Biocontrol: Bacillus penetrans and Related Parasites of Nematodes[1]

R. M. Sayre[2]

Abstract: Bacillus penetrans Mankau, 1975, previously described as *Duboscqia penetrans* Thorne 1940, is a candidate agent for biocontrol of nematodes. This review considers the life stages of this bacterium: vegetative growth phase, colony fragmentation, sporogenesis, soil phase, spore attachment, and penetration into larvae of root-knot nematodes. The morphology of the microthallus colonies and the unusual external features of the spore are discussed. Taxonomic affinities with the actinomycetes, particularly with the genus *Pasteuria*, are considered. Also discussed are other soil bacterial species that are potential biocontrol agents. Products of their bacterial fermentation in soil are toxic to nematodes, making them effective biocontrol agents.
Key Words: Duboscqia, Pasteuria ramosa, Pseudomonas denitrificans, Clostridium butyricum, Desulfovibrio desulfuricans, Bacillus thuringiensis, rickettsia.

Nematodes and bacteria are two important members of the total biota in soil habitats. The diversity of the species, and their ubiquity and abundance in soils, for thousands of years have provided opportunity for the evolution of intimate and complex interactions between the two groups of organisms. Theoretically, repeated analysis of soil habitats for bacteria-nematode interactions should reveal all of the several possible interactions that could occur between the two species. Several have been suggested by Odum and Odum (22): neutralism, competition, mutualism, proto cooperation, commensalism, predation, parasitism, and amensalism. Only two of these interactions, parasitism and amensalism, are considered here. These interactions are most pertinent to the biological control of plant-parasitic nematodes, the general topic of this paper.

The first interaction, parasitism, will emphasize the bacterium *Bacillus penetrans* Mankau, 1975 (16), and its interactions with a few plant-nematode hosts. Aspects of this interaction may seem atypical because of the bacterium's unusual morphology. However, it is currently the best documented example of an interaction between a plant nematode and parasitic bacterium (5,7,11,14,15,16,17,19,20,25,30,32,33). Additional data on two other bacterial parasites of nematodes are presented briefly. The second interaction, amensalism, considers the inhibitory or antibiotic effect of some species of bacteria on species of plant-parasitic nematodes.

When Thorne (30) described *Duboscqia penetrans* as a protozoan, he could not have realized its bacterial nature because electron-microscope techniques were not available to him, and the concept of the prokaryotic cell had not been introduced. Later, Williams (32) studied the same organism in a population of root-knot females taken from sugarcane, presented an interpretation of its life stages, and indicated some reservations about Thorne's identification. Nevertheless, he used Thorne's designation *Duboscqia*. His drawings agree well with recent electron micrographs of the organisms (Fig. 1). Canning (3) also doubted the identification as a protozoan and stressed the organism's fungal characteristics. Electron-microscope studies of Mankau (16) and Imbriani and Mankau (11) established the prokaryotic and bacterial nature of the organism.

Bacillus penetrans—life cycle

A) *Spore germination:* Germination occurs about 8 days after the spore-encumbered nematode enters the root and begins feeding in the host. The germ tube of the spore emerges through the central opening of the basal ring (Fig. 2) and penetrates the cuticle of the nematode. After the hypodermal tissue is entered, a spherical vegetative hyphal colony is formed (Fig. 3). While spore germination and colony formation seem more typical of fungi than of

Received for publication 10 October 1979.

[1]Symposium paper presented at the annual meeting of the Society of Nematologists, Salt Lake City, Utah, U.S.A., 23-26 July 1979.

[2]Research Plant Pathologist, Nematology Laboratory, Plant Protection Institute, Agricultural Research, Science and Education Administration, U.S. Department of Agriculture, Beltsville Agricultural Research Center (West), Beltsville, MD 20705.

The author gratefully acknowledges the help of Dr. W. P. Wergin, Mr. R. B. Ewing, and Ms. A. Ostericher in preparing the figures.

