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Isolates of the *Pasteuria penetrans* Group from Phytoparasitic Nematodes in Bermudagrass Turf[1]

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Abstract: A survey was conducted between 1985 and 1989 of isolates of the *Pasteuria penetrans* group on phytoparasitic nematodes in bermudagrass (*Cynodon* spp.) turf in southern Florida. Six different isolates of the *P. penetrans* group were observed from five different species of phytoparasitic nematode hosts. Five of the bacterial isolates were different ($P \leq 0.01$) in sporangium diameter, endospore width, and ratio of sporangium diameter to endospore width. All locations surveyed had one or more isolates present, suggesting that the *Pasteuria penetrans* group is widespread in its distribution in southern Florida. Three survey sites had high densities of *Belonolaimus longicaudatus*, with more than 60% of the host population encumbered with a large-spored isolate of *Pasteuria* (mean sporangium diameter = 6.10 μm). One of these sites was monitored for 16 months during which the proportion of nematodes encumbered with this *Pasteuria* isolate remained constant. Soil infested with this isolate was not suppressive to *Pasteuria*-free populations of *B. longicaudatus* grown on bermudagrass for 6 months after controlled soil inoculation. However, the proportion of spore-encumbered and parasitized *B. longicaudatus* after 6 months was 73%, which was similar to the 74% level observed at the field site. The ultrastructure of mature sporangia of the large-spored isolates of *Pasteuria* from *B. longicaudatus* and *Hoplolaimus galeatus* is described and compared with ultrastructural descriptions of *P. penetrans* sensu strictu and *P. thornei* from the literature. These *B. longicaudatus* and *H. galeatus* isolates of *Pasteuria* appear to be distinct from the known species and may warrant new species status.

Key words: bacterial parasite, *Belonolaimus longicaudatus*, bermudagrass, biological control, *Helicotylenchus microlobus*, *Hoplolaimus galeatus*, *Meloidogyne* spp., *Pasteuria penetrans* group, *Tylenchorhynchus annulatus*, ultrastructure.

Currently, management of phytoparasitic nematodes for perennial crops such as turfgrass relies largely on postplant application of organophosphate or carbamate pesticides (6). Nematicides labeled for use on turfgrass in 1989 are nematostatic at the concentrations achieved in the field and usually require multiple applications for short-lived suppression of phytoparasitic nematode populations (5,6). Chronic exposure of nematodes and the soil microflora to sublethal doses of nematicides can selectively encourage microbial decomposition of pesticides and (or) nematicide resistance in nematodes (22).

Releases of members of the *Pasteuria penetrans* group, obligate nematode endoparasitic bacteria, may provide an alternative or supplement to chemical control (3,18). These endospore-forming actinomycetes attach to, and infest, the nematode host via the cuticle. The parasitized nematode is incapable of reproduction and eventually becomes filled with developing endospores of the bacterium, which are released into the environment upon host disintegration (1). *Pasteuria thornei* Starr & Sayre can complete its life cycle in larvae or adults of *Pratylenchus brachyurus* (Godfrey) Filipjev & Shuurmans Stekhoven, whereas *P. penetrans* sensu strictu (Thorne) Sayre & Starr can complete its life cycle only in adult root-knot nematodes, *Meloidogyne* spp. (17). Spores of the *Pasteuria penetrans* group are resistant to heat (21), desiccation, and exposure to nematicides (11) and have been reported adhering to, or infesting, 205 species of nematodes from

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51 countries worldwide (17,20). Only two species of the *P. penetrans* group are well characterized (13), however, and little is known about the ecology of the group in native or managed soil systems.

The purpose of this study was to survey soil from managed hybrid bermudagrass (*Cynodon* spp.) in golf course fairways in southern Florida for the natural occurrence of phytoparasitic nematodes that were encumbered or parasitized with morphometrically distinct isolates of the *P. penetrans* group. The ultrastructure of two morphometrically distinct large-spored isolates of the *P. penetrans* group from *Belonolaimus longicaudatus* Rau and *Hoplolaimus galeatus* (Cobb) Thorne was examined. In addition, a greenhouse experiment was conducted to determine if soil infested with the large-spored isolate of the *P. penetrans* group from *B. longicaudatus* suppressed *B. longicaudatus* populations parasitizing bermudagrass.

Materials and Methods

Survey: The survey was initiated in December 1985. Samples were taken at the following locations in southern Florida: Banyan Golf Club (BGC), Palm Beach County; Plantation Golf Club (PGC), Broward County; the Royal Poinciana Country Club (RPCC), Collier County; and an experimental fairway at the Ft. Lauderdale Research and Education Center (FLREC), Broward County. BGC and RPCC are intensively managed golf courses fertilized yearly with 40–60 g N/m^2. In contrast, PGC and the FLREC fairway area receive only about 10 g N/m^2 each year. All locations had been treated with nematicides and other pesticides before the survey. These applications were discontinued several months before and during the survey. Fairways were established areas of bermudagrass, *Cynodon transvaalensis* Burt-Davy × *C. dactylon* (L.) Pers. cv. Tifway (= T-419), in all locations except FLREC, which was an established area of *C. magenissii* Hurcombe cv. Tifgreen (= T-328). Fairways were mowed at 10–13 mm two or more times per week and the clippings were removed. All soils were classified as a fine sand with the upper 15 cm containing > 96% sand, < 3% silt, 1% clay; pH 5.1–8.5, and < 4% organic matter. The cation exchange capacities of these soils were always less than 10 meq/100 g.

Soil samples were taken at one or two locations on at least one fairway at each golf course. Survey sites were circular (radius of 3 m) and marked with metal bars implanted below the soil surface at the center. The sites were then relocated with a metal detector on subsequent sampling dates. Color slides of each sample site were taken on each sampling date at a 1.5-m height with a scale and green paint color reference card to help standardize the turfgrass evaluations. Turfgrass quality scores were given to projected slides of each site at the end of the survey using a 1–10 rating scale where 1 = bare ground or 100% coverage with weeds, 6 = acceptable golf course fairway grass, and 10 = excellent quality turfgrass.

Each soil sample consisted of 14–18 randomly located cores taken to a depth of 10 cm (total volume 1.0–1.5 liters of soil). A 100-cm^3 subsample was extracted for nematodes using the sugar flotation technique (8), and the remaining roots and plant matter were placed on a Baermann funnel in an intermittent mist chamber (1 minute mist for every 10 minutes) for 7 days. Nematodes in the sample (combined sugar flotation and mist chamber fractions, except where otherwise noted) were heat killed, fixed in 2.5% formalin-glycerol (15), and stained with crystal violet.

