ADVENTURES IN BIOCONTROL

Fig. 1. Soil antagonists of plant-parasitic nematodes: (A) Spores of *Bacillus penetrans* on a root-knot nematode larva. (B) Larva ensnared by the amoeba *Theratromyxa weberi*. (C) Tardigrade species, *Hypsiblus myrops*, preying on larva. (D) Turbellarian species, *Adenoplea* sp., attacking larva. (E) Nematode-trapping fungus, *Arthrobotrys* sp., capturing larva. (F) *Mononchus* sp. preying on larva.

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Promising Organisms for Biocontrol of Nematodes

"Picture these ferocious little mononchs engaged in a ruthless chase in the midst of stygian darkness. We may imagine them taking up the scent of various small animals upon which they feed, among which almost anything they can lay mouth to seems not to come amiss, and pursuing them with a relentless zeal that knows no limit but repletion. How many acres have their organic balance determined by their millions of prowling mononchs?"

Written 63 years ago (2), these picturesque words were drawn upon to present the whimsical view of soil life and recreate the "stygian" world (Fig. 1) of N. A. Cobb, the founder of U.S. nematology. The words apply equally well to that wider and constant struggle among all soil microorganisms competing for food and space. Cobb's unusually worded rhetorical question concerning the role mononchs (predacious nematodes) play in the "organic balance" of a soil remains unanswered. The information gathered in search of the answer would greatly help current efforts to find suitable biocontrol agents for nematodes.

Reviews covering the many antagonists of nematodes present evidence that biocontrol occurs naturally in soil (1,4–7,14,16,17,20). Phytonematologists face several questions: Are some antagonists, coupled with other edaphic factors, already reducing populations of nematodes to low levels? If so, can a soil ecosystem be manipulated to take an even greater toll of the nematode populations and thereby further reduce crop losses? What are the chances of achieving biocontrol?

What Is the Promise of Soil Amendments?

In 1937, Linford (11) observed that root-knot populations were significantly reduced during the decomposition of soil organic amendments. This research sparked an effort and an interest in the use of amendments to control nematodes. The effort, even though largely empirical, has persisted because inherent in the