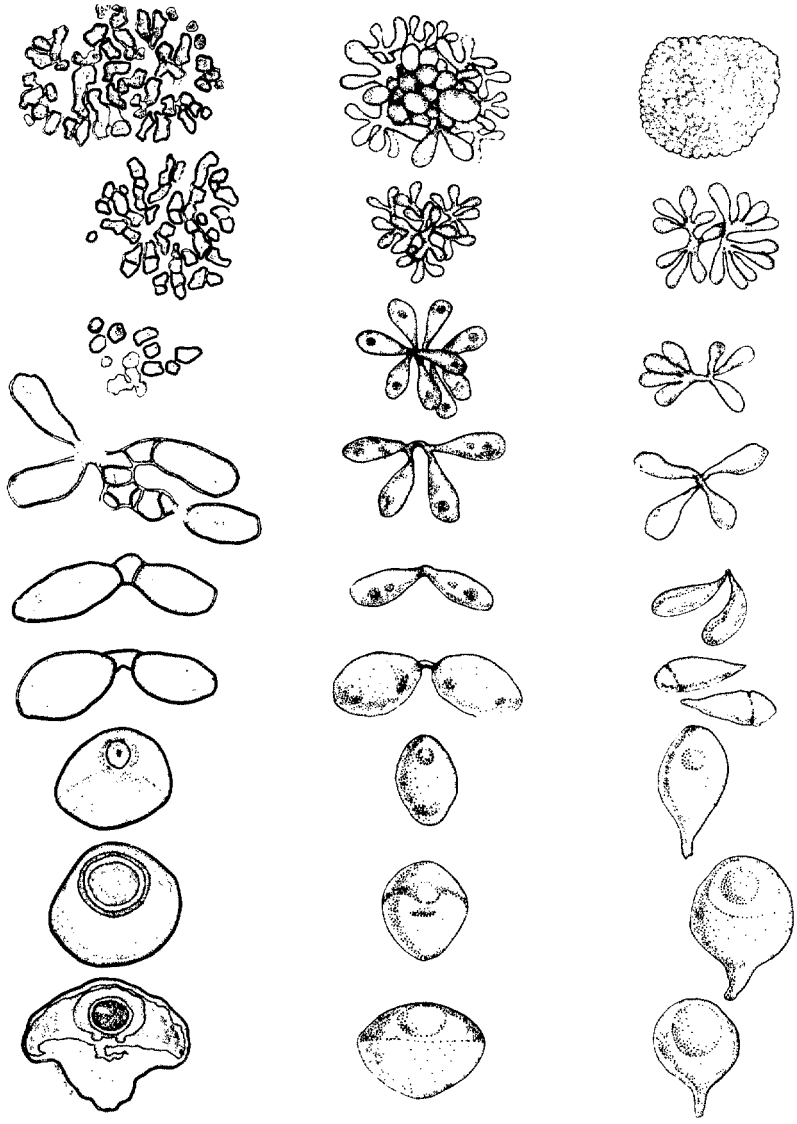

Fig. 1. Drawings of *Bacillus penetrans* from *Meloidogyne incognita* (left column) are compared with those of Williams (32) for *Dubosqia penetrans* from *M. incognita* and *M. javanica* (center column), and with those of Metchnikoff (21) for *Pasteuria ramosa, Daphnia pulex* and *D. magna* (right column). Life stages of *B. penetrans,* based on electron micrographs, start at the top of the column with the vegetative colony, followed by daughter colonies, quartets of sporangia, doublets, single sporangium, and finally the mature endospore within the old sporangial wall at the bottom. Drawings of *D. penetrans,* selected from the original publication, are arranged arbitrarily to show the similarities in morphology to *B. penetrans,* its synonomous species. Drawings of *P. ramosa* are placed in order of their occurrence in the life cycle of the parasite as reported by Metchnikoff (21).

bacteria, close examination of the vegetative cell reveals bacterial characteristics.

B) *Vegetative stage:* Mankau (15) found the vegetative cells to be prokaryotic, recognized the organism's bacterial characteristics, and named the organism *B. penetrans.* In a later study involving all life stages occurring in the nematode host, *Meloidogyne incognita,* Sayre and Wergin (25) also found organelles characteristic only of the prokaryotic cell. In neither of these studies were nuclear membranes, plastids, mitochondria, or any other exclusively eukaryotic cell characteristic found.

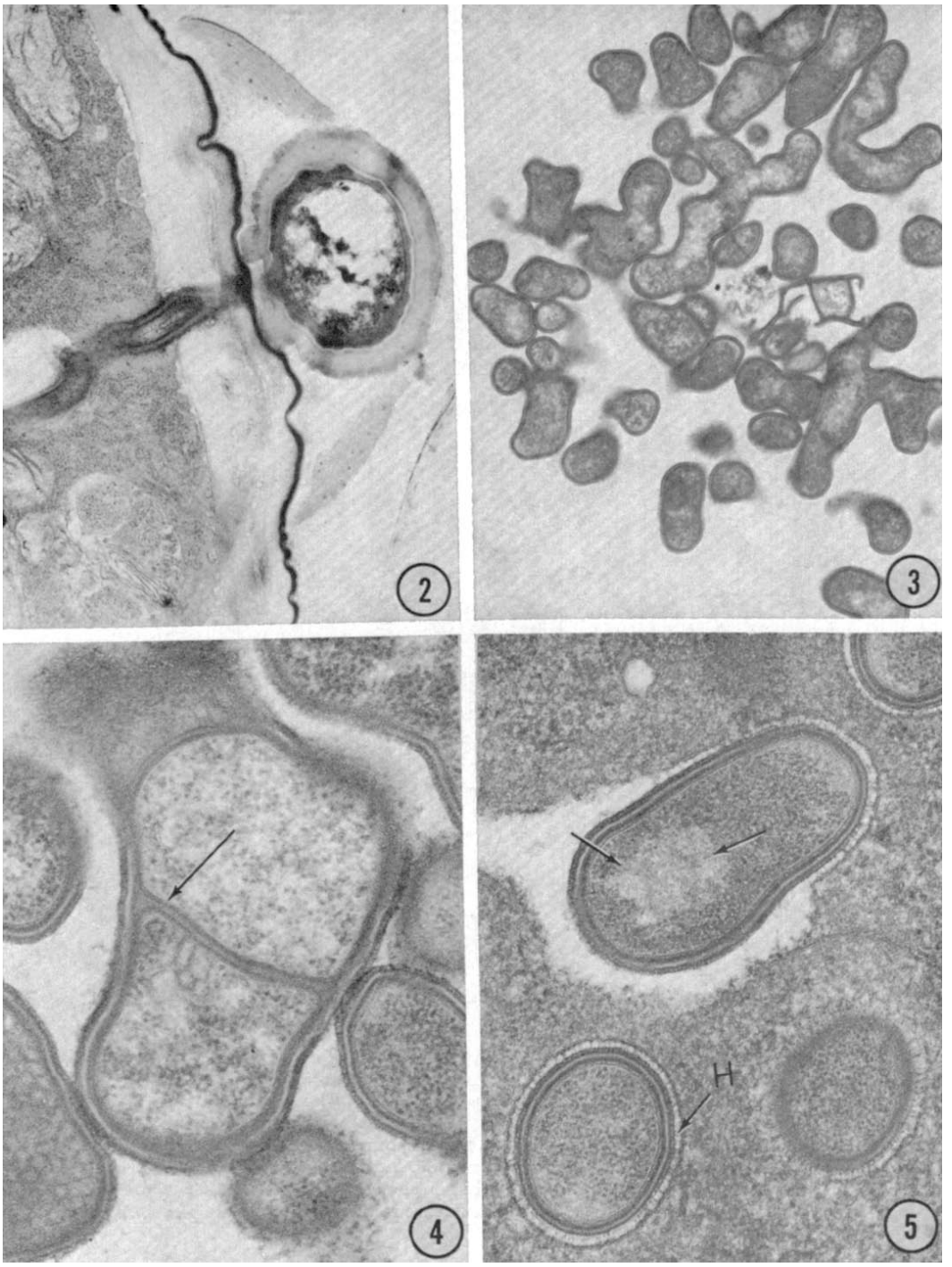

Fig. 2. Cross-section through a germinated spore. The penetrating germ tube follows a sinuous path as it traverses the cuticle and hypodermis of the nematode. ×20,000.

