Journal of Nematology 49(3):311–315. 2017.

Vertical Distribution of *Pasteuria penetrans* Parasitizing *Meloidogyne incognita* on *Pittosporum tobira* in Florida

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Abstract: Pasteuria penetrans is considered as the primary agent responsible for soil suppressiveness to root-knot nematodes widely distributed in many agricultural fields. A preliminary survey on a *Pittosporum tobira* field where the grower had experienced a continuous decline in productivity caused by *Meloidogyne incognita* showed that the nematode was infected with *Pasteuria penetrans*. For effective control of the nematode, the bacterium and the host must coexist in the same root zone. The vertical distribution of *Pasteuria penetrans* and its relationship with the nematode host in the soil was investigated to identify (i) the vertical distribution of *P. penetrans* endospores in an irrigated *P. tobira* field and (ii) the relationship among *P. penetrans* endospore density, *M. incognita* J2 population density, and host plant root distribution over time. Soil bioassays revealed that endospore density was greater in the upper 18 cm of the top soil compared with the underlying depths. A correlation analysis showed that the endospore density was positively related to the J2 population density and host plant root distribution. Thus, the vertical distribution of *P. penetrans* was largely dependent on its nematode host which in turn was determined by the distribution of the host plant roots. The *Pasteuria* was predominant mostly in the upper layers of the soil where their nematode host and the plant host roots are abundant, a factor which may be a critical consideration when using *P. penetrans* as a nematode biological control agent.

Key words: bioassay, endospore, *Meloidogyne incognita*, *Pasteuria penetrans*, *Pittosporum tobira*, soil profile, vertical distribution.

Pasteuria penetrans is widely distributed in agricultural soils globally, and it is considered as the primary agent responsible for soil suppressiveness to root-knot nematodes in many fields (Dickson et al., 1992, 1994; Weibelzahl-Fulton et al., 1996; Chen and Dickson, 1998; Freitas et al., 2000). The suppression of *Meloidogyne* spp. by *P. penetrans* develop after years of monoculture with a susceptible host. In soil, when J2 of *Meloidogyne* spp. migrate, endospores of *P. penetrans* attach to their cuticle and germinate when the nematode establishes a feeding site within the host vascular tissues (Sayre, 1993). A germ tube grows into the pseudocoelom of the nematode, resulting in microcolonies and thalli which develop into endospores causing a degeneration of the reproductive tissues and reducing fecundity of the nematode. Endospores are released into the surrounding soil on decay of the female cadavers and root tissue.

The vertical distribution of *Pasteuria penetrans* and its relationship with the nematode host in the soil is an important factor affecting its use as a biocontrol agent. For effective parasitism of the nematode, the bacterium and the host must coexist in the same root zone. However, agronomic practices such as irrigation could impact the vertical distribution of *P. penetrans* endospores in the field, and hence its ability to control nematodes (Bird and Brisbane, 1988; Chen et al., 1994; Weibelzahl-Fulton et al., 1996; Cetintas and Dickson, 2005). Endospores of *P. penetrans* move downward in soil when water is applied (Oostendorp et al., 1991; Mateille et al., 1996; Kamra et al., 1998). The percolation of *Pasteuria* endospores by rainwater or irrigation affects their vertical distribution and could displace them from the upper 30 cm of soil to 75-cm-deep or 122-cm-deep in the field soil after irrigation (Dickson et al., 1994; Cetintas and Dickson, 2005) while most plant-parasitic nematodes often occur within the top 15–20 cm of the soil (Barker and Nusbaum, 1971; Barker and Campbell, 1981; McSoreley and Dickson, 1990).

A preliminary survey of a *Pittosporum tobira* field where the grower had experienced a continuous decline in productivity caused by *Meloidogyne incognita* showed that the nematode was infected with *Pasteuria penetrans*. The nematode population density was estimated at 174.5 J2/100 cm^3 of the soil of which nearly 80% were encumbered with *Pasteuria* sp. The *Pasteuria* sp. was identified as *P. penetrans* based on the 16S ribosomal profile (Duan et al., 2003; Waterman et al., 2006). It was logical to expect nematode suppression in this 10-yr-monoculture field, but the nematodes were present at damaging levels making the field unproductive. The study was, therefore, carried out to investigate whether the irrigation practices by the grower had displaced the *P. penetrans* endospores away from the nematodes. The specific objectives of this work were to identify (i) the vertical distribution of *P. penetrans* endospores in an irrigated *P. tobira* field and (ii) the relationship among *P. penetrans* endospore density, *M. incognita* J2 population density, and host plant root distribution over time.

