JOURNAL OF NEMATOLOGY

VOLUME 29 MARCH 1997 NUMBER 1

Journal of Nematology 29(1):1–8. 1997.

Suppression Mechanisms of *Meloidogyne arenaria* race 1 by *Pasteuria penetrans*[1]

Z. X. CHEN,[2] D. W. DICKSON,[3] D. J. MITCHELL,[4] R. MCSORLEY,[3] AND T. E. HEWLETT[5]

Abstract: The biological control of *Meloidogyne arenaria* on peanut (*Arachis hypogaea*) by *Pasteuria penetrans* was evaluated using a six × six factorial experiment in field microplots over 2 years. The main factors were six inoculum levels of second-stage juveniles (J2) of *M. arenaria* race 1 (0, 40, 200, 1,000, 5,000, and 25,000 J2/microplot, except that the highest level was 20,000 J2/microplot in 1995) and six infestation levels of *P. penetrans* as percentages of J2 with endospores attached (0, 20, 40, 60, 80, and 100%). The results were similar in 1994 and 1995. Numbers of eggs per root system, J2 per 100 cm^3 soil at harvest, root galls, and pod galls increased with increasing nematode inoculum levels and decreased with increasing *P. penetrans* infestation levels ($P \leq 0.05$), except that there was no effect of *P. penetrans* infestation levels on J2 per 100 cm^3 soil in 1994 ($P > 0.05$). There were no statistical interaction effects between the inoculum levels of J2 and the infestation levels of *P. penetrans* ($P > 0.05$). When the infestation level was increased by 10%, the number of eggs per root system, root galls, and pod galls decreased 7.8% to 9.4%, 7.0% to 8.5%, and 8.0% to 8.7% in 1994 and 1995, respectively, whereas J2 per 100 cm^3 soil decreased 8.8% in 1995 ($P \leq 0.05$). The initial infestation level of *P. penetrans* contributed 81% to 95% of the total suppression of pod galls, whereas the infection of J2 of the subsequent generations contributed only 5% to 19% suppression of pod galls. The major suppressive mechanism of *M. arenaria* race 1 by *P. penetrans* on peanut is the initial endospore infestation of J2 at planting.

Key words: Arachis hypogaea, bacterium, biological control, endospore, *Meloidogyne arenaria, Pasteuria penetrans,* peanut, root-knot nematode.

Pasteuria penetrans (Thorne) Sayre & Starr is an obligate mycelial and endospore-forming bacterial parasite of root-knot nematodes (Sayre and Starr, 1985). The first report of an organism resembling *P. penetrans* was made by Cobb (1906), who found numerous highly refractile spores infecting specimens of the nematode *Dorylaimus bulbiferous.* He erroneously viewed these spores as "perhaps monads" of a parasitic sporozoan. This error persisted for nearly 70 years. A more precise but still incorrect placement, *Duboscqia* Perez 1908, was suggested by Micoletzky (1925). Later, Thorne (1940) described in detail a new parasite from *Pratylenchus pratensis* (de Man) Filipjev and, on the assumption that the organism was similar to the nematode parasite described by Micoletzky, named it *Duboscqia penetrans.* It was not until the nematode parasite was examined under the electron microscope that its affinities with bacteria rather than protozoa were observed, and it was then named *Bacillus penetrans* by Mankau (1975). Sayre and Starr (1985), who studied the ultrastructure of *B. penetrans,* drew attention to its resemblance to the actinomycete *Pasteuria ramosa* (Metchnikoff, 1888) and subsequently renamed it *Pasteuria penetrans.* Currently, the following three species of *Pasteuria* have been described from plant-parasitic nematodes: *P. penetrans* from *Meloidogyne* spp., *P. thornei* from *Pratylenchus* spp., and *P. nishizawae* from *Heterodera* spp. and *Globodera* spp. (Sayre and Starr, 1989).

Pasteuria penetrans has shown great potential as a biological control agent of root-knot nematodes (Dickson et al., 1994). It occurs in nematode-suppressive soils (Dickson et

Received for publication 14 June 1996.