Fig. 3. Portion of a mycelial colony in the pseudocoelom of the nematode. The hyphae, which are septate, appear to bifurcate at margins of the colony. ×7,500.

Fig. 4. Hyphal cells are bounded by a compound wall consisting of a double membrane (arrow). ×23,000.

Fig. 5. Section through vegetative hyphae. The hyphae contain numerous ribosomes and amorphous areas (arrows) that may contain genetic material. Short projections, which become evident on the outer surface of hyphae that lie within an electron-opaque matrix, result in the appearance of a clear surrounding "halo" (H). ×11,000.

Clearly, the research findings indicate that the use of the generic protozoan designation of *Duboscqia* is not warranted.

The bacterial hyphal cells comprising the colony are septate and bounded by a compound wall (Fig. 4). The outer wall membrane frequently contains short projections, resulting in a clear space or halo about the mycelium (Fig. 5). The inner membrane forms the septations and delineates individual cells. In addition, mesosomes are often found associated with the inner membranes. A lighter, amorphous area in the cells may contain the genetic materials (Fig. 6).

C) *Fragmentation of colonies:* Daughter colonies are formed when intercalary cells lyse, allowing a separation to occur within the mother colony.The process of internal lysis occurs periodically during the parasite's vegetative development. Gradually, daughter colonies contain fewer, but larger, vegetative cells. Eventually, quartets of developing sporangia predominate in the nematode's psuedocoloem. These structures are followed by doublets of sporangia, and finally the single sporangial stages that give rise internally to single endospores (Fig. 1).

D) *Sporogenesis:* The external morphology of the endospore of *B. penetrans* is unique; but its internal stages of spore formation are typical of other endogenous sporeforming bacteria. The spore stages consist of 1) septum formation in the anterior of the spore mother cell; 2) condensation of a forespore from the anterior protoplast; 3) formation of multilayered walls about the forespore; 4) lysis of the old sporangial wall; and 5) release of an endospore that resists heat and desiccation, and survives for long periods in storage (Fig. 7). When the vegetative and sporangial stages of *B. penetrans* are examined, they pose a problem in systematics. The vegetative stages, being hyphalike, suggest actinomycetous affinities, while the sporangial stages, identical to spore development in the genus *Bacillus* and *Clostridium* sp., suggest affinities with members of the Bacillaceae. The problem would be partially resolved if endogenous spore formation were to occur in the Actinomycetales. Good evidence suggests that endospores are found in some groups of the actinomycetes. Cross (4) presented evidence for true endogenous spore formation in several genera of the Actino-

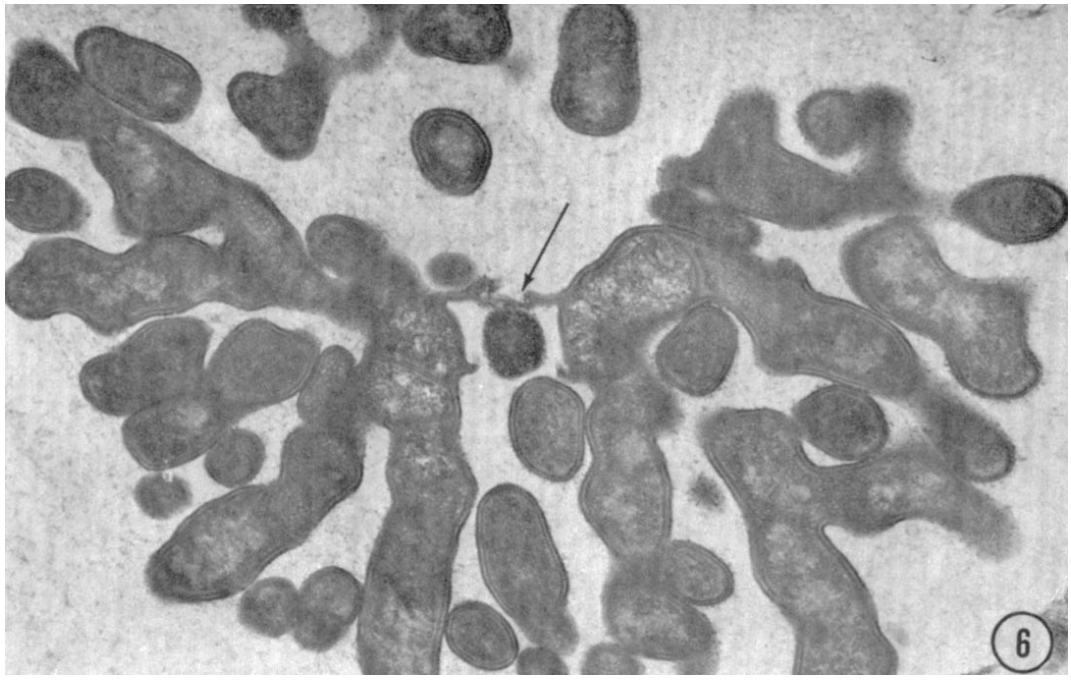

Fig. 6. Section through microcolony. A few intercalary cells lyse or separate from one another (arrow), and this allows for the formation of daughter colonies. These processes can be found in all stages of development; ultimately only separate sporangial cell are found in the mature parasitized root-knot female. ×8,250.