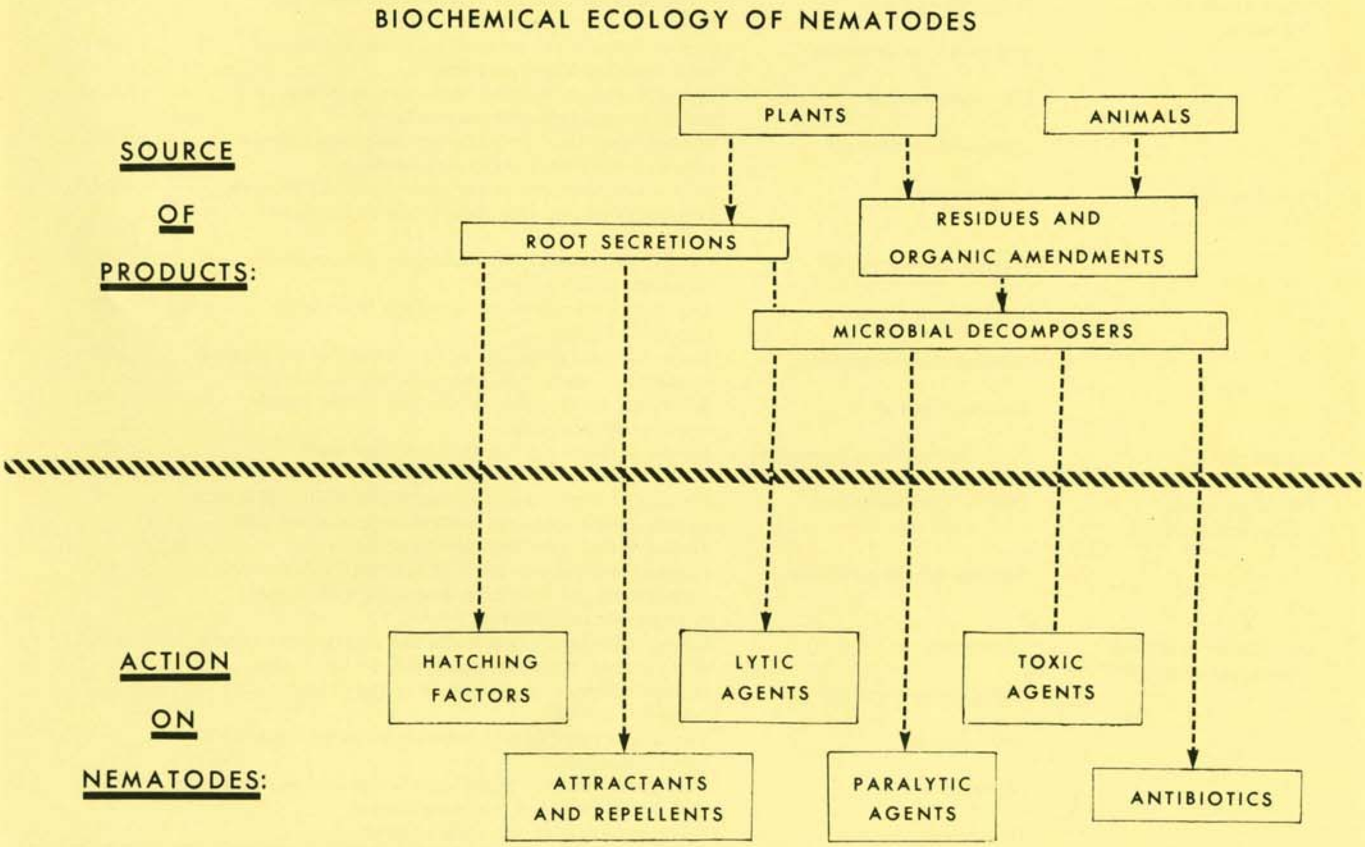

Fig. 2. Sources of soil organic amendments and their actions against nematodes.

approach is the promise of biocontrol of nematodes. An understanding of how organic soil amendments are able to reduce damage caused by nematodes should ultimately provide the basic information needed for biocontrol programs.

Two hypotheses generally suggested to explain the effectiveness of soil amendments are: 1) The decomposition products released from amendments into the soil are directly toxic to plant nematodes. 2) Manipulation of soil microbial populations by addition of amendments initiates a succession of events favoring the buildup of bacteria, microbivorous nematodes, nematode-trapping fungi, and other soil antagonists that destroy plant-parasitic nematodes. Other hypotheses have been suggested, but these two will be discussed in detail.

Products of Decomposition

Many soil microorganisms participate serially in decomposing plant and animal residues. A succession of these organisms facilitates the stepwise degradation of soil organic matter. The numerous products released during this succession vary from the most complex to the simplest molecules. Some products accumulate in soil to levels that have toxic, antibiotic, or inhibitory effects on plant-parasitic nematodes (Fig. 2).

Organisms closely related to or known to elaborate natural nematicidal compounds have been investigated and may be considered biocontrol agents. Johnston (8) found that populations of *Tylenchorhynchus martini* Fielding, 1956, were reduced by accumulation of volatile fatty acids in water-saturated soil. He isolated a bacterium, *Clostridium butyricum* Prazmowska, 1880, that produced a mixture of formic, acetic, propionic, and butyric acids in a culture filtrate that was toxic to nematodes. Similar work led to the first published example of a biological control method in a field situation. Rodriguez-Kabana et al (16) found that amounts of hydrogen sulfide in flooded rice fields were sufficient to control some nematodes. They tentatively isolated and identified *Desulfovibrio desulfuricans* (Beijerinck, 1895) Kluyver and van Neil, 1936, which occurred in the "reduced" soil zone, as the bacterium responsible for the release of hydrogen sulfide.