Phytoparasitic nematode densities were quantified and subsamples were used to estimate the proportion of *Pasteuria*-encumbered species and the number of spores attached to each nematode. Endospore widths and sporangium diameters for bacteria attached to the different species of nematodes were measured with a camera lucida. Endospore widths and sporangium diameters were statistically compared with the SAS (10) general linear models procedure and means were separated with a Student-Newman-Keuls multiple-range test

($\alpha = 0.01$). Bivariate scattergrams of endospore width versus sporangium diameter were plotted and 95% equal frequency ellipses for the observations were calculated (14). Discriminant analysis was applied to the endospore width, sporangium diameter, and ratios of endospore width to sporangium diameter from different host isolates of *P. penetrans*. A discriminant function was developed, and the posterior probability that an observation belonged to each class was tested (10).

Greenhouse experiment: High densities of *B. longicaudatus* were established on Tifgreen II bermudagrass as follows. Nematode-free bermudagrass sod was established from aerial sprigs and transplanted over autoclaved sand in polypropylene beakers (14.8 cm d, 14 cm high). Each beaker was inoculated with 125–625 *B. longicaudatus* from greenhouse cultures on green beans (*Phaseolus vulgaris* L.). Beakers were suspended in a water bath (1.0 × 2.0 × 0.3 m) in which soil temperature was controlled at 27 ± 2 C with a model CFF-500 Remcor liquid circulator (Remcor Products Co., Franklin Park, IL). The bermudagrass was maintained with weekly or more frequent watering to field capacity and mowed to 1.3 cm. After 9 months the nematode populations appeared stabilized and mean densities of 485 ± 177 *B. longicaudatus*/100 cm^3 were recorded from 16 beakers on 20 July 1989, the date of inoculation with *P. penetrans*-infested soil.

Soil infested with *P. penetrans, B. longicaudatus,* and *Tylenchorhynchus annulatus* (Cassidy) Golden was collected from PGC site 1 on 14 July 1989. Soil was either autoclaved at 120 C at 103 Kpa for 1 hour for sterilization (control) or heat treated for 48 hours at 47 C to kill the nematodes but leave the *P. penetrans* isolates viable (treated). For introduction of control or treated soil, the soil column was removed from the 16 beakers with *B. longicaudatus*-bermudagrass cultures and cut in half vertically. Half of the column was returned to the beaker and the empty volume was filled with 1 kg moist sterilized fine sand from FLREC and topped off with either 500 g autoclaved control soil from PGC site 1 (eight beakers) or 500 g heat-treated soil from PGC site 1 (eight beakers). The beakers were randomly placed in the temperature controlled water bath in the greenhouse. After 6 months, three soil cores were removed from the disturbed half of the beaker in which the bermudagrass had become established and extracted (8) to determine *B. longicaudatus* densities and proportions of nematodes encumbered or filled with the large-spored *P. penetrans* isolate from *B. longicaudatus* from PGC site 1.

As a protocol check, an additional experiment was done to compare how many spores would attach to *Pasteuria*-free *B. longicaudatus* that were introduced and immediately extracted or allowed to remain in the *P. penetrans*-infested soil for 48 hours before extraction. One hundred *B. longicaudatus* were handpicked from *P. penetrans*-free green bean cultures and mixed thoroughly into 100 cm^3 heat-treated soil from PGC site 1 for 48 hours before sugar flotation extraction and compared with immediate extraction after inoculation and mixing. The soil was mixed and stored at 27 C in 120-cm^3 sealed plastic specimen containers. This experiment was replicated at least three times, and at least 10 nematodes from each replicate were examined for attached spores.

Transmission electron microscopy (TEM): Belonolaimus longicaudatus and *Hoplolaimus galeatus* filled with the endospores of their respective large-spored isolates of *P. penetrans* from the FLREC were heat killed (17), cut, fixed in 2.5% glutaraldehyde + 0.1 M sodium phosphate buffer (pH 7.4) overnight at 4 C, embedded in 3% agarose, and cut into small blocks. The glutaraldehyde was rinsed from the blocks with five rinses of phosphate buffer, and the tissue was postfixed in 2% osmium tetroxide in phosphate buffer. The tissue was rinsed, dehydrated in an ethanol-acetone series, and infiltrated with Spurr's epoxy resin (16). The tissue was then placed in molds in a 60-C vacuum oven (6.8-kg vacuum) for 18 hours for resin polymerization. Thin-sections (80 nm) were cut with glass knives on

TABLE 1. Summary of the pooled measurements of sporangium diameter and endospore width of different isolates of *Pasteuria penetrans* group from different nematode species.

Nematode species	Sporangium diameter (μm)			Endospore width (μm)			Ratio†		
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Hoplolaimus galeatus (large spore)	7.26 a	0.36	6.53–8.05	3.54 a	0.24	2.72–3.54	2.06 bc	0.16	1.79–2.62
Belonolaimus longicaudatus (large spore)	6.10 b	0.39	4.71–7.00	2.93 b	0.24	2.33–3.78	2.10 b	0.17	1.65–2.67
Tylenchorhynchus annulatus	4.55 c	0.33	3.98–5.38	2.53 c	0.17	2.21–2.95	1.80 e	0.13	1.47–2.09
Helicotylenchus microlobus	3.87 d	0.39	2.78–4.73	1.97 d	0.22	1.39–2.50	1.98 d	0.20	1.50–2.67
H. galeatus (small spore)	3.87 d	0.33	3.19–4.64	1.95 d	0.22	1.45–2.45	2.00 d	0.19	1.55–2.42
Meloidogyne spp.	3.42 e	0.27	2.91–4.19	1.58 e	0.16	1.22–1.98	2.19 a	0.26	1.67–2.86

Means in a column followed by the same letters were not different ($P < 0.01$) with a Student-Newman-Keuls multiple-range test.

† Ratio = sporangium diameter/endospore width.

a LKB ultramicrotome, stained with uranyl acetate and lead citrate, and viewed with a Philips 201 TEM (60 kv).