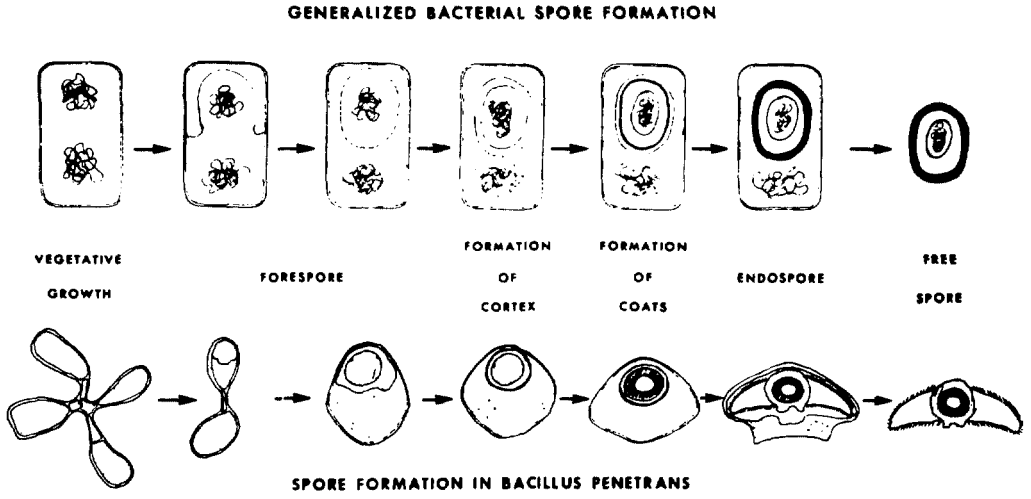

Fig. 7. Drawings showing generalized bacterial spore formation (upper row) are compared with the sporogenous stages of *Bacillus penetrans* in the lower row. Aside from differences in parasporal structures, stages in the development of *B. penetrans* result in spores having characteristics very similar to those of the other endogenous sporeforming bacteria.

mycetales. *B. penetrans* also has some additional characteristics consistent with the Actinomycetales: 1) Vegetative cells measure less than 0.2 μm in diameter and are gram-positive. 2) Hyphal-like colonies are formed and segmented during vegetative growth to yield individual club-shaped sporangia. 3) Ultrastructure of the cell walls reveals a double-tracked membrane. The outer membrane bears hairlike projections similar to those of some species of actinomycetes described by Slack and Gerencser (28). 4) Germination of spores of *B. penetrans* is like that of some endospores found in the Actinomycetales (Fig. 8).

While the above characteristics are indicative that *B. penetrans* is related to members of the Actinomycetales, the only positive way to establish such a relationship must be based on standard bacteriological

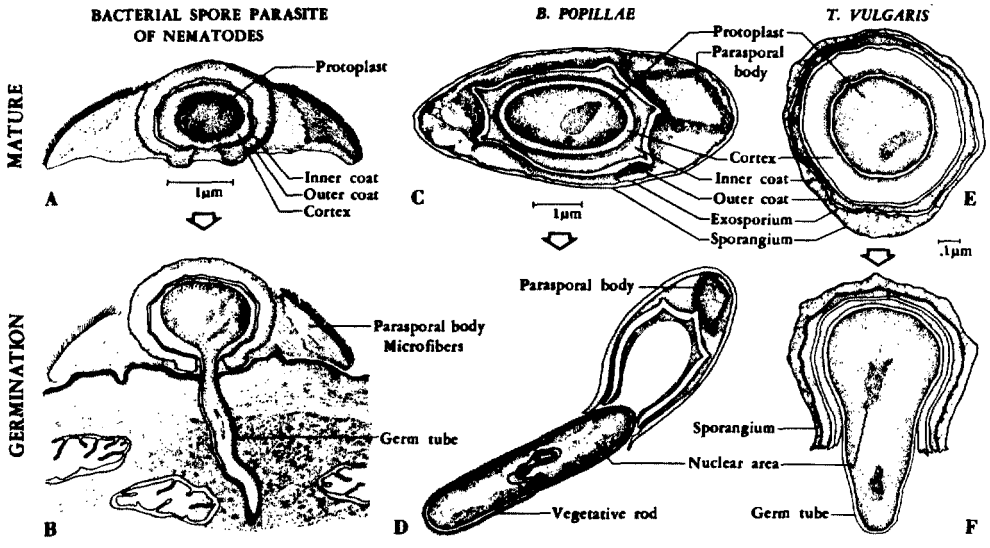

Fig. 8. Comparison of coat morphology and germination of mature spores of *B. penetrans* (A), *B. popillae* (C), and *Thermoactinomyces vulgaris* (E). *B. penetrans* (B) and *T. vulgaris* (F) differ in size, but both form a filamentous tube on germination. *B. popillae* (D) germinates as a vegetative rod.

tests. These tests depend on *in vitro* cultivation of the organism, which will have to be a future research goal.

The rediscovery of *Pasteuria ramosa* Metchnikoff, 1888, by Sayre et al. (26) and its morphological similarities to *B. penetrans* add more difficulty to systematic placement of *B. penetrans*. *B. penetrans* and *P. ramosa* form microcolonies that give rise by fragmentation to quartets, then sporangia, and finally an endogenous spore within the old mother cell wall (Fig. 1). The life stages in the two organisms, when examined by scanning and transmission electron microscopy, are remarkably similar in sequence, suggesting a generic relationship. However, another neotype already bears the name *P. ramosa* (ATCC No. 27377). It has been placed by Staley (29) in the budding-bacteria group. The amended description of the *P. ramosa* neotype encompasses a nonparasitic flagellated bacterium. Consequently, the current neotype species of *P. ramosa* is unlike the original bacterium figured and described by Metchnikoff (21). This systematic problem involving the two type strains of *P. ramosa* must be resolved if the generic name *Pasteuria* is ever to be applied to the nematode parasite.

