Journal of Nematology 24(1):29–35. 1992.
© The Society of Nematologists 1992.

Parasitism of *Helicotylenchus lobus* by *Pasteuria penetrans* in Naturally Infested Soil

A. Ciancio,[1] R. Mankau,[2] and M. Mundo-Ocampo[2]

Abstract: The population density of *Helicotylenchus lobus* and the percentage of the population with spores of *Pasteuria penetrans* were determined for 10 monthly intervals in naturally infested turfgrass soil at Riverside, California. The percentage of nematodes with attached spores ranged from 40% to 67%. No relationship was found between nematode density and the percentage of nematodes with spores. The mean and maximum numbers of spores adhering per nematode with at least one spore ranged from 2 to 8 and 7 to 66, respectively. The mean number of spores per nematode (based on total number of *H. lobus*) was correlated with the percentage of nematodes with spores. Spores adhered to both adult and juvenile *H. lobus*. Between 9% and 32% of the nematodes with spores had been penetrated and infected by the bacterium. Many infected nematodes were dead, but mature spores were also observed within living adult and juvenile *H. lobus* that exhibited no apparent reduction in viability and motility. Spore and central endospore diameters of this *P. penetrans* isolate were larger than those reported for the type isolate from *Meloidogyne incognita*, but transmission and scanning electron microscopy did not reveal significant morphological differences between the two isolates. Spores of the isolate associated with *H. lobus* did not adhere to juveniles of *M. incognita*.

Key words: biocontrol, *Helicotylenchus lobus*, host specificity, infection, nematode, parasitism, *Pasteuria penetrans*, population density, ultrastructure.

The obligate nematode parasite *Pasteuria penetrans* is considered one of the most promising antagonists of several important nematode pests. Little is known, however, about its population biology in soil or its effect on host population density. We recently found a natural infestation of *P. penetrans* parasitising the plant ectoparasitic nematode *Helicotylenchus lobus* in a lawn of perennial turfgrasses on the central campus quadrangle of the University of California, Riverside. In the present study, we describe the temporal changes in nematode density and in parasitism by *P. penetrans* over a 10-month period and provide additional data on parasitism biology and spore ultrastructure.

Materials and Methods

Nematode population study: H. lobus naturally infected by *P. penetrans* was found in irrigated ornamental turfgrass under the canopy of a white oak (*Quercus Garryana* Hook) on the campus of the University of California, Riverside. Soil samples (ca. 350 cm^3) were collected from two opposite loci (20 cm × 20 cm) equidistant from the tree at monthly intervals from June 1988 to March 1989, at 10–15 cm depth with a 2-cm-d Oakfield tube. The soil from both samples was mixed and nematodes were extracted from one 75-cm^3 subsample by Cobb's sieving decanting technique (13), with 1.4-mm and 45-μm pore diameter sieves. The nematodes in the extract were counted with a Hawksley counting chamber. At each month, an average of 50 *H. lobus* adults and juveniles were examined at 400× to determine the number of spores adhering to each nematode and the number of nematodes penetrated and infected by *P. penetrans*. Other nematode species were extracted and counted together with *H. lobus*, fixed in formalin, dehydrated, and mounted in glycerol for species identification (13). Nematophagous fungi were isolated as described by Mankau (8).

Light microscopy: Adult *H. lobus* with spores of *P. penetrans* were fixed in 5% formalin, dehydrated by the slow method, and mounted in glycerol (13). Some specimens also were stained during fixation by adding a few drops of a 5% aqueous ethanol solution of 1 mg/ml Coomassie Blue G (Sigma Chemical Co., St. Louis, MO) (1).

Received for publication 21 February 1991.

[1] Istituto di Nematologia Agraria AA.VV., CNR, Via Amendola 165/A, 70126 Bari, Italy.

[2] Professor and Staff Research Associate, Department of Nematology, University of California, Riverside, CA 92521.

The authors thank Dr. J. W. Seinhorst for kindly commenting on the manuscript.

Transmission electron microscopy (TEM): P. penetrans-infected *H. lobus* were fixed in 3% glutaraldehyde in 0.05% phosphate buffer (pH 6.8), rinsed in several changes of phosphate buffer, post-fixed in 2% OsO_4 aqueous solution for 2 hours, rinsed in phosphate buffer, and dehydrated in a hexylene glycol series from 10% to 100% before infiltration with Spurr's medium (16). Grey–silver (60–90 nm) and thicker sections were prepared with a Sorvall MT-2B ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a Hitachi Electron Microscope operating at 75 kV.

