first 20 J2 observed per sample.

^b Main treatments were established in 1991 and were left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were removed for planting peanut; however, the rhizomal peanut could not be removed until 2000 because of heavy thatch layer. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

^{c,d} Data were transformed with $\arcsin(\sqrt{x})$ and $\log_{10}(x + 1)$ before analysis, respectively, but untransformed arithmetic means are presented.

Data are means of four replications with 20 J2 per replication. Means followed by the same letter within a column are not different according to Duncan's multiple-range test ($P \leq 0.05$).

depths in weed fallow plots, followed by bahiagrass and rhizomal peanut, which corresponded to the increased numbers of J2 in the soil. In 2002, the final year of study, there were no differences in percentages of J2 with endospores attached or the average number of endospores per J2 among the treatments, except for

weed fallow and bahiagrass at 16 to 30-cm depth ($P \leq 0.05$) (Table 3).

In general, the number of *Meloidogyne* spp. detected was relatively low among all treatments and depths (Table 4). Over the course of the 4-year study, there were few differences in the root-knot nematode densi-

TABLE 4. Numbers of second-stage juveniles (J2) of *Meloidogyne* spp.^a per 100 cm³ at five soil depths taken at harvest for each peanut season 1999–2000 following 9 years of bahiagrass, rhizomal peanut, and weed fallow.

Cropping system ^b	Soil depth (cm)					Soil depth (cm)				
	0–15	16–30	31–45	46–60	61–75	0–15	16–30	31–45	46–60	61–75
1999										
Bahiagrass	40 a	87 a	36 a	42 ab	43 a	1 a	1 a	0 a	0 a	0 a
Rhizomal peanut	0 b	0 b	11 a	1 b	9 a	0 a	0 a	0 a	0 a	0 a
Weed fallow	17 a	71 a	49 a	72 a	65 a	4 a	7 a	6 a	11 a	5 a
2000										
Bahiagrass	52 a	125 a	29 a	50 a	181 a	22 a	68 a	38 a	11 a	26 a
Rhizomal peanut	90 a	126 a	32 a	82 a	98 a	20 a	42 ab	38 a	50 a	95 a
Weed fallow	24 a	112 a	63 a	64 a	194 a	18 a	11 b	16 a	17 a	51 a

^a *Meloidogyne arenaria* race 1 is the dominant species infecting peanut at this site; however, *M. javanica* also was discovered infecting peanut in this field in autumn 2001.

^b Main treatments were established in 1999 and were left with minimum disturbance until 1999. In summer 1999, bahiagrass and weed fallow were removed for planting peanut; however, the rhizomal peanut could not be removed because of heavy thatch layer. Glyphosate herbicide was sprayed over the rhizomal peanut in summer 1991 to kill the plants. Bahiagrass and weed fallow plots were planted with peanut during summers 1999 to 2002, whereas rhizomal peanut plots were planted during summers 2000 to 2002.

Data are means of four replications with 20 J2 per replication and were transformed with $\log_{10}(x + 1)$ before analysis. Actual numbers are presented. Means followed by the same letter within a column in the same year are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

TABLE 5. Analysis of variance for the effects of soil depths, inoculum densities, number of water applications, and number of trials on endospore numbers observed on second-stage juveniles (J2) of *Meloidogyne arenaria* race 1, based on soil and water bioassays.

Source of variation	df	Endospores/J2 ^a (soil)	F value	Endospore/J2 ^b (water)	F value
Soil depth (D)	3	* ^c	83.9	—	—
Inoculum density (I)	1	*	3.8	*	5.0
Number of water applications (A)	3	ns	1.0	*	5.0
Trials (T)	1	ns	0.8	ns	0.1
D × I	7	*	40.1	—	—
D × A	15	*	20.2	—	—
D × T	7	*	39.0	—	—
I × A	7	ns	0.5	*	5.5
I × T	3	ns	0.7	ns	1.2
A × T	7	ns	0.2	ns	1.6
D × I × T	15	*	20.6	—	—
D × A × T	31	*	10.2	—	—
A × I × D	31	*	11.2	—	—
I × A × T	15	ns	0.3	ns	1.8
D × I × A × T	63	*	5.8	—	—

— = No statistical analysis conducted.

^a Based on first 20 J2 observed in the soil bioassay.

^b Based on first 20 J2 of bioassay of water collected from the bottom of each tube.

ns = not significant ($P > 0.05$).

ties among the treatments within the depths sampled (Table 4). The highest density of root-knot nematode recorded was 194/100 cm³ in weed fallow plots in 2001 and at 61 to 75 cm at the deepest depth.

Movement of endospores: The interaction between soil depths, inoculum levels, number of water applications, and trials was significant ($P \leq 0.05$) with regard to the number of endospores per J2 detected from the soil ($P \leq 0.05$) (Table 5). Also, the interaction between inoculum density, number of water applications, and trials was significant regarding the number of endospores per J2 detected in the water collected from the bottom of cylinders ($P \leq 0.05$) (Table 5).