[1] Florida Agricultural Experiment Station Journal Series No. R-05303. Supported in part by a grant from the Southern Region IPM Grant No. 93-34103-8396. A portion of a Ph.D. dissertation by the first author.

[2] Graduate Research Associate, [3] Professors, and [5] Senior Biologist, Entomology and Nematology Department, [4] Professor, Plant Pathology Department, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL 32611.

E-mail: zchen@gnv.ifas.ufl.edu

al., 1994) and has suppressed nematodes in greenhouse (Brown and Smart, 1985; De Leij et al., 1992; Stirling, 1984) and field microplot experiments (Brown et al., 1985; Chen et al., 1996b; Dube and Smart, 1987; Oostendorp et al., 1991; Stirling, 1984). Recently, we have demonstrated that application of 10,000 endospores of *P. penetrans* per gram of soil effectively suppressed the peanut root-knot nematode on peanut (Chen et al., 1996a; 1996b).

In this study, a two-factor factorial experiment was conducted over a 2-year period under field conditions. The objective was to determine the suppressive relationships between *P. penetrans* infestation levels and *M. arenaria* inoculum densities.

Materials and Methods

Pasteuria penetrans isolate: An isolate of *P. penetrans* designated P-20 (Oostendorp et al., 1990), originating from *M. arenaria* race 1 from a peanut field in Levy County, Florida, was propagated on *M. arenaria* growing on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) in a greenhouse. Second-stage juveniles (J2) up to 5 days old with approximately five endospores attached were obtained by centrifugation (Hewlett and Dickson, 1993). Tomato plants (45 to 60 days old) were inoculated with the endospore-encumbered J2 at a rate of 3,000 J2/pot. The tomato plants were grown in 15-cm-diam. pots and fertilized weekly with 1 g of 20-20-20 (N-P-K)/pot. Insecticide applications and water were provided as needed. Root systems were harvested 45 to 60 days after inoculation, rinsed, and incubated in a 12.5% cytolase PCL5 solution (Genencor International, Rochester, NY) for 3 days. Following incubation, the material was decanted onto a sieve with 600-µm-pore openings nested in a sieve with 75-µm-pore openings and subjected to a high-pressure spray of water to dislodge the females (Hussey, 1971). Endospore-filled females, conspicuous by an opaque and white appearance when illuminated from above, were hand-picked using a pipette with the aid of a microscope at ×20. The females containing endospores were ground in deionized water using a 15-ml glass tissue grinder. The suspension containing endospores was decanted into a flask and diluted to a volume of 50 ml. Concentrations of endospores in the suspensions were determined by counting five 0.1-µl aliquots using a hemocytometer at ×450.

Nematode isolate: An isolate of *M. arenaria* race 1 from Levy County, Florida, was cultured on tomato in a steam-pasteurized potting soil. Eggs of *M. arenaria* were extracted from roots using 0.5% sodium hypochlorite (Hussey and Barker, 1973) and caught on a sieve with 25-µm-pore openings, rinsed, and placed on Baermann funnels. The roots and plant debris were caught and separated from eggs by a sieve with 75-µm-pore openings nested on a sieve with 25-µm-pore openings. Second-stage juveniles that hatched on the first day were discarded to avoid using nematodes that may have acquired endospores in the potting soil. Juveniles up to 5 days old were collected by decanting the water suspension from Baermann funnels onto a sieve with 25-µm-pore openings, rinsed, and transferred into a beaker. The number of J2 in the suspension was determined by counting five 1-ml aliquots with the use of a compound inverted light microscope.

Field testing: The experiment was conducted in 1994 and 1995. Microplots of clay flue liners were installed in rows of nine with a distance of 0.9 m in and between rows in an Arredondo fine sand (94% sand, 1% silt, 5% clay; <0.1% organic matter; pH 5.6) (McSorley et al., 1992) on the Green Acres Agronomy Farm, University of Florida, Gainesville. The soil profile consisted of sand (≥90% sand) to a depth of >1.2 m. The flue liner dimensions were 25 cm × 40 cm with a length of 100 cm, and each flue liner was inserted 90 cm deep into the soil. The microplots were fumigated in both 1994 and 1995 with 1,200 kg of a mixture of 98% methyl bromide and 2% chloropicrin per hectare applied under a 0.076-mm-thick polyethylene plastic mulch 3 months before adding inocula.