RESULTS AND DISCUSSION

Survey: Five significantly different sized *P. penetrans* group isolates were associated with five different species of phytoparasitic nematodes in fairway cultured bermudagrass in southern Florida (Table 1, Fig. 1). The isolate from *Helicotylenchus microlobus* Perry and the small-spored isolate from *Hoplolaimus galeatus* were not significantly different from each other in sporangium diameter, endospore width, or the ratio of sporangium diameter to endospore width (Table 1). Eighty-nine percent of the large-spored isolate of the *P. penetrans* group from *H. galeatus* was correctly classified by discriminant analysis and 11% of the spores were misclassified as the *B. longicaudatus* isolate. Ninety-eight percent of *P. penetrans* group spores from *B. longicaudatus* were correctly classified, whereas only about 1% of the spores were misclassified as the large-spored *H. galeatus* isolate. Seventy-three percent of the spores measured from *T. annulatus* were correctly classified, with 20% misclassified as the *H. microlobus* isolate and 7% misclassified as the *B. longicaudatus* isolate. Eighty percent of the spores from *H. microlobus* were correctly classified, whereas 19% were misclassified as the *Meloidogyne* spp. isolate and 1% were misclassified as the *T. annulatus* isolate. The small-spored isolate from *H. galeatus* was never correctly classified by discriminant analysis. Seventy-seven percent of small spores from *H. galeatus* were misclassified as the isolate from *H. microlobus*, 20% were misclassified as the isolate from *Meloidogyne* spp., and 3% were misclassified as the *T. annulatus* isolate. Ninety-five percent of the spores from *Meloidogyne* spp. were correctly classified and 5% were misclassified as the *H. microlobus* isolate.

A small proportion (less than 8%) of *B. longicaudatus* from RPCC sites 2A and 3A and PGC site 2 (Table 2) were encumbered with a few spores of *P. penetrans* (Table 3) that were significantly smaller than the large-spored isolate. These spore morphometrics were not included in the discriminant analyses. The small-spored isolate from *B. longicaudatus* from PGC site 2 (Table 2) had a mean sporangium diameter of 3.97 and a mean endospore width of 1.74 (n = 3), whereas the mean sporangium diameter and endospore width measured 4.72 and 1.93 for the RPCC isolate (n = 6). Internal endospore formation was not observed in the small-spored isolates from

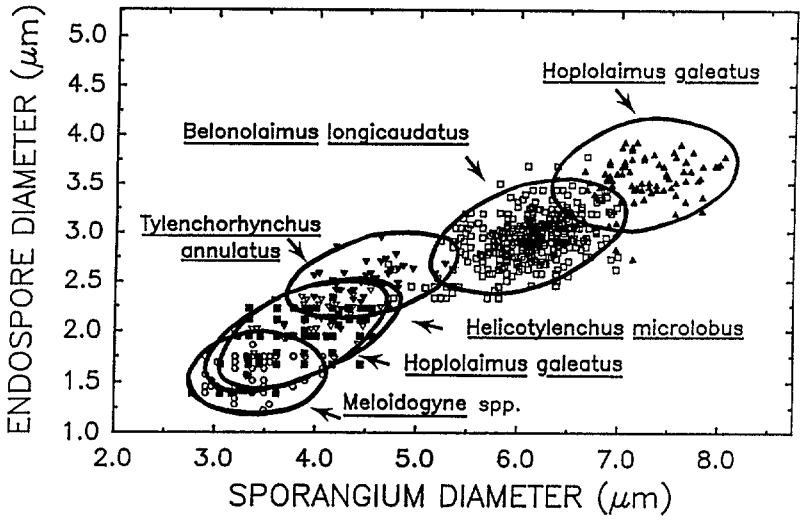

FIG. 1. Bivariate scattergram comparing endospore and sporangium diameter of different phytoparasitic nematode host isolates of the *Pasteuria penetrans* group recovered from bermudagrass turf in southern Florida. The ellipses are the 95% frequency regions for spores measured from each designated nematode host. Open box = *B. longicaudatus*, n = 412; filled triangle = large-spored isolate of *H. galeatus*, n = 80; filled box = small-spored isolate of *H. galeatus*, n = 92; open circle = *Meloidogyne* spp., n = 182; filled inverted triangle = *T. annulatus*, n = 41; open inverted triangle = *H. microlobus*, n = 220.

B. longicaudatus; their morphometrics suggest that they may be from parasitized and co-occurring *H. microlobus* or *H. galeatus* (Table 2). This suggests that some misclassification of morphometrically similar isolates of the *P. penetrans* group may have occurred in morphometric zones of overlap (Fig. 1) because different *P. penetrans* host-isolates may have attached to co-occurring nonhosts.

Host-associated spore size disparity was consistent for different localities and different sampling times. The host-specific morphometrics for most of the isolates we observed were similar to previously reported values for *P. penetrans* from their respective nematode hosts from other locations (12,13). The isolate from *Meloidogyne* spp. in this survey was morphometrically similar to an isolate reported from *Pratylenchus scribneri* Steiner 1943 and *M. javanica* (Treub) Chitwood from Maryland (13). The isolate from *T. annulatus* in this survey was morphometrically similar to an isolate reported from *T. maximus* Allen from Maryland (13). The isolate from *H. microlobus* was morphologically similar to an isolate reported from *H. microlobus* from *Zoysia japonica* Steud. in southern Florida (4). The large-spored isolate from *B. longicaudatus* was morphometrically similar to an isolate reported from *B. gracilis* Steiner (12). The large-spored isolate from *H. galeatus* was morphometrically similar to isolates from *H. galeatus* from Pennsylvania and Florida (7,13).

All sites surveyed were positive for at least one isolate of the *P. penetrans* group except BGC sites 2A and 2B. PGC site 1 showed the most diversity of any of the sites surveyed, with three morphometrically different *P. penetrans* isolates parasitizing three different phytoparasitic nematodes—*B. longicaudatus, T. annulatus,* and *Meloidogyne* spp. (Table 2). *Criconemella ornata* (Raski) Luc & Raski was observed at all but three survey sites, and none of the 195 nematodes examined were observed with spores of the *P. penetrans* group. *Hemicycliophora* sp. was observed at four survey sites, and none of the 73 nematodes examined were encumbered with spores of *P. penetrans*. *Hemicriconemoides* sp. was observed at seven sites, and none of the 77

TABLE 2. Turfgrass evaluations, ranges in mean densities of phytoparasitic nematodes per 100 cm³ soil across sampling dates at different survey sites, and mean proportion of nematodes encumbered with isolates of the *Pasteuria penetrans* group.