Materials and Methods

Field description: The study was carried out on a grower's farm in De Leon Springs, FL, at coordinates 29.1400°N, 81.3689°W. This site was a 3-yr-old planting of commercial *P. tobira* naturally infested with *M. incognita*. It was previously planted with *P. tobira* from 1998 to 2009. It was replanted in 2010 with *P. tobira* which

Received for publication September 17, 2016.
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This study was supported by the United States Federal Government through USDA Specialty Crop Block Grant Program. The authors are also grateful to Lonnie Clifton of Select Growers, Florida, for allowing the use of his field for this research.
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This paper was edited by Andrea M. Skantar.

subsequently grew poorly and exhibited severe root galling. A survey of the field revealed *M. incognita* as the causal agent of the galling and poor growth. The soil at the site is classified as an 'Astatula' fine sand single grained, well-drained soil (>95% sand).

Soil sampling: Soil samples for endospore detection were collected over a 2-yr period in March 2013, November 2013, March 2014, and November 2014. The *P. tobira* field was divided into four plots which were sampled separately by taking one sample in each plot at each date. Each sample was taken up to 36 cm depth consisting of twelve 3-cm-thick layers using a 5-cm-diameter AMS multi-stage sluge and sediment sampler (AMS, American Falls, ID). The sampler was inserted with a plastic sleeve cut into 12 circular plastic rings, each measuring 3-cm-deep and driven into the ground to collect a cylindrical soil core of 36-cm-long bound by the rings. The 36 cm soil core was carefully removed and cut into 12 layers of 3 cm each. The following individual layers were collected: 0 to 3 cm, 3 to 6 cm, 6 to 9 cm, 9 to 12 cm, 12 to 15 cm, 15 to 18 cm, 18 to 21 cm, 21 to 24 cm, 24 to 27 cm, 27 to 30 cm, 30 to 33 cm, and 33 to 36 cm and were used for *Pasteuria* bioassay. On the last sampling date, November 2014, an additional set of soil samples was taken. One set was used for *Pasteuria* bioassay while the other was processed for *P. tobira* root extraction by sieving and nematode extraction using a centrifugal sugar flotation method (Jenkins, 1964). The plant root weight and the nematode population density at each layer of the soil were determined.

Soil bioassay: The *Meloidogyne incognita* line used for the soil bioassay was raised from a single egg mass that was isolated from an infested Pittosporum root and maintained on tomato plants (*Solanum esculentum* cv. Rutgers) in a greenhouse (temperature range of 25°C to 28°C and 14-hr daylight) at the Entomology and Nematology Department, the University of Florida, Gainesville. The eggs were extracted from the tomato root using the NaOCl method (Hussey and Barker, 1973) and placed in distilled water at 28°C to hatch.

The 12 soil layers in four replications were air-dried for 90 d at room temperature to kill all the innate *M. incognita* J2. After 90 d, 10 g of dried soil was placed in a petri dish and rehydrated by adding sterile water until fully saturated (field capacity). To determine the abundance of *P. penetrans*, 200 freshly hatched (1 to 3-d old) *M. incognita* J2 were added to each petri dish containing the rehydrated soil. The petri dishes were partially covered and kept at room temperature for 72 hr (Brown and Smart, 1984; Cetintas and Dickson 2004, 2005). The J2s were then extracted by the centrifugal sugar flotation method (Jenkins, 1964). The