Meloidogyne arenaria J2 were attached with endospores of *P. penetrans* using centrifuga-

tion at 150,000 endospores/ml (Hewlett and Dickson, 1993). After centrifugation, the suspension was decanted on a 25-μm-pore sieve to remove the unattached endospores. The endospore-encumbered J2 were then rinsed and transferred into a beaker. Five subsamples, with 20 J2 in each subsample, were taken to determine the number of endospores attached per J2. All J2 had at least one endospore attached, based on the total count of 100 J2 in both 1994 and 1995. The number of endospores attached per J2 was 3.5 ± 0.3 for 1994 and 5.4 ± 1.0 for 1995. The uninfected J2 were treated similarly except they were not exposed to endospores. Five hundred J2 in five subsamples taken from the uninfested nematodes were examined at ×450, and no endospore attachment to any of the J2 was observed.

The experimental design was a six × six factorial with four replications for a total of 144 microplots; six levels of *P. penetrans* infestation and six levels of population densities of *M. arenaria* juveniles were established. The population densities of *M. arenaria* were 0, 40, 200, 1,000, 5,000, and 25,000 J2 per microplot in 1994 and 1995, except that the highest level was 20,000 J2 per microplot in 1995. Six *P. penetrans* infestation levels were established by combining 0, 20, 40, 60, 80, and 100% of endospore-encumbered J2 with uninfested J2 for each population density of *M. arenaria.* The experiment was arranged in a randomized complete block design with a total of four blocks. Microplots were inoculated on 20 May 1994 and 23 May 1995. The six levels of J2 with the designed percentages of *P. penetrans* infestations were suspended individually in 300 ml water contained in a 500-ml bottle. Fifteen uniformly spaced holes, five each with depths of 7, 14, and 21 cm, were made in each microplot with a template. The inoculum suspensions were sprinkled uniformly over the soil and into the holes and mixed into the soil by using a potato rake. A 10-day-old peanut seedling (*Arachis hypogaea* L. cv. Florunner) was transplanted into a 3-cm-deep hole in each microplot immediately after inoculation. After transplanting, 10 g of inoculum of *Rhizobium* spp. (The Nitragin Company, Milwaukee, WI) was applied into each microplot. Microplots were drip-irrigated and hand-weeded as needed. Insecticides and fungicides were applied as recommended by the University of Florida Extension Service (Whitty et al., 1975). During winter, the microplots were planted with wheat (*Triticum aestivum* L. cv. Fla 304) as a cover crop.

After 125 days, the peanut plants were harvested. Soil samples consisting of three cores (2.5-cm-diam. × 20-cm-deep) were taken in each microplot. Juveniles were extracted from 100 cm^3 soil using centrifugal flotation (Jenkins, 1964) and counted. Endospore attachment at harvest was recorded on 20 J2 for each year of the study. Pod and root galls were counted individually in 1994 and were indexed on a 0-to-10 scale based on the percentage of roots or pods galled in 1995 (0 = no galls; 1 = 1 to 10% galled; 2 = 11 to 20% galled; . . ., 10 = 91 to 100% galled) (Barker et al., 1986). Eggs were extracted from roots using 0.5% sodium hypochlorite (Hussey and Barker, 1973), and the number of eggs per root system was determined by counting a 1-ml aliquot from a 20-ml suspension. Dry weights of foliage and pods were determined after drying the tissue at 60 °C for 1 week.

Before data analysis, rating data were transformed with arcsin $(\sqrt{\chi/10})$ and nematode densities and numbers of eggs per root system with $\log_{10}(\chi + 1)$. Means presented in the text were back-transformed. The two-way analysis of variance was used to determine the effects of each factor and the interactions. Regression analysis also was used to establish the relationships between the means and the levels of each main factor. An equation was provided if the correlation was significant at $P \leq 0.05$. Percentage suppression per each 10% increment of the initial *P. penetrans* infestation level was calculated by $b/a \times 10 \times 100\%$, where a is the intercept of the linear equation and b is the slope.