Scanning electron microscopy (SEM): Adult and juvenile *H. lobus* infected by *P. penetrans* were fixed in 5% formalin, dehydrated and slowly infiltrated with glycerin, and mounted on stubs (12). Specimens were coated with 20-nm gold palladium and examined with a JOEL 35-C at 5 kV. Some specimens were crushed on the stubs before coating for SEM examination of spores.

Host range test: Approximately 5,000 healthy second-stage juveniles (J2) of *Meloidogyne incognita* in 50 ml water were poured on each of three modified Baermann funnels containing 150 cm^3 of soil from which the *H. lobus* had been extracted and air-dried. The juveniles were collected after 48 hours at room temperature and were examined with a compound microscope at 400×.

Results

No relationship was found between the monthly density of *H. lobus* and the percentage with spores (Fig. 1). The *H. lobus* population increased from June, 1988, through September, 1988, and subsequently decreased through March, 1989. The percentage of the population with spores of *P. penetrans* was 49.4 ± 9.7 (mean ± SD) over all sample times.

The mean and maximum numbers of *P. penetrans* spores adhering per nematode with spores increased during the 10 months of the study (Fig. 2). The mean number of spores per nematode (total

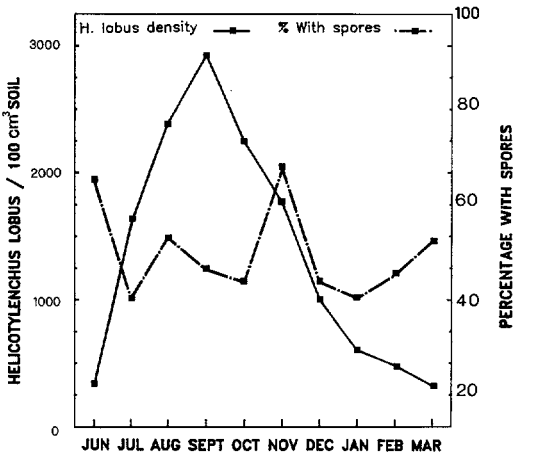

FIG. 1. Population density of *Helicotylenchus lobus* and percentage of *H. lobus* with spores of *Pasteuria penetrans* in naturally infested soil. Data are from one soil sample per month.

number of spores recorded per total number of nematodes examined) was correlated with the percentage of nematodes with spores ($r = 0.642$; $P < 0.05$) and the mean number of spores per infected nematode ($r = 0.884$; $P < 0.001$).

Some adult and juvenile *H. lobus* were filled with *P. penetrans* spores at each monthly sampling (Fig. 3E). The percentage of nematodes that had spores attached and that were partially or totally filled with sporangia ranged from 9% (November, 1988) to 32% (August, 1988).

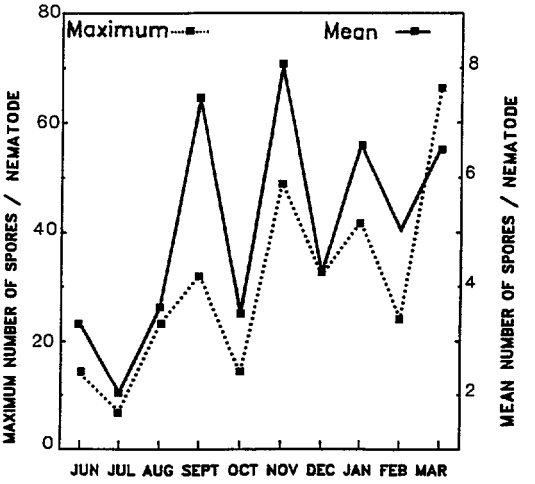

FIG. 2. Maximum and mean numbers of *Pasteuria penetrans* spores per *Helicotylenchus lobus* with spores in a naturally infested soil. Data are from one soil sample per month.