Location†	Sample dates‡	Visual ratings (range)§	*Belonolaimus longicaudatus*			*Hoplolaimus galeatus*			*Helicotylenchus microlobus*			*Tylenchorhynchus annulatus*			*Meloidogyne* spp.		
			Den.	%	n	Den.	%	n	Den.	%	n	Den.	%	n	Den.	%	n
RPCC site 1	Dec 1985		84 (large spore)	86	15	89	0	20							39	11	9
RPCC site 2A	J, F, M, A, O, Ap	3.0–6.0	89–397 (small spore)	4	56				67–222	19	52				0–33	50	4
RPCC site 2B	J, F, M, A, O, Ap	8.0–9.0	0–5	0	0				75–344	32	54	0–50	0	11	2–733	22	54
RPCC site 3A	J, F, M, A, O, Ap	2.5–4.5	72–851 (small spore)	8	159				0–139	32	28	0–8	0	0	0–200	7	41
RPCC site 3B	J, F, M, A, O, Ap	7.5–9.0	0–7	0	1				66–600	50	118	1–574	0	20	3–833	6	67
PGC site 1	J, F, M, A, My, Ju, Ag, S, O, Dc, Ap, Jul	1.5–5.5	92–295 (large spore)	65	210				82–421	0	132	4–200	53	77	3–1,867	5	614
PGC site 2	J, F, M, A, O, Ap	3.5–5.0	43–167 (large spore)	4	48	33–116 (large spore)	12	41	33–200	0	13	10–167	0	14	22–533	9	55
			6 (small spore)			66 (small spore)											
BGC site 1A	J, F, M, A, O, Ap	6.5–8.0	32–133	0	24				1–100	0	7				19–1,500	18	149
BGC site 1B	J, F, M, A, O, Ap	8.0–9.0							0–200	10	29				225–6,533	2	656
BGC site 2A	J, F, M, A, O, Ap	6.0–7.0	54–500	0	58	0–22	0	1	30–244	0	49				0–174	0	19
BGC site 2B	J, F, M, A, O, Ap	8.5–9.5							22-372	0	24	6–433	0	29			
FLREC site 1A	May, Jun, Jy	3.5–6.0	72–150 (large spore)	17	29	190–451 (large spore)	26	80							50–170	0	10
FLREC site 1B	Jan, Feb	2.5–4.5	60–120 (large spore)	83	12	670–1,440 (large spore)	2	134							0–30	0	3
FLREC site 1C	Dec 1989					30 (large spore)	67	18	14	0	14						

Den. = range in mean densities of nematodes/100 cm³ soil across all sampling dates; % = mean proportion of nematodes encumbered with *P. penetrans*; n = number of nematodes examined for *P. penetrans*.

† RPCC = Royal Poinciana Country Club, Collier County, FL; PGC = Plantation Golf Club, Broward County, FL; BGC = Banyan Golf Club, Palm Beach County, FL; FLREC = Ft. Lauderdale Research and Education Center, Broward County, FL.

‡ J, F, M, A, My, Ju, Ag, S, O, Dc = January, February, March, April, May, June, August, September, October, December 1986, respectively; Ap, May, Jun, Jy = April, May, June, July 1987, respectively; Jan, Feb = January and February 1988; Jul = July 1989.

§ Range in mean visual ratings across all sampling dates.

TABLE 3. Proportions of phytoparasitic nematodes encumbered with different numbers of or filled with spores of the *Pasteuria penetrans* group.

Species	Spore size	Location†	Sample dates‡	n§	Number of spores attached‖ 1–5	6–10	11–15	16–20	>20	Filled
Belonolaimus	large	RPCC site 1	Dec 1985	13	54	23	15	0	0	8
longicaudatus	mall	RPCC site 3A	J, F, M, A, O, Ap	11	91	9	0	0	0	0
(juveniles and adults)	large	PGC site 1	J, F, M, A, My, Ju, Ag, S, O, Dc, Ap, Jul	147	61	17	7	5	7	3
	large	FLREC site 1B	Jan, Feb	10	50	20	0	0	0	30
Hoplolaimus galeatus	small	PGC site 2	J, F, M, A, O, Ap	27	63	7	7	12	4	7
(juveniles and adults)	large	FLREC site 1A	May, Jun, Jy	20	85	5	0	5	5	0
	large	FLREC site 1C	Dec 1989	12	50	8	0	0	25	17
Helicotylenchus		RPCC site 2A	J, F, M, A, O, Ap	10	60	0	0	0	20	20
microlobus		RPCC site 2B	J, F, M, A, O, Ap	17	71	24	0	0	0	5
(juveniles and adults)		RPCC site 3A	J, F, M, A, O, Ap	8	87	0	0	0	0	13
		RPCC site 3B	J, F, M, A, O, Ap	58	60	16	7	0	9	8
Tylenchorhynchus		PGC site 1	J, F,M, A, My, Ju, Ag, S, O, Dc, Ap, Jul	41	78	12	2	0	5	3
annulatus (juveniles and females)										
Meloidogyne		RPCC site 2B	J, F, M, A, O, Ap	12	100	0	0	0	0	0
spp.		PGC site 1	J, F, M, A, My, Ju, Ag, S, O, Dc, Ap, Jul	28	75	4	0	0	0	21
(2nd stage juveniles)		BGC site 1A	J, F, M, A, O, Ap	27	48	15	11	0	26	0
		BGC site 1B	J, F, M, A, O, Ap	12	58	17	0	8	17	0

† BGC = Banyan Golf Club, Palm Beach County, FL; FLREC = Ft. Lauderdale Research and Education Center, Broward County, FL; PGC = Plantation Golf Club, Broward County, FL; RPCC = Royal Poinciana Country Club, Collier County, FL.

‡ J, F, M, A, My, Ju, Ag, S, O, Dc = January, February, March, April, May, June, August, September, October, December 1986; Ap, May, Jun, Jy = April, May, June, July 1987, respectively; Jan, Feb = January and February 1988; Jul = July 1989.

§ n = pooled number of nematodes examined which were encumbered with spores of the *P. penetrans* group.

‖ Data are proportions (%) of encumbered nematodes (n) with the designated number of spores attached.

nematodes examined were encumbered with spores.

