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Population Development of *Pasteuria penetrans* on *Meloidogyne arenaria*[1]

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Abstract: A microplot study on the influence of cropping sequences with peanut in summer and bare fallowed or cover crops of rye or vetch in winter on the population development of *Pasteuria penetrans* was initiated in the spring of 1987. The number of spores of *P. penetrans* attached per second-stage juvenile of *Meloidogyne arenaria* race 1 increased from 0.11 in the fall of 1987 to 7.6, 8.6, and 3.6 in the fall of 1989 in the rye, vetch, and fallowed plots, respectively. Higher ($P \leq 0.05$) levels of *P. penetrans* occurred in the rye and vetch plots than in fallowed plots. No influence of *P. penetrans* on peanut, rye, or vetch yield was observed in 1987 and 1988, but in 1989 peanut yield was 64% higher ($P \leq 0.05$) in plots infested with *P. penetrans* than in plots without *P. penetrans*. Numbers of *M. arenaria* in plots without *P. penetrans* were influenced by the cropping sequences in the spring of 1988 and 1989 but not in the fall following the peanut crop. In the spring the plots with rye had the lowest nematode numbers in either year ($P \leq 0.05$). Nematode numbers were lower ($P \leq 0.05$) in plots with *P. penetrans* than in plots without *P. penetrans* in the spring of 1989 (vetch) and the fall of 1989 (rye, vetch, and fallowed).

Key words: Arachis hypogeae, biological control, *Meloidogyne arenaria*, microplot, *Pasteuria penetrans*, peanut, peanut root-knot nematode, population dynamics.

The endospore-forming parasite of root-knot nematodes, *Pasteuria penetrans* (Thorne) Sayre & Starr, is a promising biological control agent. It was observed in nematode suppressive soils (1,10, Dickson, unpubl.) and was successfully tested in greenhouse (5) and field microplot experiments (3,13). However, *Pasteuria penetrans* has not been grown successfully on artificial media. It must be reared on nematodes in the greenhouse (14) or in a nematode-excised root system (16). Neither procedure provides for the production of sufficient inoculum for large-scale applications.

The population dynamics of the antagonist are not yet understood. It has been associated with relatively high (11) and with low (1,15) nematode population densities. A field microplot experiment was established to determine whether the cropping sequence of peanut (*Arachis hypogeae* L.) and winter cover crops influences the infection of *Meloidogyne arenaria* (Neal) Chitwood by *P. penetrans* and whether the presence of the antagonist improves peanut growth over time.

Materials and Methods

The population densities of *P. penetrans* and of *M. arenaria* race 1 with and without the antagonist were determined from spring 1987 to fall 1989 in microplots. The experiment was a split-plot design with randomized main plots. The main factor was the inoculum, which consisted of three treatments: no nematodes or bacteria, *M. arenaria* alone, and *M. arenaria* plus *P. penetrans*. The three summer–winter crop sequences of peanut–rye (*Secale cereale* L.), peanut–vetch (*Vicia villosa* Roth), and peanut–bare fallow were subplots. Each combination of inoculum and crop sequence was replicated 10 times.

Ninety microplots were arranged in 10 rows of nine plots with a distance of 1.5 m between plots in an Arredondo fine sand (94% sand, 1% silt, 5% clay; pH 5.6, < 1% organic matter). The 76-cm-d microplots were encircled with 60-cm-wide fiberglass sheets inserted 50 cm deep into the soil (8). The plots were treated with methyl bromide (98% a.i.) at 977 kg/ha under 3-mil polyethylene plastic 3 months before inoculation. No plant-parasitic nematodes

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were detected in soil sampled before inoculation.