The percentage of suppressiveness was compared with that of a related, previously reported experiment of using soil application of *P. penetrans* endospores (Chen et al., 1996b). Suppression mechanisms of *P. penetrans* against root-knot nematodes are dis-

cussed as the relative effectiveness of initial infestation levels of *P. penetrans* at planting verses that of subsequent generations of nematodes that occur during the cropping season.

Results

Results were similar in 1994 and 1995 (Tables 1,2). In peanut microplots without *P. penetrans* the nematode inoculum density was positively correlated with the number of eggs per root system, J2 per 100 cm^3 soil at harvest, galls on roots or root galling index, and galls on pods or pod galling index in both 1994 and 1995, and root weight in 1995 ($P \leq 0.05$) (Tables 1,2). In peanut microplots infested with *P. penetrans,* their levels were inversely correlated with the number of eggs per root system, galls on roots and galls on pods in 1994, and the number of eggs per root system, J2 per 100 cm^3 soil at harvest, root galling index, and pod galling index in 1995 ($P \leq 0.05$) (Tables 1,2).

Based on the regression parameters, the number of eggs per root system, galls on roots, and galls on pods were suppressed by 7.8%, 7.0%, and 8.7%, respectively, per each 10% increment of *P. penetrans* infestation level in 1994 (Table 1). The same 10% increment of *P. penetrans* infestation level suppressed the number of eggs per root system, J2 per 100 cm^3 soil, root gall index, and pod gall index by 9.4%, 8.8%, 8.5%, and 8.0% in 1995, respectively (Table 2).

Table 1. Effects of *Pasteuria penetrans* on population densities and galling by *Meloidogyne arenaria* race 1 and on peanut yield per microplot in 1994.[a]

Treatment	Levels of treatment	Eggs per root system	J2 per 100 cm^3 soil	Galls per root system	Number of galls on pods	Root weight (g)	Pod weight (g)	Foliage weight (g)
Nematode (J2/plot)	0	9 a	2 a	0.2 a	0.2 a	3.4	40	80
	40	22 a	6 a	7.8 a	1.1 a	5.9	66	145
	200	57 a	7 ab	25.3 a	0.5 a	4.0	42	88
	1,000	229 a	22 b	38.2 a	0.9 a	4.3	46	96
	5,000	697 b	244 c	121.3 b	3.7 b	10.4	38	95
	25,000	1,290 c	489 c	186.0 c	6.7 c	6.9	35	82
Regression parameters	a	0	0	0	0			
	b	0.0553	0.021	0.00814	0.000287			
	r	0.91	0.95	0.84	0.88			
	t-test	**	**	*	*			
P. penetrans (%)	0	653	18	98.3 a	4.3 a	6.1	47	98
	20	559	38	83.8 ab	2.8 ab	5.8	53	113
	40	332	34	62.1 ab	1.8 b	8.9	42	93
	60	353	22	61.8 bc	2.1 ab	5.0	42	99
	80	283	32	45.5 bc	1.6 b	5.3	44	99
	100	125	19	27.3 c	0.5 b	3.8	38	82
Regression parameters	a	630.4		96.7	3.78			
	b	−4.92		−0.67	−0.0329			
	r	0.96		0.98	0.93			
	t-test	**		***	**			
ANOVA	Block	NS	NS	NS	*	NS	NS	NS
	Nematode	***	***	***	***	NS	NS	NS
	P. penetrans	NS	NS	*	*	NS	NS	NS
	Interaction	NS	NS	NS	NS	NS	NS	NS

[a] This was a six × six factorial experiment. Each mean of the main effect averaged from 24 microplots. Data of numbers of eggs per root system and numbers of the second-stage juveniles (J2) per 100 cm^3 soil at harvest were transformed with $\log_{10}(x + 1)$ before subjected to ANOVA and presented as back-transformed. Means followed by different letters are different ($P \leq 0.05$) according to Duncan's multiple-range test. Regression equations are linear ($Y = a + bx$). The *t*-test was applied to *r*. *, **, *** represent $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively; NS = not significant at $P > 0.05$. Root weight, pod weight, and foliage weight were the fresh weight (g) per microplot and could be transformed to dry weight by multiplying 0.26, 0.57, and 0.26, respectively.