Ammonia also occurs naturally in soils. During the decomposition of plant residues, ammonifying bacteria produce enough NH_3 to adversely affect nematodes. Mankau and Minteer (13) suggested that NH_3 produced during decomposition of a fish meal amendment was probably responsible for the decline of root-knot nematode populations. Similarly, Walker (21) found that decomposition of nitrogenous substances during ammonification and nitrification was probably responsible for the decrease in *Pratylenchus* populations. Although no specific bacteria have been isolated and identified, apparently some involved in the nitrogen soil cycle have a role in biocontrol of plant-parasitic nematodes.

Natural Enemies of Nematodes

The second hypothesis, offered in explanation of the natural biocontrol of nematodes resulting from use of soil organic amendments, is that natural enemies of phytonematodes increase in numbers. Several of the different soil species that prey or are parasitic on

Table 1. Partial list of microorganisms antagonistic to plant-parasitic nematodes

Kind of organism	Scientific name	Mode of antagonism	References
Bacteria	*Bacillus penetrans*	Spores of bacterium attach to larvae, penetrate, and convert contents of maturing female totally to new spores	12,15
Fungal predators of larvae	*Stylopage hadra*	Nematodes are captured by adhesive nonseptate hyphae	1,4,6,7
	Arthrobotrys oligospora	Species forms sticky network for capturing larvae; most common of trapping fungi	1,4–7
	Dactylaria candida	Species forms three-celled nonconstricting rings and adhesive knobs that ensnare larvae	1,4,5,7
	Dactylaria brochopaga	Species forms three-celled constricting rings that are triggered when larvae enter ring openings	1,4–7
Fungal parasites of larvae	*Catenaria* sp.	Chytridiomycete species are weakly parasitic on many nematodes species; zoospores attacking larvae are uniflagellate	1,4,17
	Myzocytium humicola	Oomycete species has sexual stages; biflagellate zoospores attach to larvae	1
	Meristacrum asterospermum	Zygomycete species produce conidia that attach to cuticle of larvae	1,7
	Harposporium anguillulae	Deuteromycete species produce conidia that are ingested by saprozoic nematodes and germinate in their gut	1,7
	Nematoctonus sp.	Basidiomycete species produces spores that attach to and penetrate hosts	1,7
Fungal parasites of eggs	*Verticillium chlamydosporium*	Species converts egg contents to hyphal mass	20
Fungal parasites of adults and cysts	*Dactylella oviparasitica*	Primarily fungal parasite of eggs but occasionally attacks mature female root-knot nematodes; associated with decline of root-knot populations on citrus	19
	Nematophthora gynophila	Fungus resting spores are found in mature *Heterodera avenae* cysts and have been associated with decline of nematode populations	9,10
Invertebrate predators on larvae and adults	*Mononchus* sp.	Genus of predatory nematodes that attack many species of plant-parasitic nematodes and other soil fauna	2,3,7
	Theratromyxa weberi	Amoeboid species that preys on larvae of many nematode species	7,17
	Adenoplea sp.	Turbellarian species that preys on larvae and vermiform adult nematodes	17
	Hypsibius sp.	Tardigrade species ("water bears") that feed on nematodes and several other soil microorganisms	7,17
	Onychiurus armatus	Collembola species that feeds on cysts	7

nematodes are listed in Table 1. The predators include turbellarians (Fig. 3A), tardigrades (Fig. 3B), enchytraeids, insects, mites, fungi (Fig. 3C), amoebae (Fig. 3D), and other nematode species (Fig. 3F). The parasites include viruses, bacteria (Fig. 3E), protozoans, and fungi.