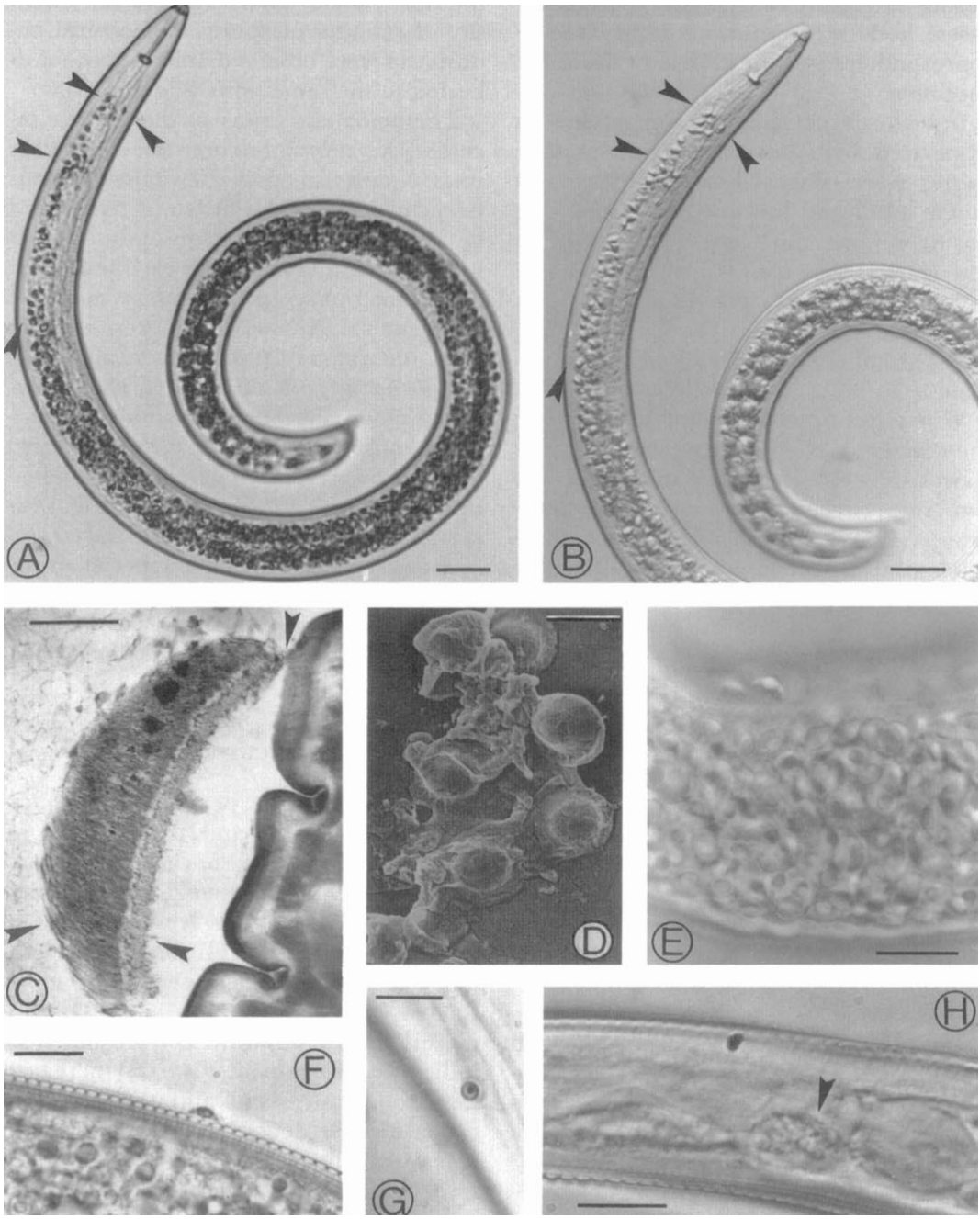

FIG. 3. Parasitism of *Helicotylenchus lobus* by *Pasteuria penetrans*. A, B) Heat-killed specimen of juvenile *H. lobus* with *P. penetrans* sporangia within the neck and anterior of the body (arrows). (A: light transmission; B: interference contrast). C) TEM micrograph of a marginal section of *P. penetrans* spore adhering to *H. lobus* showing the inner and outer layers of parasporal fibers (arrows). D) SEM micrograph of *P. penetrans* infective spore covered by the exosporium. E) Light micrograph of *H. lobus* filled by sporangia. F, G) Spores adhering to *H. lobus* stained by Coomassie blue during fixation. H) Vegetative thallus (arrow) of the parasite within the esophageal region of parasitised *H. lobus*. (Scale bars: A, B = 20 μm; C = 1 μm; D = 5 μm; E, H = 10 μm.)