TABLE 2. Effects of *Pasteuria penetrans* on population densities and galling by *Meloidogyne arenaria* race 1 and on peanut yield per microplot in 1995.[a]

Treatment	Levels of treatment	Eggs per root system	J2 per 100 cm^3 soil	Root gall index	Pod gall index	Root weight (g)	Pod weight (g)	Foliage weight (g)
Nematode (J2/plot)	0	5 a	1 a	0 a	0 a	7.9 a	98	308
	40	33 b	3 a	0.2 ab	0.3 a	8.2 a	109	333
	200	84 b	3 a	0.2 ab	0.2 a	8.0 a	96	277
	1,000	561 c	19 b	0.6 b	0.1 a	6.8 a	60	210
	5,000	4,830 d	274 c	4.8 c	3.0 b	14.7 b	88	304
	20,000	14,420 d	740 c	7.2 d	5.4 c	18.6 b	67	253
Regression parameters	a	0	0	0	0	8.26		
	b	0.592	0.038	0.000396	0.000289	0.000557		
	r	0.989	0.991	0.90	0.944	0.92		
	t-test	***	***	**	**	**		
P. penetrans (%)	0	804	46 ab	3.1 a	1.8 a	12.3	83	276
	20	675	53 a	2.2 ab	1.4 a	11.7	75	258
	40	281	11 bc	1.1 bc	1.1 ab	11.7	104	350
	60	144	26 abc	1.8 ab	1.0 ab	11.2	99	338
	80	134	13 bc	1.1 bc	0.7 ab	11.5	105	306
	100	217	9 c	0.3 c	0.3 b	5.9	53	158
Regression parameters	a	711.2	47.0	2.79	1.74			
	b	−6.71	−0.414	−0.0237	−0.0139			
	r	0.87	0.81	0.90	0.99			
	t-test	*	*	*	***			
ANOVA	Block	NS	NS	NS	NS	NS	NS	NS
	Nematode	***	***	***	***	***	NS	NS
	P. penetrans	NS	*	***	*	NS	NS	NS
	Interaction	NS	NS	NS	NS	NS	NS	NS

[a] This was a six × six factorial experiment. Each mean of the main effect averaged from 24 microplots. Data of numbers of eggs per root system and numbers of the second-stage juveniles (J2) per 100 cm^3 soil at harvest were transformed with $\log_{10}(z + 1)$ before analysis. The root gall index and pod gall index were graded from 0 to 10 based on the percentage of roots and pods galled (0 = no galls; 1 = 1 to 10% galled; 2 = 11 to 20% galled; . . . , 10 = 91 to 100% galled). The grading data were transformed with arcsin $(\sqrt{x/10})$ before subjected to ANOVA and presented as back-transformed. Means followed by different letters are different ($P \leq 0.05$) according to Duncan's multiple-range test. Regression equations are linear ($Y = a + bx$). The *t*-test was applied to *r*. *, **, *** represent $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$, respectively; NS = not significant at $P > 0.05$. Root weight, pod weight, and foliage weight were the fresh weight (g) per microplot and could be transformed to dry weight by multiplying 0.30, 0.59, and 0.26, respectively.

Based on two-way analysis of variance, the effects of nematode treatments were significant for the eggs per root system, J2 per 100 cm^3 soil at harvest, galls on roots or root galling index, and galls on pods or pod galling index in both 1994 and 1995 ($P \leq 0.05$). The effects of *P. penetrans* treatments were significant for the number of galls on roots and on pods in 1994, and J2 per 100 cm^3 soil at harvest, root galling index, and pod galling index in 1995 ($P \leq 0.05$). There were no statistical interaction effects between nematode treatments and *P. penetrans* treatments in either year ($P > 0.05$).