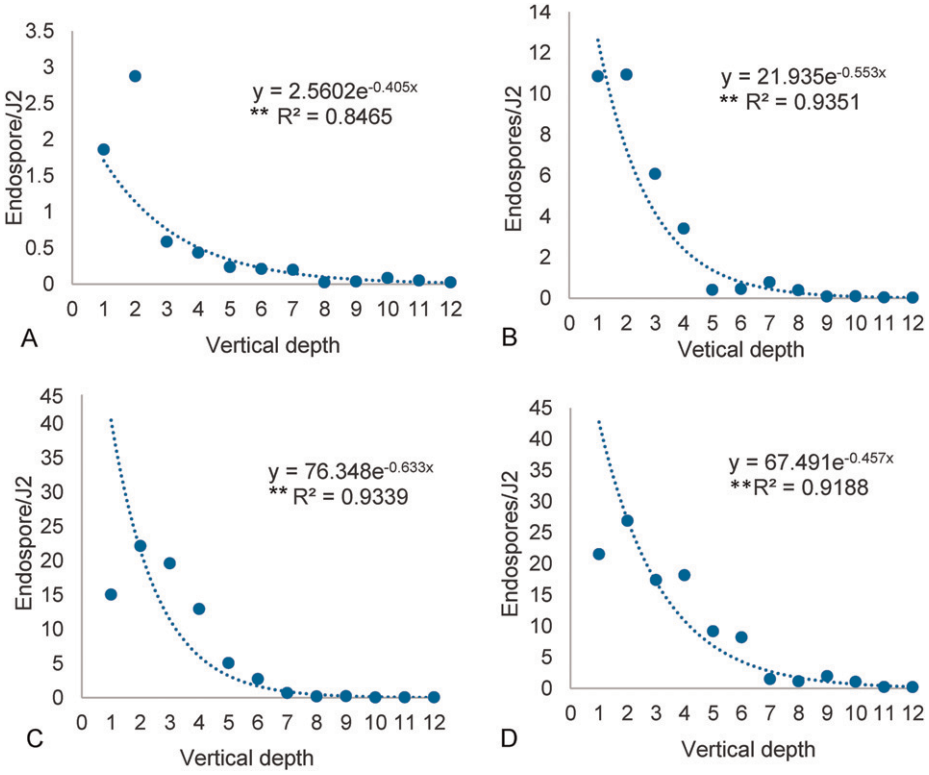

FIG. 1. *Pasteuria penetrans* endospores attached per *Meloidogyne incognita* J2 after 72 hr of infestation of the soil taken at different depths (1 = 0 to 3 cm, 2 = 3 to 6 cm, and 3 = 6 to 9 cm. . . 12 = 33 to 36 cm) with 200 *M. incognita* as determined by the soil bioassay. Data are the mean number of attachment on 20 J2 replicated four times in each of four consecutive sampling seasons: A (March 2013), B (November 2013), C (March 2014), and D (November 2014). Points are the actual *Pasteuria* numbers, and the dotted line is the fitted regression line or the predicted *Pasteuria* numbers. **Significant R^2 ($P < 0.05$).

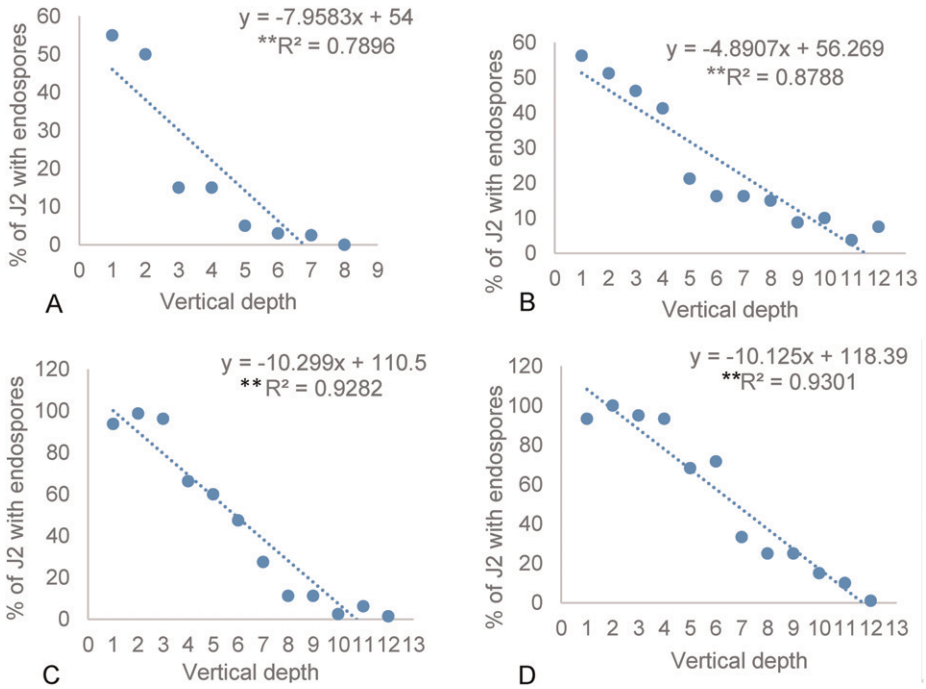

FIG. 2. Percentage attachment of *P. penetrans* endospores on *M. incognita* J2 after 72 hr of infestation of the soil taken at different depths (1 = 0 to 3 cm, 2 = 3 to 6 cm, 3 = 6 to 9 cm... 12 = 33 to 36 cm) with 200 *M. incognita* J2 as determined by soil bioassay. The data are the mean percent attachment of 20 juveniles replicated four times in each of four consecutive sampling seasons: A (March 2013), B (November 2013), C (March 2014), and D (November 2014). Points are the actual data and dotted line is the fitted regression line or the predicted % of J2 with endospores attached. **Significant R^2 ($P < 0.05$).

endospores attached to the J2 were enumerated for each soil layer under an inverted light microscope (×200 magnification). The average number of endospores attached per J2 and the percentage of J2 with endospores attached were determined on the first 20 nematodes (J2) observed.