A few *H. lobus* that were filled with spores also had ungerminated spores adhering externally on the cuticle. In some cases, both refractile and nonrefractile spores adhered to the cuticle of the same specimen.

Infection by the parasite was not always associated with host mortality: mature spores were observed within living and motile adult and juvenile *H. lobus*. Living juveniles were also observed with only a few *P. penetrans* spores inside the body, usually in the esophageal region (Fig. 3A, B). In a few cases, clearly visible sporangia only partially filled the body of mature females.

When examined by light microscopy, the diameters of spores and central endospores were 4.6 ± 0.3 μm and 2.0 ± 0.1 μm, respectively. SEM and TEM micrographs (Fig. 4B, D) did not reveal any morphological differences between spores of this population of *P. penetrans* and those of the type isolate described from *M. incognita*. In the host-range test, however, no *M. incognita* juveniles became spore-encumbered when passed through soil infested with the *H. lobus* parasite. The spores were coated with the typical exosporium (Figs. 4C, 3D) and displayed an inner basal enlargement (Fig. 4E) and two distinct layers of parasporal fibers (Fig. 3C). Spores adhered to the host along the entire cuticle of *H. lobus*, from the lip region to the tail mucron, although more spores adhered to the cephalic region (Fig. 4A). In a few cases, spores were attached upside down, by the convex side, or only by a marginal portion of the parasporal fibers. Some *H. lobus* juveniles were observed losing ungerminated spores with the cuticle during a molt.

Despite the large numbers of nematodes counted, penetration pegs were seldom seen. The vegetative thallus (Fig. 3H) appeared to grow intracellularly, digesting the surrounding tissues and occupying the resulting cavity. Similar internal cavities containing fully formed sporangia were observed when *P. penetrans* only partially filled the host's body.

Coomassie blue in the fixative stained spores, as well as labial and vulvar lips, on the host cuticle. Differences in the intensity of staining of spores and central endospores were observed among spores adhering to the same hosts (Fig. 3F, G).

The nematode fauna of the test site included *Xiphinema californicum, Coslenchus* sp., *Criconema* sp., and a few miscellaneous tylenchids, none of which was parasitised by *P. penetrans*. Predacious mononchids were found at low levels at each sampling. The nematophagous fungi *Monacrosporium thaumasia* and *Arthrobotrys* sp. were isolated from the soil, and the endoparasitic fungi *Catenaria anguillulae, Haptoglossa heterospora, Lagenidium* sp., and other unidentified zoosporic fungi were isolated from diseased or parasitized nematodes. The influence of these antagonists on the *H. lobus* population was not quantified. *Helicotylenchus lobus* was the dominant species in the fauna; *H. lobus*, miscellaneous tylenchs, microbiovores, and predators represented 25%, 15%, 55%, and 5% of the nematodes, respectively.

Discussion

Nematode density, percentage of nematodes with spores, and the number of spores per nematode appeared to vary independently. The reasons for this lack of relationship are unclear. A number of factors may reduce the efficacy of *P. penetrans*. Temperature has been shown to influence *P. penetrans* parasitism of *M. javanica* (17), and climatic conditions may indirectly affect parasitism as increased nematode movement and reproduction can reduce the density of free spores available for further hosts. A high and constant level of food source such as the turf lawn may balance nematode mortality by enhancing nematode reproduction (11). Spore density and distribution could also be influenced by the restricted host movement and parasite reproduction observed for some infected individuals with large spore burdens. Some *H. lobus* juveniles lose adhering ungerminated spores during a