A local population of *M. arenaria* race 1 that is maintained in our culture collection in the greenhouse on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) and an isolate of *P. penetrans* designated P-20, which was isolated from *M. arenaria* race 1 from a peanut field in Levy County, Florida, were used for the experiment. *Pasteuria penetrans* was harvested from dried, nematode-infected peanut hulls collected from the field. They were incubated in Pectinol 59L (Genencor, South San Francisco, California) overnight and washed on a 600-μm sieve nested in a 75-μm sieve. Spore-filled females were selected under a stereoscope and ground in water in a glass-tissue grinder. The resulting spore suspension was mixed with a suspension of 30,000 freshly hatched *M. arenaria* second-stage juveniles (J2) and agitated on a shaker. After about 3 hours, 80% of the J2 had one or more spores attached and each of 30 tomato plants was inoculated with 1,000 of these J2. Simultaneously, each of a second set of 30 tomato plants was inoculated with 1,000 J2 of the same population but not exposed to *P. penetrans*. Thirty noninfected tomato plants were grown for the control treatment. After 85 days the soil and roots of each set of plants were mixed, and 800 cm^3 of the soil preparation was placed in a hole (10 × 20 cm) in the center of each microplot. The inoculum levels were 22,000 eggs plus 52,000 J2 per plot without *P. penetrans* and 14,000 eggs plus 80,000 J2 per plot with *P. penetrans*. Forty percent of the latter J2 had one or more adhering spores.

Seeds of peanut cv. Florunner were dusted in a commercial *Rhizobium* sp. preparation, and four pairs of seeds were placed in 4-cm-deep holes in equally spaced patterns in each microplot on 2 June 1987, 10 May 1988, and 18 April 1989. Each plot was covered with a 2.5-cm-mesh wire screen to protect seeds and emerging plants from birds. After emergence, one of each pair of the seedlings was removed. A 90-cm-high wire-mesh fence was placed around each plot to confine the peanut plants. Climatological data were recorded daily by a weather station adjacent to the plot area.

Rye (2.6 g seed/plot) and vetch (1.3 g seed/plot) were broadcast seeded as cover crops in mid-November each year, after harvest of peanuts. Vetch and peanut are hosts for *M. arenaria* race 1, but rye is not. Fertilization and other agronomic practices for peanut, rye, and vetch followed recommended practices. The plots were weeded, irrigated by overhead sprinklers, and sprayed for pest control as needed. The soil in each plot was mixed between crops. In the fall of 1988, the control plots without *M. arenaria* or *P. penetrans* were retreated with methyl bromide because contamination by *M. arenaria* had occurred in several plots.

One peanut plant from each plot was destructively sampled 38–42 days after planting to determine the number of nematodes that penetrated the roots. Roots were washed, weighed, stained (2), and macerated in 250 ml water in a blender for 20 seconds. The number of nematodes in a 50-ml aliquot of each sample was counted.

The number of *M. arenaria* J2 per 125 cm^3 soil was estimated at harvest of peanut (128–136 days after planting) and each of the winter cover crops. Each sample consisted of a composite of ten 2.5-cm-d cores per plot taken 25 cm deep in the root zone. The soil was mixed and processed by sugar flotation centrifugation (7). The number of *P. penetrans* spores attached per J2 was counted on 20 specimens from each plot with an inverted microscope at 450×. The nematodes in the roots were stained and counted.

Data were subjected to analysis of variance. If the main plot or the subplot effects were significant ($P \leq 0.05$), means were separated by Duncan's multiple-range test. If a significant main plot × subplot interaction ($P \leq 0.05$) was observed, main plot means for each subplot and subplot means for each main plot were separated by Duncan's multiple-range test using the error term formulas given by Cochran and Cox (4).