In trials 1 and 2, *P. penetrans* endospores were observed to disperse through the soil with water (Table 6). After one application of water, some endospores were detected as deep as 25 to 37.5 cm in both the first and second trials. Endospores were present at the greatest depth, 37.5 to 50 cm, after the third application of water in both trials. The number of endospores of *P. penetrans* detected among soil depths varied for both trials (Table 6). The number of water applications in the same inoculum density affected the number of endospores detected at various depths ($P \leq 0.05$) (Table 5). In trial 1, at 25 to 37.5-cm depth, the application of water three and four times moved a greater number of endospores than one or two water applications at the 20,000 inoculum density ($P \leq 0.05$) (Table 6). In the same trial, at 37.5 to 50-cm depth, after four water applications there were a greater number of endospores than one to three water applications at the 20,000-inoculum density ($P \leq 0.05$) (Table 6). In trial 2, at 37.5 to 50-cm depth following three and four water applications, there were a greater number of endospores than one or two water applications at the 20,000-inoculum density ($P \leq 0.05$) (Table 6). The water application affected the number of endospores detected in the water collected from the bottom of cylinders ($P \leq 0.05$) (Table 5). The number of endospores detected in the water collected from the bottom of the tubes was 0, 0, 0, and 0.8 for one to four applications of water, respectively, at the inoculum density of 10,000 endospores. The number of endospores detected in the water collected from the bottom of the cylinders was 0, 0, 1, and 7.8 for one to four applications of water, respectively, at the inoculum density of 20,000 endospores.

DISCUSSION

Pasteuria penetrans endospores were detected down to 75 cm deep in field soil. Endospore-encumbered J2

TABLE 6. Percolation of *Pasteuria penetrans* endospores added at two densities (10,000 and 20,000/g soil). PVC tubes filled with field soil were subjected to one to four applications of water over a period of 55 days.

Soil depths (cm)	Endospores/J2 ^a							
	One application ^b		Two applications		Three applications		Four applications	
	10K	20K	10K	20K	10K	20K	10K	20K
Trial one								
0–12.5	12.5 Aa	18.2 Aa	10.1 Aa	15.3 Aa	8.5 Aa	10.8 Aa	8.7 Aa	10.5 Aa
12.5–25.0	2.0 Ab	6.3 Ab	1.5 Ab	3.0 Ab	3.2 Ab	0.5 Ac	4.9 Ab	1.5 Ac
25.0–37.5	1.8 Ab	0.1 Bc	2.0 Ab	0.5 Bc	3.0 Ab	3.1 Ab	3.3 Ac	3.5 Ab
37.5–50.0	0.0 Bc	0.0 Bc	0.0 Bc	0.0 Bc	1.2 Abc	0.5 Bc	0.8 ABd	1.8 Ac
Trial two								
0–12.5	9.3 Aa	14.8 Aa	5.7 Aa	12.8 Aa	6.0 Aa	8.4 Aa	7.7 Aa	7.5 Aa
12.5–25.0	8.2 Aa	3.1 Ab	4.4 Aa	3.8 Ab	3.8 Ab	5.2 Ab	4.0 Ab	3.5 Ab
25.0–37.5	0.0 Bb	0.7 Bc	0.5 ABb	3.0 Ab	1.0 ABc	1.8 ABc	1.3 Ac	3.5 Ab
37.5–50.0	0.0 Ab	0.0 Bc	0.0 Ab	0.0 Bc	0.7 Ac	2.1 Ac	1.1 Ac	2.0 Ab

^a Five hundred second-stage juveniles of *Meloidogyne arenaria* race 1 were used to assay endospores of *Pasteuria penetrans* in each sample.

^b One hundred and fifty-five milliliters of water was added to each tube per water application.

Data are means of three replications with 20 J2 per replication. Means followed by the same letter within a row in the same inoculum density (uppercase) and within column in the same trial (lowercase) are not different according to Duncan's multiple-range test ($P \leq 0.05$).

have been reported down to 122 cm deep in a sandy field soil in Florida (Dickson et al., 1994), but this is the first report of their vertical distribution to 75 cm deep. Thus, it is believed that *Pasteuria* endospores become widely dispersed throughout the soil profile naturally, at least in sandy soils. Their downward dispersal with percolating water could result in their numbers being depleted in the top 15 to 20 cm of soil if they are not being continuously amplified in that zone. Downward movement with percolating water may be restricted, however, by the characteristics of the soil such as small-pore space, higher clay content, organic matter, and (or) other unknown factors (Kamra et al., 1998; Mateille et al., 2002). The soil in these studies was ca. 93% sand, which suggests that the endospores could move readily in the pore spaces between the sand particles.

Outcroppings of clay pockets occurred across the field site were used in this study; however, these pockets are easily seen when sampling and were avoided. Although not mapped, their existence affects root-knot nematode distribution (McSorley and Frederick, 2002); therefore, they affect endospore densities across the field.

Second-stage juveniles of *Meloidogyne* spp. were recovered from soil taken at each of the different depths down to 75 cm deep. The numbers were low in all plots and depths in 2000 because of drought conditions at time of sampling.

Some endospores of *P. penetrans* P-20 isolate were found more than 37.5 cm deep in the laboratory experiment after three or four applications of water. This corroborates the results by Oostendorp et al. (1990), who reported that in a small vertical soil-filled chamber, some endospores of P-20 isolate moved downward as deep as 3.2 cm after a single application of water. After 3 days, they detected endospores at the greatest distance of 6.4 cm from the surface. No endospores were observed in the water collected from the bottom of cylinder. However, in our study, we found endospores attached to J2 in the bottom of the cylinders (50 cm deep). A study conducted under a water drip supply showed that 67.7% and 10.6% of endospores percolated with water in a sandy soil and a clay soil, respectively (Mateille et al., 1996). In another study endospores were found 12 cm deep after a single application of water (Kamra et al., 1998). These results suggest that some endospores could be washed down by rainfall or irrigation events, thereby distributing them throughout the soil profile. Such distribution is likely helpful in

aiding *P. penetrans* as a highly effective biological control agent. However, if large numbers are flushed from the soil's A horizon, this may be detrimental to their long-term persistence.

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