A common trend at RPCC was for *H. microlobus* and *Meloidogyne* spp. to be infected with their respective *P. penetrans* group isolates. Interestingly, the small-spored isolate of *H. galeatus,* which was not morphometrically distinguishable from the *H. microlobus* isolate (Table 1), did not parasitize *H. microlobus* where the two species co-occurred (PGC site 2). This suggests that other more discriminating methods, i.e., molecular diagnostics, may be preferred over morphometric comparisons for designation of biological differences in some isolates of the *P. penetrans* group.

Belonolaimus longicaudatus was parasitized by a large-spored isolate of the *P. penetrans* group at RPCC site 1 where *H. galeatus* co-occurred without an isolate of the *P. penetrans* group. However, there were three locations (FLREC sites 1A and 1B and PGC site 2) where both *B. longicaudatus* and *H. galeatus* co-occurred encumbered with their respective large-spored isolates of the *P. penetrans* group (Table 2). At PGC site 2, *H. galeatus* was observed to be encumbered with both the large-spored and small-spored isolates of the *P. penetrans* group (Fig. 2).

Turfgrass performance generally rated lowest where there were high densities of *B. longicaudatus,* regardless of the presence of its associated large-spored isolate of the *P. penetrans* group (Table 2). *Belonolaimus longicaudatus* cycled through high densities during the spring (March through May) at the PGC site 1 (Fig. 3) and reached an equilibrium density of about 120 nematodes/100 cm^3 soil for the remainder of the year.

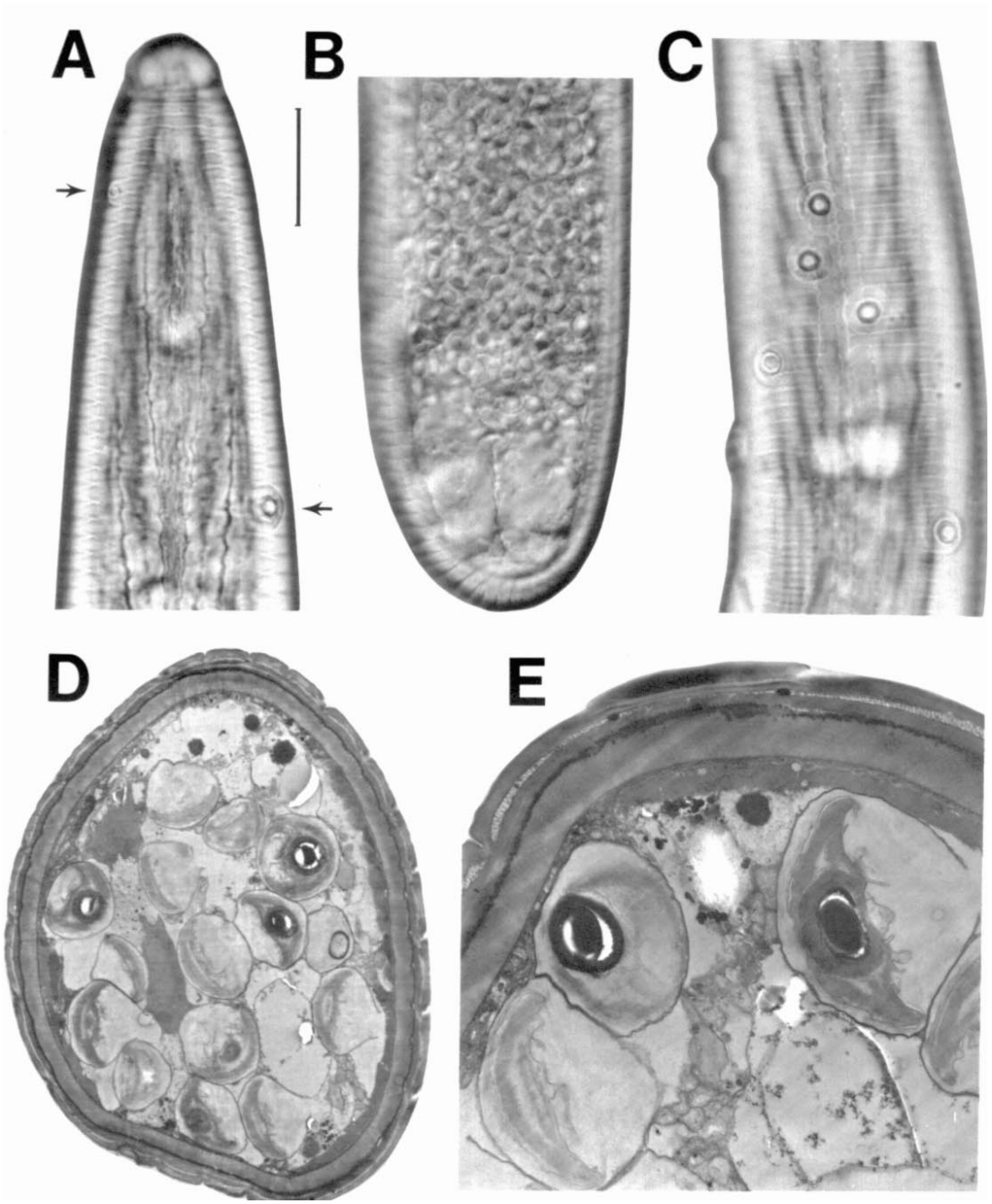

FIG. 2. Light and TEM photomicrographs of *Pasteuria*-encumbered or parasitized *Hoplolaimus galeatus* from bermudagrass. A) Head region of a female encumbered with the small-spored and large-spored isolates of the *Pasteuria penetrans* group (arrows). B) Tail region of a juvenile filled with spores of the small-spored isolate of *P. penetrans*. C) Anterior region of an adult male with the large-spored isolate of *P. penetrans*. D) Cross-section of a female filled with spores of the large-spored isolate of *P. penetrans*. E) Mature sporangium of the large-spored isolate of *P. penetrans* from internally infested female. Scale bars: A–C = 20 μm, D, E = 5 μm.