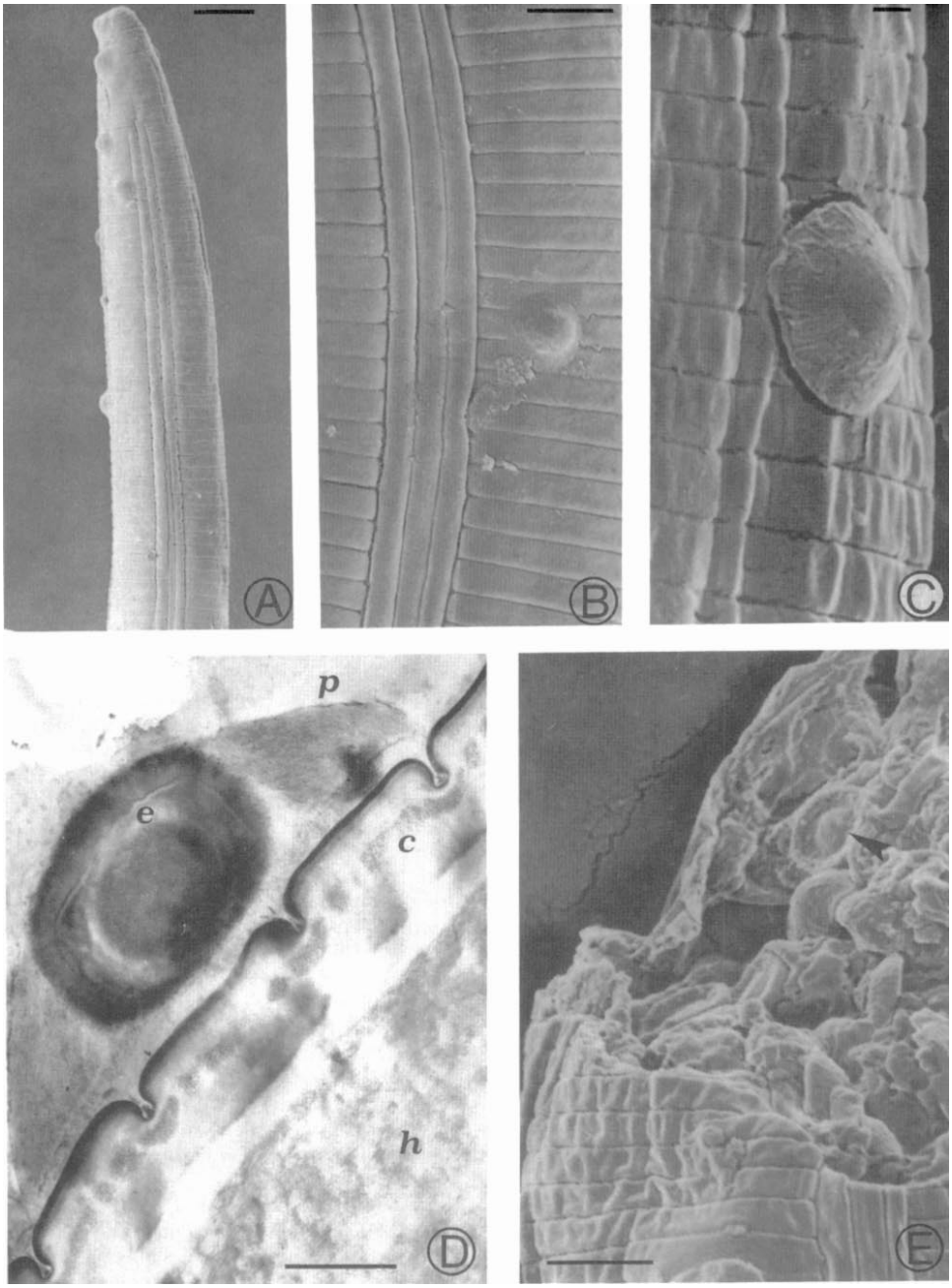

FIG. 4. Scanning (SEM) and transmission electron (TEM) micrographs of *Pasteuria penetrans* parasitising *Helicotylenchus lobus*. A–C) SEM images of *P. penetrans* spores adhering to the cuticle of *H. lobus*. A) Anterior region of *H. lobus*. B) Spore not covered by exosporium. C) Spore covered by remnants of the exosporium. D) TEM image of a cross section through a *P. penetrans* spore adhering to host's cuticle (c); (p: parasporal fibers; e: central endospore; h: hypodermis). E) *P. penetrans* sporangia inside an infected *H. lobus* showing the inner basal enlargement (arrow). (Scale bars: A = 10 μm; B = 5 μm; C, D = 1 μm; E = 5 μm.)

molt. Finally, in laboratory experiments with the root-knot isolate of *P. penetrans*, spore adhesion to the host cuticle did not always induce infection, as not all spores germinated after adherence (4).