Three nematode antagonists deserve consideration because of their unique biology and because they appear to be functioning as biocontrol agents. They are: 1) *Nematophthora gynophila* Kerry and Crump, n. sp. (10), an oomycetous fungus that heavily parasitizes the cysts of *Heterodera avenae* Filipjev, 1934; 2) *Dactylella oviparasitica* Stirling and Mankau, 1978, a nematode-trapping fungus that parasitizes the eggs of *Meloidogyne* sp.; and 3) *Bacillus penetrans* Mankau, 1975, a bacterial parasite of *Meloidogyne* sp.

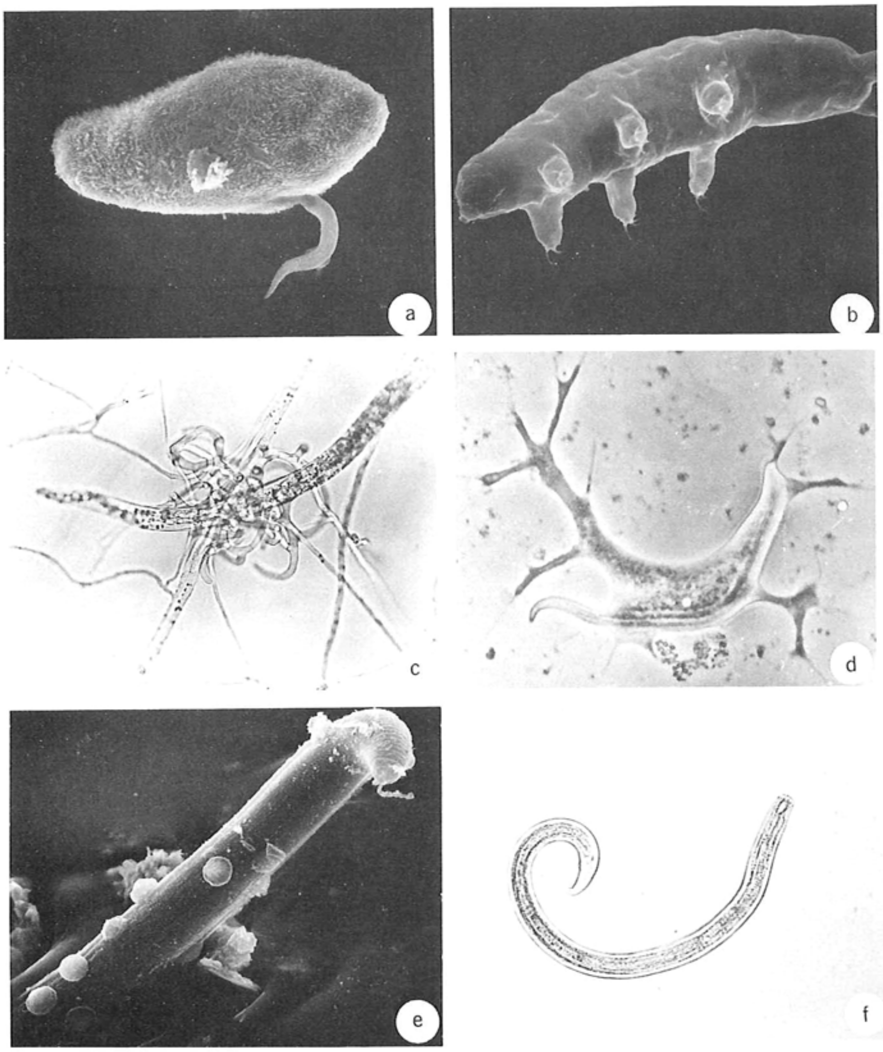

Fig. 3. Soil antagonists of plant-parasitic nematodes: (A) Nematode larva being ingested by a turbellarian. (B) A tardigrade. (C) Network trap of nematode-destroying fungus with ensnared and partially digested larva. (D) Larva ensnared by an amoeba. (E) Larva encumbered by bacterial spores. (F) A mononch nematode species predacious on other nematodes.