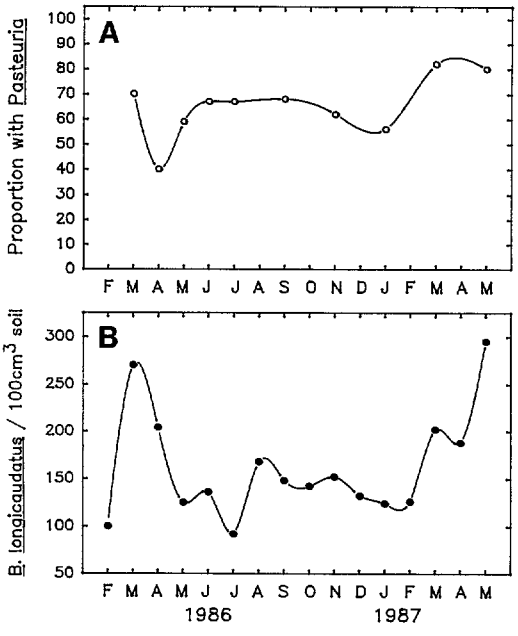

FIG. 3. Monthly trends in population densities from PGC site 1. A) Proportion of *B. longicaudatus* with *Pasteuria* attached. B) Total number of *B. longicaudatus* per 100 cm³ of soil.

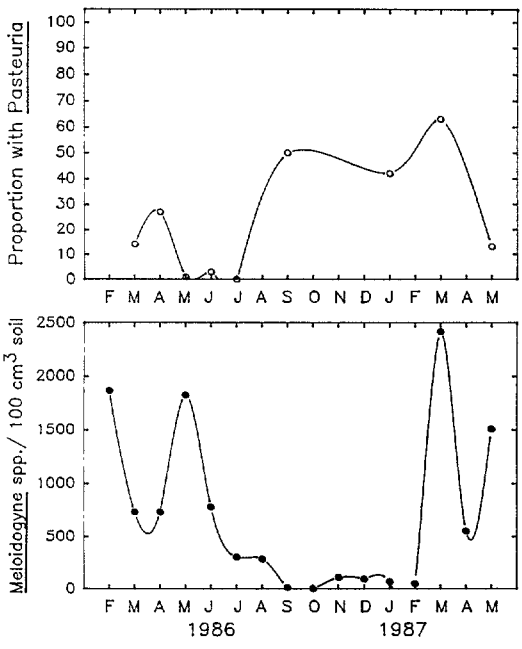

FIG. 4. Monthly trends in the densities of *Meloidogyne* spp. and the proportion with *Pasteuria penetrans* from PGC site 1. A) Proportion of *Meloidogyne* spp. with *Pasteuria* attached. B) Total number of *Meloidogyne* spp. per 100 cm³ of soil over time.

The increase in population density for *B. longicaudatus* corresponded to the period of bermudagrass green-up in the spring in southern Florida which coincided with the seasonal increase in daily solar radiation.

The proportion of *B. longicaudatus* encumbered with its large-spored isolate of the *P. penetrans* group remained fairly constant over the 16 months of survey work at PGC site 1 (Fig. 3). This suggests that the nematode host-bacterial pathogen association may be at equilibrium at the PGC site 1. The poor bermudagrass performance and consistently high *B. longicaudatus* densities at PGC site 1 (Table 2, Fig. 3) suggests that this large-spored *P. penetrans* isolate does not control *B. longicaudatus* to acceptable levels and may be inappropriate for use in inoculative biocontrol on golf course fairways.

Endospore-filled second-stage juveniles of *Meloidogyne* spp. were recovered in one survey location (Table 3). This is interesting because *P. penetrans* reportedly completes its life cycle only in adult root-knot nematodes (17). Apparently, this isolate of the *P. penetrans* group was able to complete its life cycle in second-stage juveniles of *Meloidogyne* spp. extracted from PGC site 1 (Table 3). The proportion of root-knot nematodes encumbered with *P. penetrans* appeared to be inversely correlated with the density of nematodes recovered from soil samples (Fig. 4).

Greenhouse experiment: The soil collected from PGC site 1 on 14 July 1989 contained 270 *B. longicaudatus*/100 cm³, of which 74% were parasitized with the large-spored isolate of the *P. penetrans* group from *B. longicaudatus*, and 17 *T. annulatus*/100 cm³, of which 66% were parasitized with its respective *P. penetrans* isolate. Of the infested *B. longicaudatus*, 63% were encumbered with 1–5 spores, 17% with 6–10 spores, 5% with 11–15 spores, 3% with 16–20 spores, 3% with more than 20 spores, and 9% were filled with endospores of the large-spored isolate of the *P. penetrans* group from *B. longicaudatus*.

There was no difference ($P \leq 0.05$) between the number of *B. longicaudatus* har-

vested from control beakers inoculated with sterilized soil from PGC site 1 (273 ± 93 [range = 140–432] per 100 cm^3 soil) compared with the number of *B. longicaudatus* harvested from beakers inoculated with heat-treated soil (324 ± 155 [range = 162–563] per 100 cm^3 soil) 6 months after inoculation. Of the 107 juveniles, 66 males, and 83 females of *B. longicaudatus* that were examined from the eight treated beakers, 73% were encumbered or infested with the large-spored isolate of *P. penetrans* from *B. longicaudatus,* compared with 0% of 209 nematodes examined from the controls. Of the infested *B. longicaudatus,* 45% were encumbered with 1–5 spores, 14% with 6–10 spores, 8% with 11–15 spores, 6% with 16–20 spores, 20% with more than 20 spores, and 7% were filled with endospores. Thus, after 6 months, *B. longicaudatus* colonized the bermudagrass that developed in the disturbed area and the *P. penetrans* in the infested soil was still viable and used the nematode as a host to about the same degree as at PGC site 1. A greater degree of infection by *P. penetrans* isolates has been obtained with *Meloidogyne* spp. in greenhouse tests, although many more spores (2.0×10^6 per female) were obtained for *Meloidogyne* spp. (2,9) than the 364–2,128 per *B. longicaudatus* observed here. Also, it did not appear as though internally infested *B. longicaudatus* quickly disintegrated to release spores. Low spore numbers and slow release of spores from host cadavers may have impeded recycling of this *P. penetrans* isolate in the field and in the greenhouse experiment.

A major drawback for working with any isolate of the *P. penetrans* group that comes from ectoparasitic nematodes, such as *B. longicaudatus,* will be in obtaining clean spore preparations for controlled experiments. Stirling and Watchel (19) capitalized on the host–parasite association of *P. penetrans* sensu strictu with endoparasitic root-knot nematodes to develop an effective method of spore mass production. However, ectoparasitic nematodes internally parasitized with spores of *Pasteuria* are relatively rare (Table 3), have few spores, and must be extracted from the soil and then handpicked from nematode suspensions.