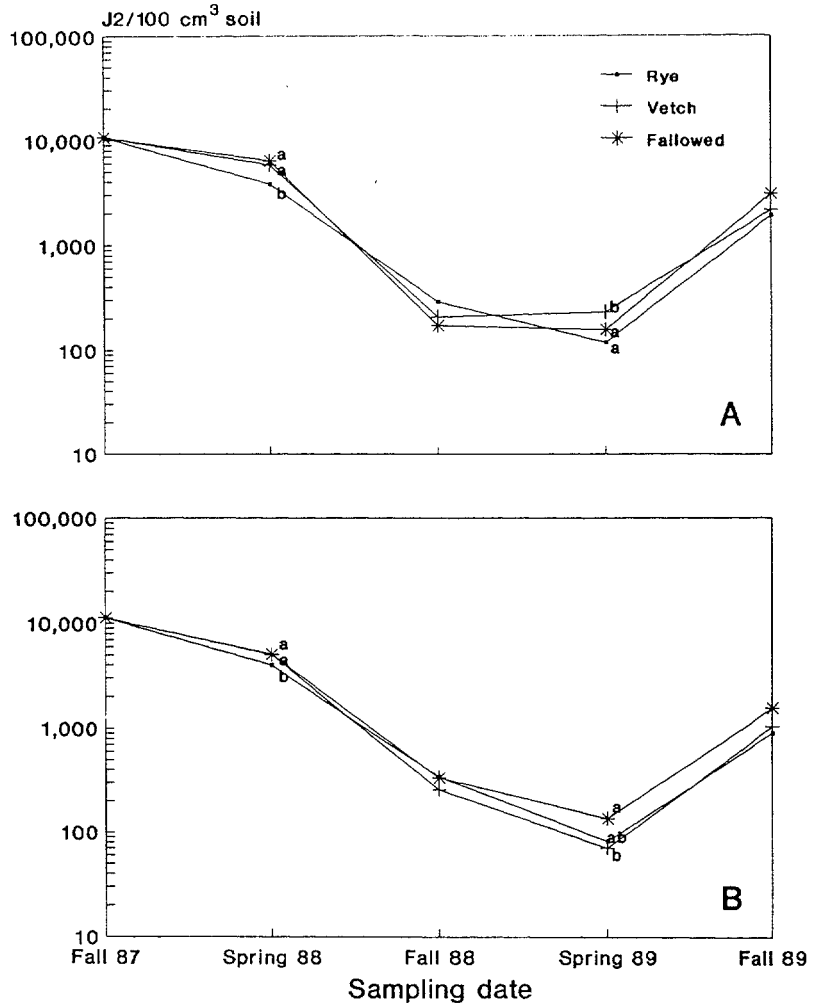

FIG. 1. Number of second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 in soil of microplots planted with peanut in summer and with rye or vetch, or bare fallowed in winter. A) Without *Pasteuria penetrans* isolate P-20. B) With *P. penetrans* isolate P-20. Means of the cropping sequences at individual sampling dates followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

RESULTS

Meloidogyne arenaria race 1 became well established in the microplots under the first peanut crop. The population levels were > 10,000 J2/100 cm³ soil at the end of the 1987 season in plots with *M. arenaria* alone (Fig. 1A) or *M. arenaria* plus *P. penetrans* (Fig. 1B). The peanut yield was greatly reduced ($P \leq 0.05$) in plots containing nematodes compared with the nematode-free control (Table 1). Penetration of peanut roots by *M. arenaria* did not differ between plots with or without *P. penetrans.*

In spring 1988, at harvest of the winter cover crops, the vetch crop was severely damaged ($P < 0.05$) by *M. arenaria* in plots with or without *P. penetrans* (Table 1). The nonhost rye crop was not affected by *M. arenaria.* In 1988 nematode penetration into roots of peanut was reduced ($P \leq 0.05$) 38 days after planting in *P. penetrans*-infested plots compared with plots of *M. arenaria* alone. At harvest, no differences ($P \leq 0.05$) in nematode penetration between these treatments were observed, and the peanut yields were reduced ($P \leq 0.05$) below those in the control in both treatments.

In spring 1989, growth of the cover crops

TABLE 1. Weight of cover crop foliage, yield of peanut crop, and number of nematodes in peanut roots in microplots without nematodes (control), with *Meloidogyne arenaria* (Ma), and with *M. arenaria* plus *Pasteuria penetrans* (Ma + Pp).

Cover crop	Treatment†	Cover crop foliage weight (g/plot)	Peanut Yield (g/plot)	Peanut Nematodes/g root 38–42 days	Peanut Nematodes/g root 129–136 days
		1987			
	Control		252 a	0 b	0 b
	Ma		65 b	20 a	71 a
	Ma + Pp		55 b	29 a	76 a
		1988			
Rye	Control	728 a	208 a	0 c	4 b
	Ma	647 a	7 b	500 a	460 a
	Ma + Pp	737 a	8 b	226 b	483 a
Vetch	Control	837 a	193 a	32 c	48 b
	Ma	79 b	2 b	321 a	324 a
	Ma + Pp	61 b	15 b	197 b	420 a
Fallow	Control	0	200 a	0 c	0 b
	Ma	0	2 b	371 a	412 a
	Ma + Pp	0	5 b	211 b	468 a
		1989			
Rye	Control	515 a	296 a	0 b	0 b
	Ma	389 b	91 c	85 a	176 a
	Ma + Pp	470 ab	183 b	7 b	172 a
Vetch	Control	265 a	246 a	0 b	0 b
	Ma	138 b	62 c	144 a	202 a
	Ma + Pp	213 ab	145 b	22 b	157 a
Fallow	Control	0	243 a	0 b	0 b
	Ma	0	147 c	55 a	180 a
	Ma + Pp	0	164 b	21 b	168 a