E) *Soil phase:* In a laboratory analysis, Mankau (16) found about 2.1×10^6 spores in each parasitized root-knot female. But the fate of these spores when released from a decomposed dead nematode is not known. Neither the distance they move nor their rate of movement in soil has been measured. If the spores are nonmotile and have no surface charge, their dispersion from point of release would depend largely on the rate of water percolation in soil, on the size of soil pore openings, on tillage practices, and to a lesser extent, on the activities of soil invertebrate populations. If these spores behave as Dutky (6) described for spores of *Bacillus popillae,* and are tightly bound to soil particles at the point of release, the relative importance of soil factors in movement of the spores would be altered.

F) *Spore attachment to nematode:* As far as known, the dispersed spores in soil attach only to nematode hosts, atlhough edaphic factors may influence the attachment process. For example, the adhesion fibers of the mature endospore must be exposed for nematode attachment to occur (Fig. 9). When mature parasitized females are manually crushed in laboratory examination, most of their spores are surrounded by a sporangial wall and a thin exosporium. These two covering layers are apparently removed or degraded in soil by an unknown process.

Because no methods are available for cultivation or isolation of *B. penetrans* from soils on a selective medium, a bioassay method is used to detect spores. Healthy larvae of the nematodes are allowed to migrate through spore-infested soil. The larvae emerging from a soil sample are examined microscopically for the spore load carried on their cuticles. The percentage of larvae encumbered with spores and relative numbers of spores on each larva serve as an indicator of the concentration of spores in soil. The raised lateral fields of the larvae are frequently observed to be the cuticular areas where spore attachement occurs most frequently (Fig. 10). Another area for future research is an explanation of the specificity of spore attachment to certain nematodes and not others.

Dutky (5) used the bioassay method of spore attachment to study the host ranges of three different isolates of the spore parasite. One isolate occurred on *M. incognita* and the other two adhered to populations of *Pratylenchus brachyurus* (Godfrey) from Florida and Maryland. The root-knot nematode isolate of the bacterium did not attach to the *Pratylenchus* sp. bacterial isolate, or vice versa. Dutky also found that spores attaching to the root-knot nematodes were significantly larger in diameter than those from *P. brachyurus*. Apparently, different strains of *B. penetrans* exist. Dutky and Sayre (7) also studied the influence of soil moisture, texture (pore space), and temperature on spore attachment. No correlation was found between the numbers of spores attached per larva and the soil moisture level, or the soil pore size. To determine the thermal inactivation point of spores in soil, *M. incognita* larvae were exposed to a spore-infested soil incubated previously for 1 hr at 40, 60, 80, 100, 120, and 140 C. The level of spore attachment was no lower in soils heated to 40 C than in unheated control soils. Heating soils to 60,

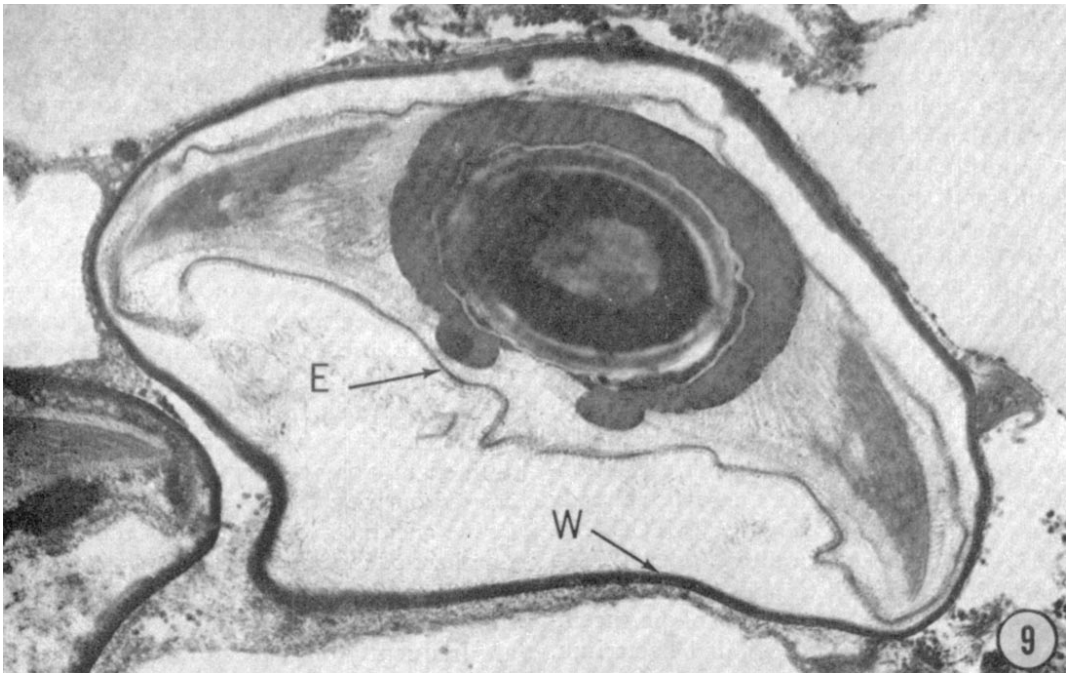

Fig. 9. Section through a sporangium undergoing the final stage of differentation. The endospore has lost its tight apposition with the wall of the sporangium. The matrix of the parasporal segment becomes finely filamentous, and parasporal fibers are found. The spore and fibers are encircled by a membrane or exosporium (E) and the old sporangial wall (w). ×1,300.