Data collection and analysis: The average number of endospores/J2 and the percentage of J2 with endospores attached for each of the sampling dates were subjected to regression analysis against depth using Microsoft Excel (Microsoft Corporation, Redmond, WA). The average number of endospores/J2, percentage of J2 with endospores attached, J2 population density, and *P. tobira* root distribution were correlated with one another. All these variables were also correlated with the soil vertical depth using Microsoft Excel.

RESULTS

Soil bioassays revealed that the number of *P. penetrans* endospores attaching to *M. incognita* J2 (Fig. 1) and the percentage of J2 with endospores attached (Fig. 2) were higher in the upper layers of soil compared with the underlying depths. The bulk of the endospores were concentrated in the upper 18 cm of the soil. The number of endospores attached to the J2 was exponentially related to the soil vertical depth whereas the percentage of J2 with endospores attached was linearly related to the vertical depth. The nematode population density was highest in the upper 12 cm of the soil (Fig. 3) whereas the *P. tobira* roots were mainly in the upper 9 cm of the soil (Fig. 4).

A correlation analysis of the various parameters measured in this study showed that the endospore density was negatively related to the soil profile depth but positively correlated with the J2 population density and host plant root distribution. Thus, the number of *P. penetrans* endospores in the soil decreased with increasing vertical depth of the soil profile. The soil and root samples collected at each depth also showed that

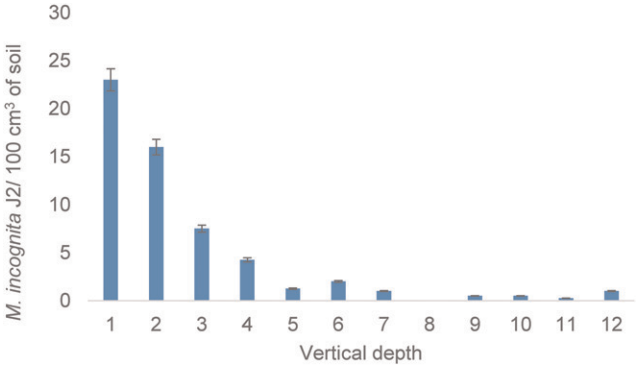

FIG. 3. Vertical distribution of *Meloidogyne incognita* J2 in the field soil collected at different depths (1 = 0 to 3 cm, 2 = 3 to 6 cm, 3 = 6 to 9 cm... 12 = 33 to 36 cm). The data are the mean number of J2 replicated four times in the last sampling date, November 2014. Bars represent standard deviation.

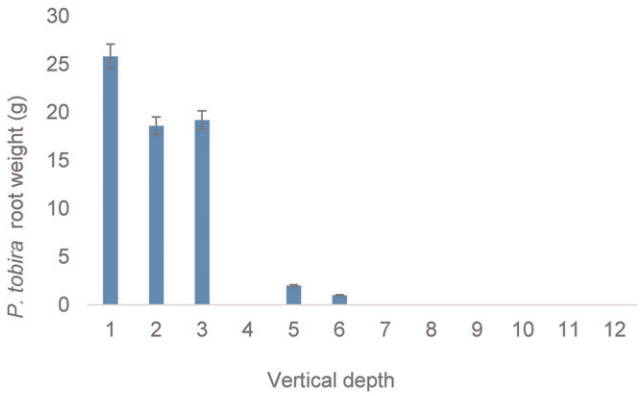

FIG. 4. Vertical distribution of *Pittosporum tobira* root in the field taken at different depths (1 = 0 to 3 cm, 2 = 3 to 6 cm, 3 = 6 to 9 cm. . . 12 = 33 to 36 cm). The data are mean root weight (g/100 cm^3 of soil) from four samples collected on a single sampling date, November 2014. Bars represent standard deviation.

nematode numbers and plant host roots decreased linearly with increasing vertical depth (Table 1).