All six of the *P. penetrans* group isolates (Table 1) were observed to produce spores within juveniles or adults of their respective host nematodes, except *Meloidogyne* spp., in which endospore formation was observed only in second-stage juveniles (adult females were not surveyed) (Table 3). The majority of the nematodes examined (48–100%) were encumbered with fewer than six spores per nematode (Table 3).

In the protocol check experiment, 24% of the *P. penetrans*-free *B. longicaudatus* (n = 50) incubated for 48 hours in heat-treated *Pasteuria*-infested soil were encumbered with the large-spored *P. penetrans* isolate and 92% of these nematodes had only one or two spores attached (n = 12). When *B. longicaudatus* was mixed and harvested immediately from heat-treated *Pasteuria*-infested soil, only one of the 33 nematodes examined was encumbered with a single spore of *P. penetrans*. Thus, the longer the time period from collection to extraction of a soil sample, the greater the chance of an artificially elevated spore encumbrance level. Most of our soil samples were extracted within 72 hours of collection, except those in the greenhouse experiment which were harvested immediately. Thus, the percentage of *P. penetrans* associated with nematodes reported in Tables 2 and 3 may be artificially elevated.

Transmission electron microscopy (TEM): Comparisons of mature sporangia of the large-spored isolates from internally infested *H. galeatus* and *B. longicaudatus* with TEM (Figs. 2, 5, 6) revealed ultrastructural differences between each other and the two currently described species of *Pasteuria* (13). *Pasteuria penetrans* sensu strictu, *P. thornei,* and the *H. galeatus* and *B. longicaudatus* isolates of *Pasteuria* all had sporangial walls with double membranes. The basal portion of the sporangium is devoid of electron-dense material in all but *P. thornei.* The sporangium appears cup shaped in *P. penetrans* sensu strictu and the *B. longicaudatus* isolate, whereas it appears rhomboidal in

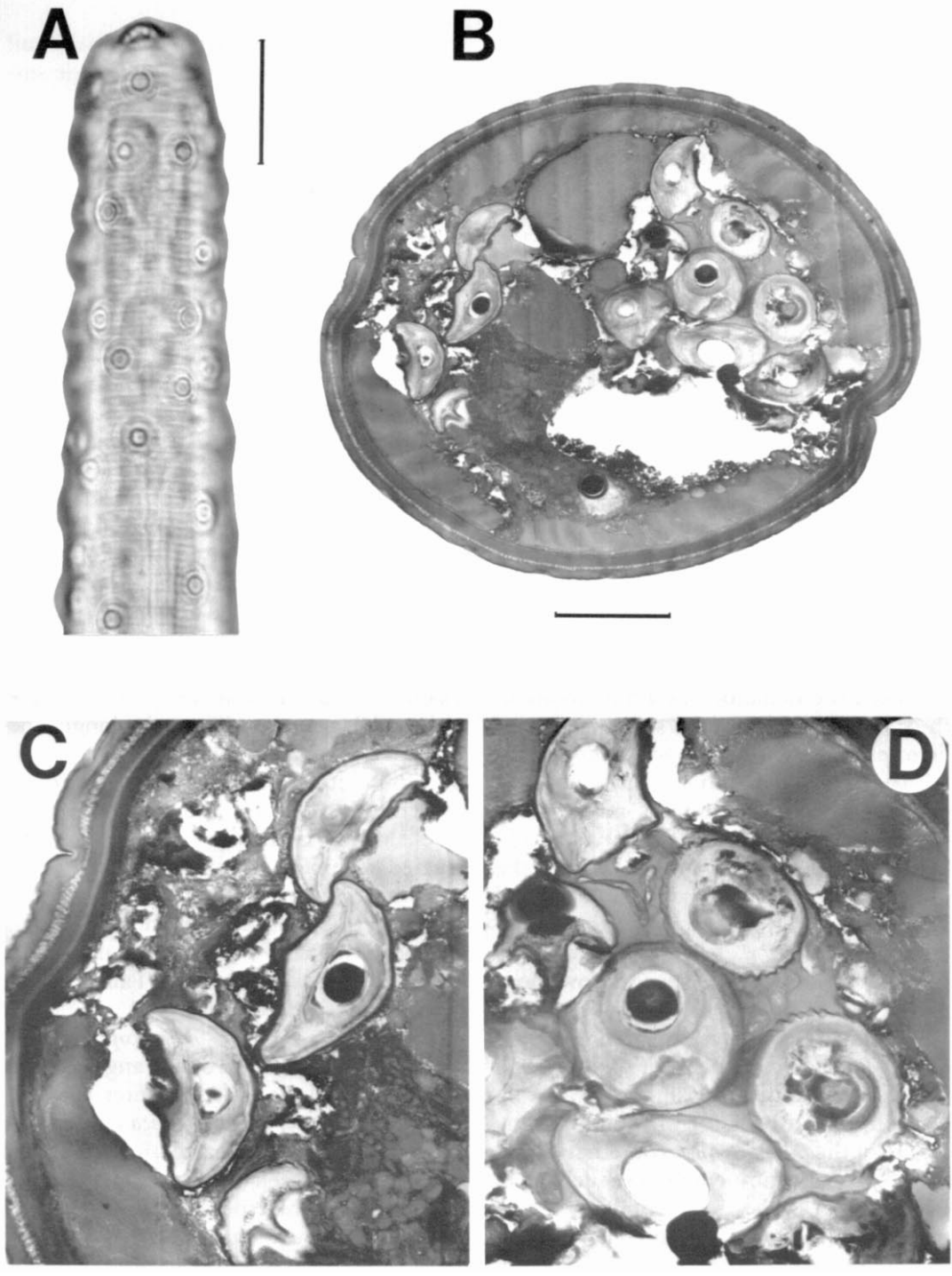

FIG. 5. *Pasteuria*-encumbered or parasitized *Belonolaimus longicaudatus* from bermudagrass. A) Light photomicrograph of the head region of a juvenile with the large-spored isolate of the *Pasteuria penetrans* group. B) TEM photomicrograph of cross-section of a juvenile filled with spores of the large-spored isolate of *P. penetrans*. C, D) Close-up of B showing mature sporangia of the large-spored isolate of the *P. penetrans* group from internally infested juvenile. Scale bars: A = 20 μm, B–D = 5 μm.