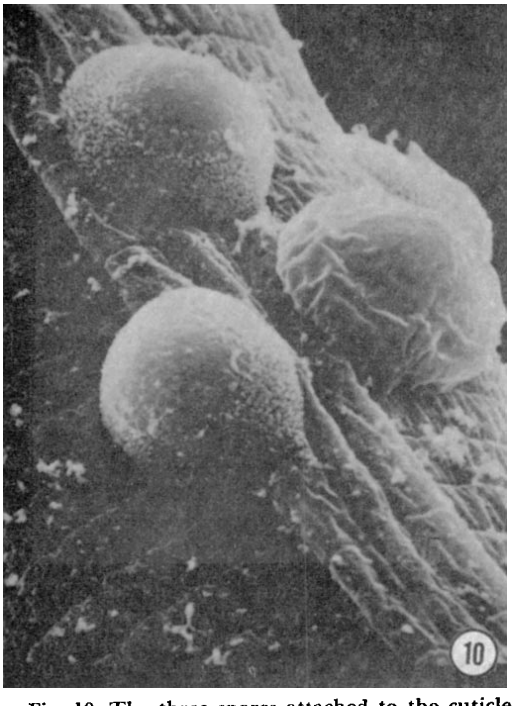

Fig. 10. The three spores attached to the cuticle of this larva renew the life cycle of *Bacillus penetrans*. The raised lateral field (F) of the larva provides a favorable cuticular area for attachment. ×1,000.

80, 100, and 120 C reduced the number of spores attached per larva and the percentage of larvae with spores attached. No spores attached at 140 C. Spores heated to 60, 80, 100, and 120 C may be able to attach to nematodes but not to penetrate or develop inside them. The true thermal inactivation point for these spores is somewhat lower than 140 C. Investigations of the influence of other factors, such as soil pH and osmotic potential on the ability of spores to infect nematodes, would also be useful.

Bacillus penetrans— geographical distribution

Parasitism of nematodes by *B. penetrans* has been reported from 12 states and 16 foreign countries (5). These reports probably reflect the presence of a nematologist in that state or country where nematode populations are periodically sampled. They suggest worldwide distribution. Thorne (30) found 66% of a South Carolina population of *Pratylenchus* infested, while only 28% of a Georgia population had succumbed to the parasite. He believed the parasite to be widespread in the South Atlantic states.

Williams (32) reported that 34% of a population of root-knot nematodes were parasitized by an organism very much like *B. penetrans*. In Florida, Esser and Sobers (9) observed 25% of a fixed population of *Helicotylenchus* to be diseased, while 80% of living populations were infested externally or internally by the parasite. Other reports were less precise. In California, Allen (2) reported that a high percentage of *Dolichodorus obtusus* Allen were infested.

Bacillus penetrans— control experiments

Mankau (16) presented data on the control of root-knot nematodes using *B. penetrans*. In greenhouse tests, air-dried soil infested with spores of *B. penetrans* was planted with tomato seedlings to which 10,000 root-knot nematode larvae had been added. After 70 days, plants in the air-dried spore-infested soil had greater dry weights, more leaves, and less root galling than plants in soil free of spores or growing in sterilized soil (Table 1).

In microplot experiments, Mankau (14) used the following treatments: A) air-dried soil containing spores was placed in holes 3 inches wide and 6 inches deep; B) seedlings were grown in spore-infested soil and then transplanted into microplots; C) 240,000 larvae encumbered with spores were added to plots to a depth of 4 inches. When the soils were bioassayed 11 months after cropping, 98% of the larvae emerging from treatment *A* were heavily encumbered with spores. In *B*, only 53% carried spores, but there were only a few spores per larva. In *C*, 7% were lightly infected. This result suggested that small amounts of spore-infested soils were an effective means for introducing the parasites into field plots.

Table 1. Effect of *Bacillus penetrans* on root-knot disease of tomato 70 days after inoculation with 10,000 *Meloidogyne incognita* larvae.[a]

Soil	Leaves/ plant	Gall rating (0-25)	Tops (g)
A. Air-dried, spore-infested	21.0(b)[b] (±2.2)	6.5(b) (±2.9)	5.32(b) (±0.8)
B. Sterilized, spore-infested	14.2(a) (±4.4)	20.5(a) (±5.2)	3.12(a) (±0.5)
C. Air-dried, uninfested (control)	13.2(a) (±4.4)	22.7(a) (±4.5)	3.42(a) (±0.95)

[a]Taken in part from data of Mankau (16).

[b]Means followed by the same letter are not significantly different from each other at the 5% level.

Mankau and Prasad (19) tested seven nematicides at recommended field doses to determine their compatibility with *B. penetrans:* 1,3-D, aldicarb, carbofuran, phenamiphos, and ethroprop had no noticeable effect on the bacterial parasite, while 1,2-dibromo-3-chloropropane was only slightly toxic.

The ability of *B. penetrans* to prevent reproduction and eventually kill root-knot nematodes as well as several other pest nematode species, offers a good possibility for biological control of a major crop pest. The resistance of these long-lived spores to heat and desiccation, and their compatibility with nematicides, are characteristics well suited for use in field soils.

Other Prokaryotic Parasites

Two additional prokaryotic species are also parasitic to plant nematodes and function as biocontrol agents. Adams and Eichenmuller (1) found the bacterium *Pseudomonas denitrificans* Bergy et al. parasitizing populations of *Xiphinema americanum* Cobb, 1913. In juveniles, the bacterium was found throughout the body, being sparse in the esophageal region. In adult females, bacteria were concentrated in the intestines and ovaries. This distribution suggested the possibility of transovarial transmission. The bacterium was widespread, and the researchers believed that it might be a cause of difficulty in rearing *Xiphinema* species in the greenhouse.

A second prokaryote is found in cyst nematode species: *Heterodera goettingiana* Liebscher from England, and *Globodera rostochiensis* Wollenweber from Bolivia, by Shepherd et al. (27); and in *H. glycines* Ichinohe from the United States, by Endo (8). This intracellular rickettsia appears to be identical in the three cyst populations from widely separated places.