Except for peanut root weight in 1995, peanut pod weight, root weight, and foliage weight were not affected by *P. penetrans* or nematode treatments in 1994 or 1995 ($P > 0.05$) (Tables 1,2). The peanut root weight correlated with the nematode inoculum levels in 1995 ($P \leq 0.05$) (Table 2). The increased root weight with increasing nematode levels in 1995 was due to root galls that contained more root tissue than healthy roots (Table 2).

The endospore attachment of J2 at harvest was generally low for both years, averaging over all microplots only 0.05 endospores/J2 in 1994 and 0.14 endospores/J2 in 1995. The percentage of J2 with at least one endospore attached was 3.3% in 1994 and 7.6% in 1995.

Discussion

Pasteuria penetrans suppressed root-knot nematodes on peanut in both years of the study. Suppression was proportional to *P. penetrans* infestation levels, regardless of the initial population densities of root-knot nematodes. Therefore, the suppression was independent of the nematode population density. This suggested that *P. penetrans* was a reliable biological control agent for managing root-knot nematodes at various population densities.

Neither the nematode inoculum levels nor the *P. penetrans* infestation levels affected peanut growth and yields. The yields, determined by weight, were not a good index because diseased tissue tended to be heavier than healthy tissue. The quality of peanut pods, here presumed to be inversely proportional to the number of galls on pods and pod gall index, was improved by increasing *P. penetrans* infestation levels, but the quality deteriorated with increasing nematode inoculum levels. In a related experiment, both yield increase and quality improvement occurred when endospores of *P. penetrans* were added directly to microplot soil (Chen et al., 1996b).

The initial infestation level of *P. penetrans* was an important factor in the suppression of root-knot nematodes on peanut. The suppressiveness correlated well with the initial *P. penetrans* levels. *Pasteuria penetrans* did not build up to a suppressive population density in soil during one tested cropping season because most endospore-filled females were removed from the microplots along with root systems, when much of the root system was harvested. This was verified by the low endospore attachment of J2 at harvest in both years. Moreover, the chance of *P. penetrans* infection of subsequent generations of nematodes during the cropping season was probably negligible, either because of the minor endospore build-up in soil or because J2 that hatched from egg masses on root surfaces quickly enter adjacent roots without being exposed to endospores in soil (Stirling, 1984). Reductions of number of galls on pods and reductions in the pod gall index were 8.0% to 8.7% per 10% increment of initial *P. penetrans* levels. Similar results were obtained in a previously reported study of soil application of *P. penetrans* endospores (Chen et al., 1996b), where the reduction of pod galls was 8.4% to 10.8% per 10% increment of endospore attachment before the season (Chen et al., 1996b). When soil is treated with *P. penetrans* endospores, the chance of bacterial infection of subsequent generations of nematodes during the cropping season should be anticipated. Reduction of pod galls in an experiment using soil application of endospores (Chen et al., 1996b), however, was only slightly higher than that in the present study. Thus, the suppressiveness contributed from the *P. penetrans* infection on subsequent generations of nematodes was minor. Again, this demonstrated that the initial infestation level of *P. penetrans* was critical for the biological control of *M. arenaria.* Supposedly the increase in suppressiveness observed in the soil endospore application experiment (Chen et al., 1996b) was contributed to the bacterial infection of subsequent generations of nematodes during the cropping season. Thus, we can calculate that the initial infestation levels contributed 81% to 95% of the total suppressiveness of the pod galls and that the infection of subsequent generations of nematodes contributed only 5% to 19%. The limited importance of the infection of subsequent generations of nematodes might result from the short migration distances for J2 that hatched on root surfaces to invade nearby roots (Stirling, 1984).