DISCUSSION

Our bioassays indicated that the presence of *P. penetrans* in the soil could be detected with great precision by detecting the endospore-encumbered J2 extracted from the soil. The endospore density as measured by the percentage of J2 with endospores attached and the average number of endospores per J2 was high in the top 18 cm of the soil profile where the *M. incognita* J2 population density and *P. tobira* roots were concentrated. This implies that the vertical distribution of the endospores within the soil profile is not only dependent on their nematode host densities (Sayre, 1993; Dickson et al., 1994) but also on the distribution of the host plant roots. Other reports indicated that the distribution of *P. penetrans* endospores may be affected by factors such as irrigation water through soil percolation (Oostendorp et al., 1991; Mateille et al., 1996; Kamra et al., 1998; Cetintas and Dickson, 2005). Nonetheless, our results indicate that the distribution of the nematode host (*M. incognita*) and the host plant root distribution are the most important determinants of the vertical distribution of the endospores in the soil in this production system.

TABLE 1. Correlation between vertical depth (Depth), J2 population (Nemcount), root mass (g) distribution (Rootwgt/g), number of endospores/J2 (Sporcount), and percentage of J2 with endospores attached (% attchmt)

	Depth	Nemcount	Rootwgt/g	Sporcount	% attchmt
Depth	1				
Nemcount	−0.78	1			
Rootwgt/g	−0.76	0.93	1		
Sporcount	−0.92	0.84	0.80	1	
% attchmt	−0.96	0.70	0.68	0.94	1

The data are from the last sampling date, November 2014. Significant R^2 ($P < 0.05$).

Although endospore distribution was predominantly determined by the availability of the nematode host and the plant root distribution in the upper layers, few endospore-encumbered J2 were detected in deeper layers (>18 cm deep), giving a sparingly scattered distribution of the *Pasteuria* across the soil profile. The presence of the *Pasteuria* in these layers might be due to seasonal migration of the J2, natural rainfall, and agronomic practices, such as irrigation, consistent with the assertion that endospores could be washed down by rainfall or irrigation events in percolating water, thereby distributing them throughout the soil profile (Mateille et al., 1996; Kamra et al. 1998; Cetintas and Dickson, 2005).

Endopsore densities were very low in March 2013, moderate in November 2013, and very high in 2014. However, *M. incognita* population levels were consistently high in both years. The exact cause of the increasing trend of endospore densities is unknown. Nevertheless, a positive correlation between densities of J2 and endospore densities was evident suggesting a density-dependent relationship between the nematode and the bacterium. Recently, a cyclic nature of Root-knot nematode (RKN)-*Pasteuria* interactions in an annual crop was demonstrated (Timper et al., 2016). When nematode densities are high, the *P. penetrans* rapidly builds up in soil to suppressive levels; however, once the nematode densities decline, so do the densities of *P. penetrans*. The RKN-*Pasteuria* interactions in perennial crops may show different dynamics: *Pittosporum tobira* is a perennial plant; hence, the galls of different ages were hardly distinguishable. The galls formed in different years typically coalesced to form giant galls within which the nematode reproduced. The nematodes embedded within these galls were beyond the reach of the bacterium which, perhaps, explains why we did not observe the suppression of *M. incognita* when endospore densities were so high in the soil. Other possible explanation for this may include (i) secondary infestation by the J2 inside/in vicinity of the roots such that there was little chance of J2 exposed to spores in the soil, (ii) the perennial plants succumbed to the nematode disease because of poor cultural practices and/or combinations, and (iii) the horizontal distributions of spores and J2 did not match because both organisms had aggregated distributions. On the other hand, suppression may have occurred, but it could not be detected on the perennial crop because of the cumulative nature of root galling.

Over the period of 3 yr of *P. tobira* in the field, there was no evidence that the endospore densities reached suppressive levels because *P. tobira* roots were heavily galled in all plots, and foliage yields appeared to be suppressed despite the surging endospore levels; the reason for this incongruence is yet to be investigated.

However, the study showed high correlations of *P. penetrans* spores, *M. incognita* J2, and *P. tobira* plant host root distribution in the soil profile. The vertical distribution of *P. penetrans* was, therefore, largely dependent on its nematode host which in turn was determined by the distribution of the host plant roots. The *Pasteuria* endospores were present mostly in the top soil where their nematode host and the plant host roots were abundant, a factor which may be a critical consideration when using *P. penetrans* as a biological control agent.

Literature Cited

Barker, K. R., and Campbell, C. L. 1981. Sampling nematode populations. Pp. 451–474 *in* B. M. Zuckerman and R. A. Rohde, eds. Plant parasitic nematodes, vol. 3. New York: Academic Press.