The Cyst Parasite

In southern England, despite the common practice of growing cereals continuously, the population of the oat cyst nematode, *H. avenae*, declined, stabilized, and caused no apparent yield losses (9). Surveys of the cereal fields disclosed four fungal parasites of the cyst nematode. *N. gynophila*, the most prevalent, produced biflagellate zoospores that penetrated the developing nematode females as they erupted through the cortex of oat roots. The infected cysts were flaccid and their protective cuticles were destroyed. The fungus killed the females and their eggs. The colonized cysts gave rise to zoosporangia and subsequently to discharge tubes that protruded from the cysts into the soil, where they released additional zoospores. During the final stages of the parasite's development, the cysts were filled with resting spores (Fig. 4).

In field studies, Kerry (9) measured the effect of the fungus on oat cyst nematode populations. Fumigating nematode-suppressive soils with formalin selectively eliminated *N. gynophila*, and the surviving cyst nematodes, freed from parasitism, increased in numbers. This finding, combined with previous observations of the direct relationship between the high incidence of fungal parasitism of cysts and the decline of field populations of *H. avenae*, led to the conclusion that the fungus was a natural biocontrol agent of the oat cyst nematode. Four other *Heterodera* spp. were also found to be susceptible to the fungus, suggesting that additional cyst species may be susceptible and that the usefulness of the fungus as a biological control agent of the cyst nematode may be expanded to other crops and geographic locations.

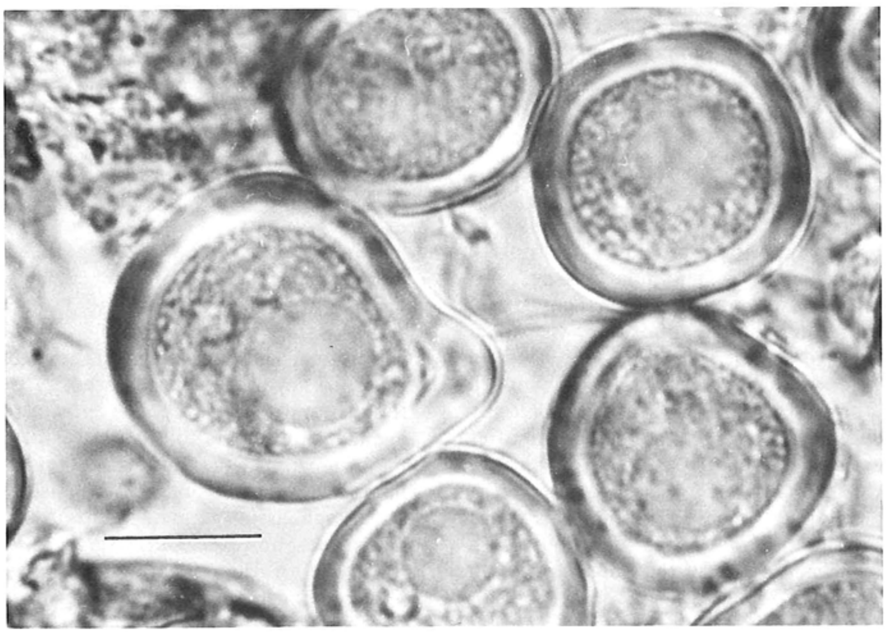

Fig. 4. Thick-walled spores of *Nematophthora gynophila* from crushed cyst of *Heterodera avenae*. Bar represents 10 μ. (Courtesy of B. R. Kerry)

The Egg Parasite

The nematode egg parasite, *D. oviparasitica*, was isolated from egg masses of root-knot collected from peach