Means of the main plots of each year followed by the same letter are not different according to Duncan's multiple-range test ($P \leq 0.05$). There were no differences among the subplots (crop sequence) or main plot × subplot interactions ($P \leq 0.05$). Cover crop foliage was weighed fresh at harvest, and weights were compared separately for each crop.

† In the fall of 1988 all control plots were retreated with methyl bromide because 26% of the control plots were contaminated with *M. arenaria*.

TABLE 2. Percentage of second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 with spores of *Pasteuria penetrans* attached to their cuticle in soil samples from microplots planted with peanut in summer and with rye or vetch or bare fallowed in winter.

Cropping sequence	Number of spores attached/J2 0	1–4	5–9	10–19	≥ 20
	Fall 87				
	93	7	0	0	0
	Spring 88				
Rye	89	10	0	0	< 1
Vetch	89	10	< 1	0	0
Fallow	93	6	< 1	0	0
	Fall 88				
Rye	75	22	2	1	0
Vetch	70	26	3	1	0
Fallow	82	17	1	0	0
	Spring 89				
Rye	70	29	1	< 1	0
Vetch	66	28	4	0	2
Fallow	68	30	1	0	< 1
	Fall 89				
Rye	28	25	14	8	25
Vetch	20	28	12	14	26
Fallow	43	31	11	8	7

Values in each row were based on 10 replicates, 20 J2 observed per replication.

was suppressed ($P \leq 0.05$) in the plots with *M. arenaria* alone relative to the control without nematodes (Table 1). Plant growth in the *P. penetrans*-infested plots did not differ from that in the other treatments. In 1989, 42 days after planting, penetration of peanut roots by *M. arenaria* in *P. penetrans*-infested plots was 8 (rye), 15 (vetch), and 38% (fallow) of that in plots with *M. arenaria* alone. At harvest, however, root penetration was not influenced by *P. penetrans*, but peanut yield was higher ($P \leq 0.05$) in *P. penetrans*-infested plots than in plots with *M. arenaria* alone.

In the spring, the population levels of *M. arenaria* were influenced by the cropping sequences (Fig. 1A and B). Lower numbers of J2 were recovered from rye than from vetch plots with nematodes alone (Fig. 1A). In the fall after harvest of peanut, no influence of the winter crops on nematode levels was observed. The nematode soil population density in plots with nematodes alone dropped continuously from more than 10,000/100 cm^3 soil in fall 1987 to about 170/100 cm^3 soil (average of the cropping sequences) in spring 1989 and increased again in the summer 1989 (Fig. 1A).

In the *P. penetrans*-infested plots (Fig. 1B), the nematode numbers showed the same overall pattern as in plots with *M. arenaria* alone (Fig. 1A). In spring 1989, fewer nematodes were found after vetch than after fallow (71 vs. 138/100 cm^3 soil) (Fig. 1B). Nematode numbers were lower in soil infested with *P. penetrans* when compared