The population build-up of root-knot nematodes decreased when initial infestation level of *P. penetrans* was increased. Numbers of eggs per root system in both years and the number of J2 in soil in 1995 were inversely correlated with the initial infestation level of *P. penetrans.* Initial infestation of *P. penetrans* reduced the numbers of J2 invading roots (Stirling, 1984). The nematode reproduction was blocked after the endospore-encumbered J2 entered roots and the bacterium developed in the nematode pseudocoel (Bird, 1986). Because of the im-

portance of the initial *P. penetrans* levels, practices such as solarization, nematicide incorporation, and other integrated pest management measures might be optimized to increase initial infestation levels of *P. penetrans* for biological control of root-knot nematodes (Brown and Nordmeyer, 1985; Maheswari et al., 1987; Walker and Wachtel, 1988).

Behavior and efficacy of *P. penetrans* do not always conform to general biological control concepts. The most successful examples of biological control of pests have been against insects of perennial crops (Bennett, 1974). Perennial crops provide a stable habitat for both insect pests and their natural enemies. Vegetational diversity also is important for encouragement and retention of effective populations of natural enemies for insect pests (Bennett, 1974). *Pasteuria penetrans,* however, has successfully suppressed root-knot nematodes on both annual and perennial crops (Chen et al., 1996b; Stirling, 1984). Monoculture and rotation with non-host crops for root-knot nematode management has no obvious adverse effects on *P. penetrans* (Dickson et al., 1994). Population densities of *P. penetrans* increased in monoculture over years (Dickson et al., 1994). Several adaptations of *P. penetrans* may account for the reduced impact of crop habitat and vegetational diversity on its effectiveness as a biological control agent. *Pasteuria penetrans* produces endospores that are dormant in soil. Although we do not know exactly how many years *P. penetrans* endospores can survive in soil, similar endospores of other bacteria probably survive in soil for more than 1,000 years (Logan, 1994). This is different from the natural enemies of insects that usually require a constant food supply to maintain their population densities. Endospores of *P. penetrans* are highly resistant to heat, desiccation, nematicides, and other adverse environmental conditions (Stirling, 1991). A short period of absence of host crops has little effect on endospore survival but may force J2 to move long distances in soil to locate host roots and facilitate attachment of endospores to J2 (Stirling, 1984). Endospore dormancy in soil and endospore resistance to different environments may be the key traits attributing to success in suppressing root-knot nematodes on both annual and perennial crops under various ecological conditions and crop regimes.

Literature Cited

Barker, K. R., J. L. Townshend, G. W. Bird, I. J. Thomason, and D. W. Dickson. 1986. Determining nematode population responses to control agents. Pp. 283–296 *in* K. D. Hickey, ed. Methods for evaluating pesticides for control of plant pathogens. St. Paul, MN: APS Press.

Bennett, F. D. 1974. Criteria for determination of candidate hosts and for selection of biotic agents. Pp. 87–96 *in* F. G. Maxwell and F. A. Harris, eds. Proceeding of the summer institute on biological control of plant insects and diseases. Jackson, MI: University Press of Mississippi.

Bird, A. F. 1986. The influence of the actinomycete, *Pasteuria penetrans,* on the host-parasite relationship of the plant-parasitic nematode, *Meloidogyne javanica.* Parasitology 93:571–580.

Brown, S. M., J. L. Kepner, and G. C. Smart, Jr. 1985. Increased crop yields following application of *Bacillus penetrans* to field plots infested with *Meloidogyne incognita.* Soil Biology and Biochemistry 17:483–486.

Brown, S. M., and D. Nordmeyer. 1985. Synergistic reduction in root galling by *Meloidogyne javanica* with *Pasteuria penetrans* and nematicides. Revue de Nématologie 8:285–286.

Brown, S. M., and G. C. Smart, Jr. 1985. Root penetration by *Meloidogyne incognita* juveniles infected with *Bacillus penetrans.* Journal of Nematology 17:123–126.

Chen, Z. X., D. W. Dickson, and T. E. Hewlett. 1996a. Quantification of endospore concentrations of *Pasteuria penetrans* in tomato root material. Journal of Nematology 28:50–55.

Chen, Z. X., D. W. Dickson, R. McSorley, D. J. Mitchell, and T. E. Hewlett. 1996b. Suppression of *Meloidogyne arenaria* race 1 by soil application of endospores of *Pasteuria penetrans.* Journal of Nematology 28: 159–168.