Barker, K. R., and Nusbaum, C. J. 1971. Diagnostic and advisory programs. Pp. 281–301 *in* B. M. Zuckerman, R. A. Rohde, and W. F. Mai, eds. Plant parasitic nematodes, vol. 1. New York: Academic Press.

Bird, A. F., and Brisbane, P. G. 1988. The influence of *Pasteuria penetrans* in field soils on the reproduction of root-knot nematodes. Revue de Nematologie 11:75–81.

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

Cetintas, R., and Dickson, D. W. 2004. Distribution and downward movement of *Pasteuria penetrans* in field soil. Journal of Nematology 37:155–160.

Cetintas, R., and Dickson, D. W. 2005. Persistence and surppresiveness of *Pasteuria penetrans* to *Meloidogyne arenaria* Race 1. Journal of Nematology 36:540–549.

Chen, S., Dickson, D. W., and Whitty, E. B. 1994. Suppression of *Meloidogyne arenaria* by *Pasteuria penetrans* in the field. Nematologia Brasileira 24:147–156.

Chen, Z. X., and Dickson, D. W. 1998. Review of *Pasteuria penetrans*: Biology, ecology, and biological control potential. Journal of Nematology 30:313–340.

Dickson, D. W., Oostendorp, M., Giblin-Davis, R. M., and Mitchell, D. J. 1994. Control of plant-parasitic nematodes by biological antagonists. Pp. 575–601 *in* D. Rosen, F. D. Bennett, and J. L. Capinera, eds. Pest management in the subtropics, biological control - A Florida perspective. Andover, Hampshire, UK: Intercept.

Dickson, D. W., Oostendorp, M., and Mitchell, D. J. 1992. Development of *Pasteuria penetrans* on *Meloidogyne arenaria* race 1 in the field. Pp. 213–218 *in* F. J. Gommers, and P. W. Th. Maas, eds. Nematology from molecule to ecosystem. Dundee, Scotland: European Society of Nematologists.

Duan, Y. P., Castro, H. F., Hewlett, T. E., White, J. H., and Ogram, A. V. 2003. Detection and characterization of *Pasteuria* 16S rRNA gene sequences from nematodes and soils. International Journal of Systematic and Evolutionary Microbiology 53:105–112.

Freitas, L. G., Dickson, D. W., Michelle, D. J., and McSorley, R. 2000. Suppression of *Meloidogyne arenaria* by *Pasteuria penetrans* in the field. Nematologia Brasileira 24:147–156.

Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp., inoculating a new technique. Plant Disease Reporter 57:1025–1028.

Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Kamra, A., Dhawan, S. C., and Kamra, A. 1998. Observations on host range, vertical movement, and soil pH on spores of *Pasteuria penetrans* infecting *Heterodera cajani*. Indian Journal of Nematology 28:192–202.

Mateille, T., Duponnois, R., Dabire, K., N'Diaye, S., and Diop, M. T. 1996. Influence of the soil on the transport of the spores of *Pasteuria penetrans*, parasite of nematodes of genus *Meloidogyne*. European Journal of Soil Biology 32:81–88.

McSoreley, R., and Dickson, D. W. 1990. Vertical distribution of plant–parasitic nematodes in sandy soils under soybean. Journal of Nematology 22:90–96.

Oostendorp, M., Hewlett, T. E., Dickson, D. W., and Mitchell, D. J. 1991. Specific gravity of spores of *Pasteuria penetrans* and extraction of spore-filled nematodes from soil. Journal of Nematology 23:729–732.

Sayre, R. M. 1993. *Pasteuria*, Metchnikoff, 1888. Pp. 101–111 *in* A. L. Sonenshein, J. A. Hoch, and R. Losick, eds. Bacillus subtilis and other gram positive bacteria: Biochemistry, physiology, and molecular genetics. Washington, DC: American Society for Microbiology.

Timper, P., Liu, C., Davis, R. F., and Wu, T. 2016. Influence of crop production practices on *Pasteuria penetrans* and suppression of *Meloidogyne incognita*. Biological control 99:64–71.

Waterman, J. T., Bird, D. M., and Opperman, C. H. 2006. A method for isolation of *Pasteuria penetrans* endospores for bioassay and genomic studies. Journal of Nematology 38:165–167.

Weibelzahl-Fulton, E., Dickson, D. W., and Whitty, E. B. 1996. Suppression *of Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in Florida. Journal of Nematology 28:43–49.