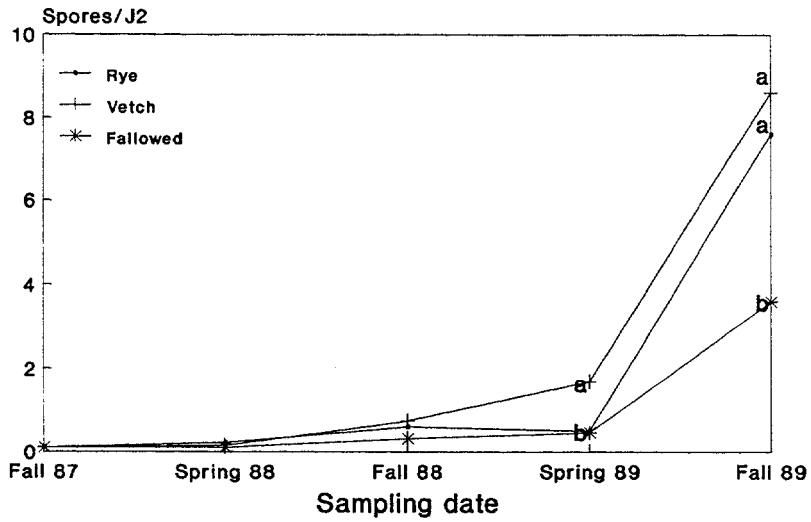

FIG. 2. Number of spores of *Pasteuria penetrans* isolate P-20 attached to second-stage juveniles (J2) of *Meloidogyne arenaria* race 1 in microplots planted with peanut in summer and with rye or vetch, or bare fallowed in winter. Means of the cropping sequences at individual sampling dates followed by the same letter are not significantly different according to Duncan's multiple-range test ($P \leq 0.05$).

with soil without *P. penetrans* in the vetch plots in spring 1989 (71 vs. 228/100 cm^3 soil) and averaged across rye, vetch, and fallowed plots in fall 1989 (1,178 vs. 2,383/100 cm^3 soil) ($P \leq 0.05$).

Numbers of spores of *P. penetrans* per J2 in *P. penetrans*-infested plots increased from 0.11 in fall 1987 to 7.6, 8.6, and 3.6 in fall 1989 in the rye, vetch, and bare fallowed cropping sequences, respectively (Fig. 2). Nematodes in vetch plots had more spores attached ($P \leq 0.05$) than did those in fallowed plots in spring and fall 1989, whereas nematodes in rye plots had more spores attached than did those in fallowed plots in fall 1989 ($P \leq 0.05$). Most of the nematodes in soil were still free of spores in spring 1989 (Table 2). In fall 1989, however, a rapid increase of *P. penetrans* was observed and up to 80% of the nematodes (vetch plots) carried one or more spores.

In the plots with *M. arenaria* alone, no *P. penetrans* spores were observed except low numbers (< 0.1 spore/J2) in two plots at the last sampling date in fall 1989. Pathogens of nematodes other than *P. penetrans* were not noticed in the soil samples.

DISCUSSION

The number of *P. penetrans* spores adhering to the J2 in soil increased continuously over the 3-year experiment and was influenced by the cropping sequence. Despite the high temperature requirements of *P. penetrans* (12), spore attachment increased during the winter season 1988–89 under vetch, a good host for *M. arenaria*. The mean soil temperature 10 cm deep for December–February 1987–88 was 14.1 and 16.9 C for 1988–89. Under rye, a poor host for *M. arenaria*, and under fallow, no increases in the densities of *P. penetrans* were observed in the winter season. Therefore, the increase under vetch in the winter of 1988–89 does not seem to be caused by cultural practices or slow release of spores from degrading peanut roots, but by the presence of a host plant. In the rye plots, the number of spores of *P. penetrans* adhering to *M. arenaria* increased greatly in the summer of 1989 and reached higher levels than in the fallowed plots in the fall of 1989. The reasons for the higher increase on rye are not understood, but may be related to an influence of the rye on microbial population balances, soil structure, organic matter, or other unknown factors.

Differences in the numbers of *M. arenaria* in plots without *P. penetrans* among the three cropping sequences were observed

only in the spring of each year, and not in the fall. This indicates that the summer crop, which was peanut in all plots, had a stronger influence on the nematode population density than did the cover crops or the fallowing in winter.

We first observed lower numbers of nematodes in the *P. penetrans*-infested plots than in the plots with *M. arenaria* alone in 1989. In spring 1989, nematode density in the soil was reduced only in plots that had been planted with vetch. In these plots, 30% of the juveniles had one or more spores attached at the preceding sampling date in fall 1988. This was equivalent to 0.75 spores/J2. It was reported previously (13) that 73% of juveniles with one *P. penetrans* spore attached developed into egg-producing females. Therefore, we expected a much smaller reduction in the nematode population density than we observed. This may indicate a high virulence of isolate P-20 or a significant increase in spore attachment between the fall sampling date and the planting of the succeeding crop. In rye and fallowed plots infested with *P. penetrans,* reductions of the nematode soil population densities were first observed in fall 1989, compared with plots without *P. penetrans.* Again, 30% of the J2 was found to have spores attached at the preceding sampling date in spring 1989.