Because this rickettsial organism is morphologically similar to a companion symbiote of a leaf hopper, there is some doubt that it is a parasite. It is unicellular and rod-shaped, and divides by binary fission (Fig. 11). The cell mean length is

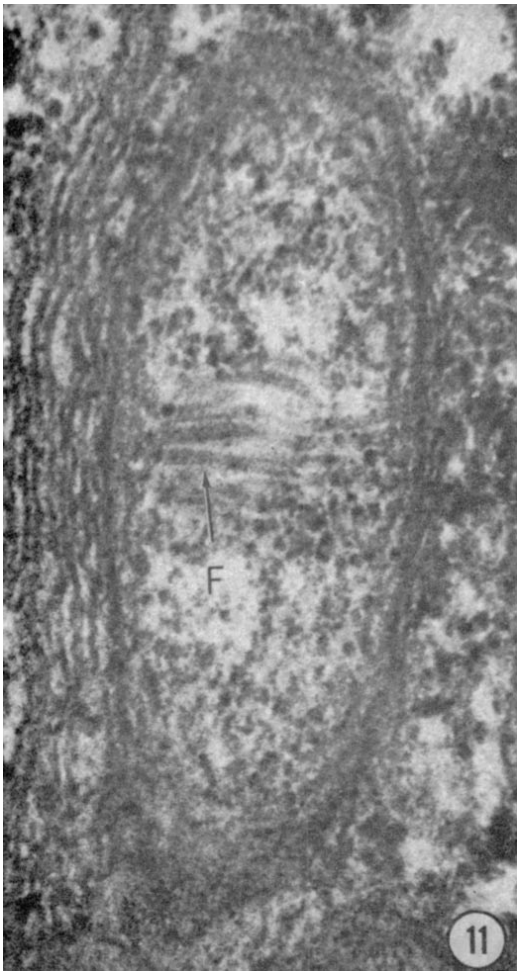

Fig. 11. Section through a rickettsia shows a portion of the fascicle (F) of rods or tubules attached by their ends to the plasma membrane. ×126,000.

1.8 μm, and its width is 0.4 μm. The rickettsial cell contains peculiar inclusions in the form of hollow rods that appear to be attached to the cytoplasmic membrane. Parallel clusters of the rods are found lying in bundles, termed fascicles. In general, the organism has only minor pathological effects on cells. Many of the microorganisms within a cell appear to be surrounded by several membranes of endoplasmic reticulum. This membrane enclosure may function to isolate the microorganism from the host cytoplast.

Amensalism—bacterial antibiosis

Many soil bacteria species are capable of decomposing plant and animal residue. A succession of these bacteria facilitates stepwise degradation of soil organic matter. The products released by the metabolic activity of the bacteria vary from complex to the simplest of molecules. Some of these many products accumulate in soils and may be toxic, antibiotic, or inhibitory to plant-parasitic nematodes.

Johnston (12) found that the reduction in populations of *Tylenchorhynchus martini* Fielding, 1956, was caused by volatile fatty acids in water-saturated soil. He isolated a bacterium, *Clostridium butyricum* Prazmowski, 1880, that produced a mixture of formic, acetic, propionic, and butyric acids in its culture filtrate, which was toxic to the nematode.

Hydrogen sulfide occurs in flooded rice fields in amounts apparently sufficient to control some nematodes of this crop (24). Rodriguez-Kabana et al. (24) identified *Desulfovibrio desulfuricans* (Beijerinck, 1895) Kluyver and van Neil, 1936, in the "reduced" soil zone as the bacterium responsible for the release of H_2S.

During the natural decomposition of plant residues, ammonifying bacteria apparently produce enough NH_3 to influence nematodes. Mankau and Minteer (18) suggested that the NH_3 produced during the decomposition of a fish amendment was probably responsible for the decline of root-knot nematodes, and Walker (31) also believed that the decomposition of nitrogenous substances during ammonification and nitrification was probably responsible for the decrease in nematode populations.

More recently (10), a thermostable toxin of *Bacillus thuringiensis* Berliner was found to be toxic to populations of *Meloidogyne, Panagrellus,* and *Aphelenchus* and prevented *M. incognita* larvae from forming galls on tomato roots. This toxin, being active also against many species of invertebrates, may lack the specificity needed for selective control of pests. Several years ago Katznelson et al. (13) found a species of *Myxobacterium* that had the ability to lyse species of *Caenorhabditis, Rhabditis,* and *Panagrellus.* When tests were conducted on plant-parasitic forms, however, the isolate was not lytic. Perhaps a bacterium will be found that will selectively lyse plant-parasitic forms. If not, genetic engineering might be used to alter bacterial cells, caus-

ing them to be more lytic to plant-parasitic nematodes, or the yield of nematicidal metabolite might be increased so that they become better biocontrol agents.

CONCLUSIONS

Many problems must be solved before microorganisms can be used to control plant-parasitic nematodes. Criteria for selection include virulence of bacterium to nematode, susceptibility of nematodes to the parasite, compatibility of the bacterium when used with other chemical treatments, storage or shelf life of the bacterium, and time of application of bacterium for maximum pest control. Obviously, judicious selection and planning is needed. The chances of success will probably be enhanced by computer technology. A computer program prepared by Perry (23) simulates a population model for the effect of parasitic fungi on numbers of the cereal cyst nematode, *H. avenae*. Work on this model suggests the importance and direct effect of rainfall on the fungus infection rate in nematodes in well-drained soils. Further work should improve parameter estimates, aid in evaluation of the model, and identify factors with the greatest effect on biocontrol. Currently, no known computer programs simulate the influence of an antagonistic bacterium on a population of a plant-parasitic nematode. The computer tool should improve our chances of success in biocontrol of plant-parasitic nematodes by allowing selective choice of the more influential factors and by reducing the number of tests required.

LITERATURE CITED

1. Adams, R. E., and J. J. Eichenmuller. 1963. A bacterial infection of Xiphinema americanum. Phytopathology 53:745 (Abstr.).