The development of a population model

for *P. penetrans* would be valuable. The model could help explain how spore density changes over time and the amount of time required to suppress a nematode host population below an economically acceptable plant damage threshold. The model requires the definition and the quantification of a variety of factors involved in the host–parasite interaction. It is unlikely, however, that all *Pasteuria*–nematode relationships will be similar because life histories and reproductive rates vary among nematodes and isolates of *P. penetrans*. After studying the interactions of *P. penetrans* and *M. incognita* in South Africa, Spaull (15) concluded that *P. penetrans* did not limit host density but only removed surplus individuals; the proportion of nematodes with spores was positively correlated with nematode density. Others (2,9), however, have reported substantial reductions of *Meloidogyne* spp. and other host populations by the parasite in microplot and field studies. In Southern Florida, a constant proportion of nematodes with *P. penetrans* spores was reported from a bermudagrass turf infested by *Belonolaimus longicaudatus*, whereas an inverse relationship between nematode densities and proportion of nematodes with spores was observed in a population of *Meloidogyne* spp. (9).

The observation of motile, active juveniles of *H. lobus* with a few *P. penetrans* spores within the body (Fig. 3A, B) suggests that sporulation can occur very rapidly after penetration of the host, that maturation of infected juveniles may be delayed, or that transovarial transmission of the thallus to the first-stage juvenile may be possible. We have also observed mature sporangia of a small spore-size parasite within motile juvenile Cephalobidae (unpubl.) and in a juvenile *Xiphinema brasiliense* parasitized by a large spore-size *Pasteuria* sp. found in Peru (3). Other *P. penetrans* isolates have been reported sporulating in J2 of *Meloidogyne* spp., *Heterodera avenae*, and *Tylenchulus semipenetrans* (5–7).

The *Pasteuria* isolate studied here did not show enough original structural features to establish a new species, although the mean diameters of spores and central endospores were larger than those reported for the type isolate in *M. incognita* (10). Morphometrics of spores from *H. lobus* do not differ statistically from those of *P. penetrans* spores found within a specimen of *H. pseudorobustus* from Messina (Italy; unpubl.) and are similar to those reported from an infected *H. krugeri* (14). Among reported *Helicotylenchus* hosts, the morphometrics of the *H. lobus* isolate differ only from those of a small spore-size isolate found on *H. dihystera* in South Africa (14). Parasitism in *H. lobus* was not previously reported, and it is possible that our isolate represents a pathotype specific to a number of *Helicotylenchus* species.

Literature Cited

1. Bird, A. F. 1988. A technique for staining the endospores of *Pasteuria penetrans*. Revue de Nématologie 11:364–365.

2. 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.

3. Ciancio, A., and R. Mankau. 1989. Note on *Pasteuria* sp. parasitic in Longidorid nematodes. Nematrópica 19:105–109.

4. Davies, K. G., B. R. Kerry, and C. A. Flynn. 1989. Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. Annals of Applied Biology 112:491–501.

5. Davies, K. G., C. A. Flynn, V. Laird, and B. R. Kerry. 1990. The life-cycle, population dynamics and host specificity of a parasite of *Heterodera avenae*, similar to *Pasteuria penetrans*. Revue de Nématologie 13:303–309.

6. Fattah, F. A., H. M. Saleh, and H. M. Aboud. 1989. Parasitism of the citrus nematode, *Tylenchulus semipenetrans*, by *Pasteuria penetrans* in Iraq. Journal of Nematology 21:431–433.

7. Giblin-Davis, R. M., L. L. McDaniel, and F. G. Bilz. 1990. Isolates of the *Pasteuria penetrans* group from phytoparasitic nematodes in bermudagrass turf. Supplement to Journal of Nematology 22:750–762.

8. Mankau, R. 1975. A semiquantitative method for enumerating and observing parasites and predators of soil nematodes. Journal of Nematology 7:119–122.

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

10. 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.

11. Seinhorst, J. W. 1966. The relationships between population increase and population density in plant parasitic nematodes. I. Introduction and migratory nematodes. Nematologica 12:157–169.

12. Sher, S. A., and A. H. Bell. 1975. Scanning electron micrographs of the anterior region of some species of Tylenchoidea (Tylenchida: Nematoda). Journal of Nematology 7:69–83.

13. Southey, J. F. 1970. Laboratory methods for work with plant and soil nematodes. Technical Bullettin No. 2, London: Her Majesty's Stationery Office.

14. Spaull, V. W. 1981. *Bacillus penetrans* in South African plant parasitic nematodes. Nematologica 27:244–245.

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

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

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