Cobb, N. A. 1906. Fungus maladies of the sugar cane, with notes on associated insects and nematodes, 2nd ed. Hawaiian Sugar Planters Association Bulletin No. 5. Honolulu, HI: Hawaiian Sugar Planters Association.

De Leij, F. A. A. M., K. G. Davies, and B. R. Kerry. 1992. The use of *Verticillium chlamydosporium* Goddard and *Pasteuria penetrans* (Thorne) Sayre & Starr alone and in combination to control *Meloidogyne incognita* on tomato plants. Fundamental and Applied Nematology 15:235–242.

Dickson, D. W., M. Oostendorp, R. M. Giblin-Davis, and D. J. Mitchell. 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. Hampshire, UK: Intercept.

Dube, B., and G. C. Smart, Jr. 1987. Biological con-

trol of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. Journal of Nematology 19:222–227.

Hewlett, T. E., and D. W. Dickson. 1993. A centrifugation method for attaching endospores of *Pasteuria* spp. to nematodes. Journal of Nematology 25:785–788.

Hussey, R. S. 1971. A technique for obtaining quantities of living *Meloidogyne* females. Journal of Nematology 3:99–100.

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

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

Logan, N. A. 1994. Bacterial systematics. London: Blackwell Scientific Publications.

Maheswari, T. U., A. Mani, and P. K. Rao. 1987. Combined efficacy of the bacterial spore parasite, *Pasteuria penetrans* (Thorne, 1940), and nematicides in the control of *Meloidogyne javanica* on tomato. Journal of Biological Control 1:53–57.

Mankau, R. 1975. *Bacillus penetrans* n. comb. causing a virulent disease of plant-parasitic nematodes. Journal of Invertebrate Pathology 26:333–339.

McSorley, R., D. W. Dickson, E. M. Candanedo-Lay, T. E. Hewlett, and J. J. Frederick. 1992. Damage functions for *Meloidogyne arenaria* on peanut. Journal of Nematology 24:193–198.

Metchnikoff, E. 1888. *Pasteuria ramosa,* un representant des bacteries a division longitudinale. Annales de l'Institut Pasteur 2:165–170.

Micoletzky, H. 1925. Die Freilebenden Susswasser und Moornematoden Danemarks. Kobenhavn: Host & Son.

Oostendorp, M., D. W. Dickson, and D. J. Mitchell. 1990. Host range and ecology of isolates of *Pasteuria* spp. from the southeastern United States. Journal of Nematology 22:525–531.

Oostendorp, M., D. W. Dickson, and D. J. Mitchell. 1991. Population development of *Pasteuria penetrans* on *Meloidogyne arenaria*. Journal of Nematology 23:58–64.

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

Sayre, R. M., and M. P. Starr. 1989. Genus *Pasteuria* Metchnikoff, 1888. Pp. 2601–2615 *in* S. T. Williams, M. E. Sharpe, and J. G. Holt, eds. Bergey's manual of systematic bacteriology. Baltimore, MD: Williams and Wilkins.

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

Stirling, G. R. 1991. Biological control of plant parasitic nematodes: Progress, problems, and prospects. Wallingford, UK: CAB International.

Thorne, G. 1940. *Duboscqia penetrans* n. sp. (Sporozoa, Microsporidia, Nosematidae), a parasite of the nematode *Pratylenchus pratensis* (de Man) Filipjev. Proceedings of Helminthology Society Washington 7:51–53.

Walker, G. E., and M. F. Wachtel. 1988. The influence of soil solarization and non-fumigant nematicides on infection of *Meloidogyne javanica* by *Pasteuria penetrans*. Nematologica 34:477–483.

Whitty, E. B., W. L. Curry, T. C. Skinner, D. S. Harrison, R. P. Cromewell, F. A. Johnson, T. A. Kucharek, and R. A. Dunn. 1975. Peanut production guide. Circular 145F, Institute of Food and Agricultural Sciences, University of Florida, Gainesville.