2. Allen, M. W. 1957. A new species of the genus Dolichodorus from California (Nematoda: Tylenchida). Proc. Helm. Soc. Wash. 24:95-98.

3. Canning, E. U. 1973. Protozoal parasites as agents for biological control of plant-parasitic nematodes. Nematologica 19:342-348.

4. Cross, T. 1970. The diversity of bacterial spores. J. Appl. Bact. 33:95-102.

5. Dutky, E. M. 1978. Some factors affecting infection of plant-parasitic nematodes by a bacterial spore parasite of nematodes. M.S. Thesis, Dept. of Botany, University of Maryland, 44 p.

6. Dutky, S. R. 1963. The milky diseases. In: Insect Pathology an Advanced Treatise Volume 2, pp. 75-114, ed. E. A. Steinhaus, Academic Press, New York, 689 pp.

7. Dutky, E. M., and R. M. Sayre. 1978. Some factors affecting infection on nematodes by the bacterial spore parasite Bacillus penetrans. J. Nematol. 10:285 (Abstr.).

8. Endo, B. Y. 1979. The ultrastructure and distribution of an intracellular bacterium-like microorganism in tissues of larvae of the soybean cyst nematode, Heterodera glycines. J. Ultrastruct. Res. 67:1-14.

9. Esser, R. P., and E. K. Sobers. 1964. Natural enemies of nematodes. Proc. Soil & Crop Sci. Soc. of Florida 24:326-353.

10. Ignoffo, C. M., and V. H. Dropkin. 1977. Deleterious effects of the thermostable toxin of Bacillus thuringiensis on species of soil-inhabiting, myceliophagous, and plant-parasitic nematodes. J. Kans. Entomol. Soc. 50:394-398.

11. Imbriani, J. L., and R. Mankau. 1977. Ultrastructure of the nematode pathogen, Bacillus penetrans. J. Invertebr. Pathol. 30:337-347.

12. Johnston, T. M. 1959. Effect of fatty acids mixtures on the rice stylet nematode (Tylenchorhynchus martini Fielding, 1956). Nature 183:1392.

13. Katznelson, H., D. C. Gillespie, and F. D. Cook. 1964. Studies on the relationship between nematodes and other soil microorganisms. Can. J. Microbiol. 10:699-704.

14. Mankau, R. 1972. Utilization of parasites and predators in nematode pest management ecology. Proc. Tall Timbers Conf. on Ecological Animal Control by Habitat Management 4:129-143.

15. Mankau, R. 1975. Prokaryote affinities of Duboscqia penetrans, Thorne. J. Protozool. 21:31-34.

16. Mankau, R. 1975. Bacillus penetrans n. comb. causing a virulent disease of plant-parasitic nematodes. J. Invertebr. Pathol. 26:333-339.

17. Mankau, R., and J. L. Imbriani. 1975. The life cycle of an endoparasite in some Tylenchid nematodes. Nematologica 21:89-94.

18. Mankau, R., and R. J. Minteer. 1962. Reduction of soil populations of the citrus nematode by the addition of organic materials. Plant Dis. Reptr. 46:375-378.

19. Mankau, R., and N. Prasad. 1972. Possibilities and problems in the use of a sporozoan endoparasite for biological control of plant parasitic nematodes. Nematropica 2:7.

20. Mankau, R., and N. Prasad. 1977. Infectivity of Bacillus penetrans in plant parasitic nematodes. J. Nematol. 9:40-45.

21. Metchnikoff, M. E. 1888. Pasteuria ramosa, un représentant des bactéries à division longitudinales. Ann. Inst. Pasteur Paris 2:165-170.

22. Odum, E. P., and H. T. Odum. 1959. Fundamentals of ecology. Philadelphia, Saunders, 546 pp.

23. Perry, J. N. 1978. A population model for the effect of parasitic fungi on numbers of the cereal cyst nematode Heterodera avenae. J. Appl. Ecol. 15:781-788.

24. Rodriguez-Kabana, R., J. W. Jordan, and J. P. Hollis. 1965. Nematodes: biological control in rice fields: role of hydrogen sulfide. Science 148:524-526.

25. Sayre, R. M., and W. P. Wergin. 1977. Bac-

terial parasite of a plant nematode: morphology and ultrastructure. J. Bacteriol. 129:1091-1101.

26. Sayre, R. M., W. P. Wergin, and R. E. Davis. 1977. Occurrence of Moina rectirostris (Cladocera: Daphnidae) of a plant morphologically similar to Pasteuria ramosa (Metchnikoff, 1888). Can. J. Microbiol. 23:1573-1579.

27. Shepherd, A. M., S. A. Clark, and A. Kempton. 1973. An intracellular microorganism associated with tissues of Heterodera spp. Nematologica 19: 31-34.

28. Slack, J. M., and M. A. Gerencser. 1975. Actinomyces, filamentous bacteria; biology and pathogenicity. Burgess Publishing Co., Minneapolis. p. 175.

29. Staley, J. T. 1973. Budding bacteria of the Pasteuria-Blastobacter group. Can. J. Microbiol. 19: 609-614.

30. Thorne, G. 1940. Duboscqia penetrans n. sp. (Sporozoa, Microsporidia, Nosematidae), a parasite of the nematode Pratylenchus pratensis (de Man) Filipjev. Proc. Helm. Soc. Wash. 7:52-53.

31. Walker, J. T. 1969. Pratylenchus penetrans (Cobb) populations as influenced by microorganisms and soil amendments. J. Nematol. 1:260-264.

32. Williams, J. R. 1960. Studies on the nematode soil fauna of sugarcane fields in Mauritius. Nematologica 5:37-42.

33. Williams, J. R. 1967. Observations on parasitic protozoa in plant-parasitic and free-living nematodes. Nematologica 13:336-342.