A reduction of nematode penetration of the roots occurred in pot experiments only if high numbers of *P. penetrans* spores attached to the nematode cuticle (13). In 1988 and 1989, we observed reduced numbers of nematodes in the peanut roots 38–42 days after planting in all three cropping sequences, although the nematode numbers in soil were similar to those in plots with *M. arenaria* alone (except the vetch plots in 1989) and only a low percentage of the J2 had more than 20 spores attached. A spore load of at least 20 is thought to be necessary to reduce nematode migration (13). This, again, may be explained by an increase in the attachment rate between the sampling dates and the actual crop planting dates. In addition, a nematode may have to migrate longer distances before root penetration under field conditions than in pots. Therefore, low numbers of attached spores may have a more detrimental effect in the field than in pots.

Peanut and vetch yields were substantially suppressed by *M. arenaria.* In spring 1988, numbers of *M. arenaria* were high. The yield of the succeeding peanut crop was greatly suppressed and nematode numbers in the fall were lower than in the spring. In 1989, with lower initial nematode numbers than in 1988, peanut yields improved and the nematode population density increased. The high increase in numbers of *P. penetrans* in summer 1989 paralleled reductions in the nematode population density and increases in peanut yield in the *P. penetrans*-infested plots.

In this study, the population density of *P. penetrans* increased over time from the relatively low levels of spores added initially. Thus, even if large-scale production of inoculum of *P. penetrans* proves to be impossible or too costly, the use of this antagonist in integrated nematode management schemes may be feasible. Studies on cereal monoculture in Great Britain showed a continuous decrease in the population density of the cereal cyst nematode, *Heterodera avenae,* after a population peak in the second year. This decrease was due to egg and cyst parasitic fungi, mainly *Nematophthora gynophila* and *Verticillium chlamydosporum* (6,9). Additional long-term experimentations are needed to determine whether nematode population densities can be similarly maintained at acceptable low levels by *P. penetrans.*

Literature Cited

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

2. Byrd, D. W., T. Kirkpatrick, and K. R. Barker. 1983. An improved technique for clearing and staining plant tissues for the detection of nematodes. Journal of Nematology 15:142–143.

3. 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.

4. Cochran, W. G., and G. M. Cox. 1957. Experimental designs, 2nd ed. New York: John Wiley & Sons.

5. Dube, B., and G. C. Smart, Jr. 1987. Biological control of *Meloidogyne incognita* by *Paecilomyces lilacinus* and *Pasteuria penetrans*. Journal of Nematology 19: 222–227.

6. Gair, R., P. L. Mathias, and P. N. Harvey. 1969. Studies of cereal nematode populations and cereal yields under continuous or intensive culture. Annals of Applied Biology 63:503–512.

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

8. Johnson, J. T., J. R. Rich, and A. W. Boatwright. 1981. A technique for establishing microplots in the field. Journal of Nematology 13:233–235.

9. Kerry, B. R., D. H. Crump, and L. A. Mullen. 1975. Studies of cereal cyst-nematode, *Heterodera avenae* under continuous cereals, 1975–1978. II. Fungal parasitism of nematode females and eggs. Annals of Applied Biology 100:489–499.

10. Minton, N. A., and R. M. Sayre. 1989. Suppressive influence of *Pasteuria penetrans* in Georgia soils on reproduction of *Meloidogyne arenaria*. Journal of Nematology 21:574 (Abstr.).

11. Spaull, V. W. 1984. Observations on *Bacillus penetrans* infecting *Meloidogyne* in sugarcane fields in South Africa. Revue de Nématologie 7:277–282.

12. Stirling, G. R. 1981. Effect of temperature on infection of *Meloidogyne javanica* by *Pasteuria penetrans*. Nematologica 27:458–462.

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

14. Stirling, G. R., and M. F. Wachtel. 1980. Mass production of *Bacillus penetrans* for the biological control of root-knot nematodes. Nematologica 26:308–312.

15. Stirling, G. R., and A. M. White. 1982. Distribution of a parasite of root-knot nematodes in South Australian vineyards. Plant Disease 66:52–53.

16. Verdejo, S., and B. A. Jaffee. 1988. Reproduction of *Pasteuria penetrans* in a tissue-culture system containing *Meloidogyne javanica* and *Agrobacterium rhizogenes*-transformed roots. Phytopathology 78: 1284–1286.