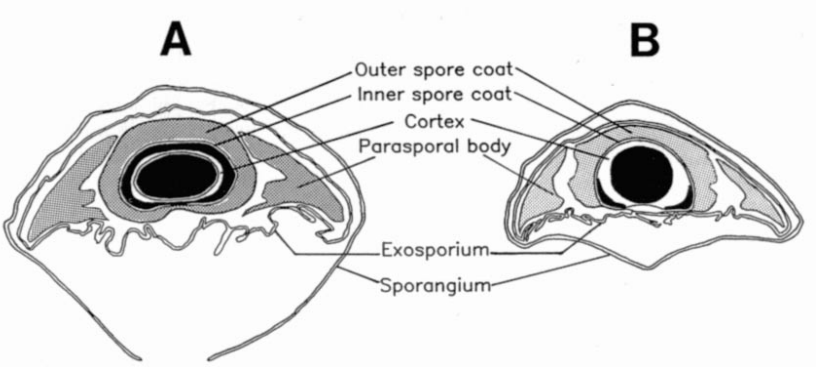

FIG. 6. Drawings from TEM photomicrographs of mature sporangia of the large-spored host isolates of *Pasteuria*. A) *Hoplolaimus galeatus* isolate from FLREC site 1A. B) *Belonolaimus longicaudatus* isolate from FLREC site 1B.

the *H. galeatus* isolate and *P. thornei*. The parasporal body was electron dense in *P. penetrans* sensu strictu and the *H. galeatus* isolate, but faint in *P. thornei* and the *B. longicaudatus* isolate. Parasporal fibers were observed in both described species and the two large-spored isolates, but were less distinct in *P. thornei* and the *B. longicaudatus* isolate. The angle of parasporal attachment to the outer spore coat was gradual in both isolates and *P. penetrans* sensu strictu, but steep in *P. thornei*. Endospore shape was elliptical in both isolates and *P. penetrans* sensu strictu, but spherical in *P. thornei*. The outer spore coat was electron dense in *P. penetrans* sensu strictu and the *H. galeatus* isolate, but faint in *P. thornei* and the *B. longicaudatus* isolate. The outer spore coat was very thin at the base in all species and isolates. *Pasteuria penetrans* sensu strictu was the only isolate or species with a ridge that encircled the ventral base of the outer spore coat. The inner spore coat was clearly defined in all of the species and isolates examined. The outer cortical wall was electron dense and present in all species or isolates examined; it was uniform in thickness in *P. penetrans* sensu strictu and the *H. galeatus* isolate and surrounded the spore sublaterally in *P. thornei* and the *B. longicaudatus* isolate.

Pasteuria spp. are obligate invertebrate parasites which appear fastidious and have not been cultured axenically (21). This has forestalled the rigorous biochemical studies that are normally part of a valid description for new species of bacteria. Therefore, the two described species of *Pasteuria* from nematodes, *P. penetrans* sensu stictu and *P. thornei*, were described and differentiated on the basis of morphological, developmental, and pathological criteria (17). A strong case could be made for separate species status for the large-spored isolates of *Pasteuria* from *H. galeatus* and *B. longicaudatus* based upon the field data, morphometrics, and ultrastructural comparisons presented in this paper. However, more developmental and pathological research is needed before a species separation for these two isolates is fully justified.

LITERATURE CITED

1. 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.

2. Davies, K. G., B. R. Kerry, and C. A. Flynn. 1988. Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. Annals of Applied Biology 112:491–501.

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

4. Esser, R. P. 1980. A bacterial spore parasite of nematodes. Nematology Circular No. 63, Florida Department of Agriculture and Consumer Services, Gainesville, Tri-ology 19:1–3.

5. Giblin-Davis, R. M., J. L. Cisar, and F. G. Bilz. 1988. Evaluation of three nematicides for the control of phytoparasitic nematodes in 'Tifgreen II' bermudagrass. Annals of Applied Nematology (Journal of Nematology 20, Supplement) 2:46–49.

6. Giblin-Davis, R. M., J. L. Cisar, and F. G. Bilz. 1988. Response of fairway managed bermudagrass to application of fertilizer and fenamiphos. Nematropica 18:117–127.

7. Jaffee, B. A., A. M. Golden, and R. M. Sayre. 1985. A bacterial parasite of *Hoplolaimus galeatus*. Journal of Nematology 17:501 (Abstr.).

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

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

10. SAS Institute. 1985. SAS user's guide: Statistics, version 5. SAS Institute, Cary, NC.

11. Sayre, R. M. 1980. Biocontrol: *Bacillus penetrans* and related parasites of nematodes. Journal of Nematology 12:260–270.

12. 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.

13. Sayre, R. M., M. P. Starr, A. M. Golden, W. P. Wergin, and B. Y. Endo. 1988. Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a related mycelial and endospore-forming bacterial parasite from *Pratylenchus brachyurus*. Proceedings of the Helminthological Society of Washington 55:28–49.

14. Sokal, R. R., and F. J. Rohlf. 1981. Biometry: The principles and practice of statistics in biological research, 2nd ed. New York: W. H. Freeman and Co.

15. Southey, J. F., editor. 1970. Laboratory methods for work with plant and soil nematodes. London: Her Majesty's Stationery Office.

16. Spurr, A. R. 1969. A low-viscosity resin embedding medium for electron microscopy. Journal of Ultrastructural Research 26:31–43.

17. Starr, M. P., and R. M. Sayre. 1988. *Pasteuria thornei* sp. nov. and *Pasteuria penetrans sensu strictu* emend., mycelial and endospore-forming bacteria parasitic, respectively, on plant-parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne*. Annales de l'Institut Pasteur/Microbiologie 139:11–31.

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

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

20. Sturhan, D. 1988. New host and geographical records of nematode-parasitic bacteria of the *Pasteuria penetrans* group. Nematologica 34:350–356.

21. Williams, A. B., G. R. Stirling, A. C. Hayward, and J. Perry. 1989. Properties and attempted culture of *Pasteuria penetrans*, a bacterial parasite of root-knot nematode (*Meloidogyne javanica*). Journal of Applied Bacteriology 67:145–156.

22. Yamashita, T. T., and D. R. Viglierchio. 1987. *In vitro* testing for nonfumigant nematicide resistance in *Xiphinema index*. Revue de Nématologie 10:75–79.