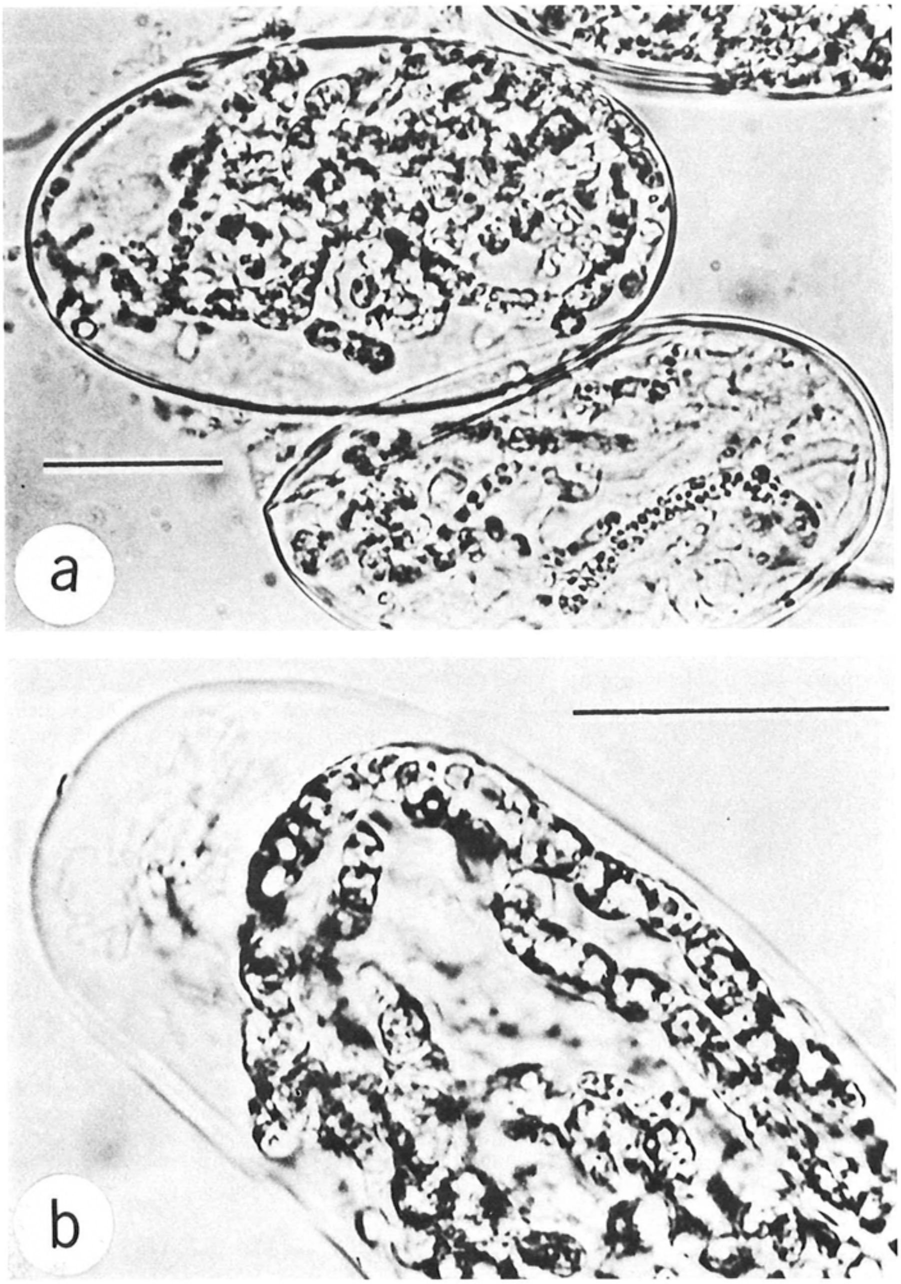

Fig. 5. (A) Eggs of *Meloidogyne* sp. invaded in the field by *Dactylella oviparasitica*. (B) Swollen (3–4 μm diameter) and convoluted hyphae inside an egg. Bars represent 25 μm. (Courtesy of R. Mankau)

orchards in the San Joaquin Valley of California (19). The field observation of unexpectedly low populations of root-knot and the amount of fungal parasitism found later in the laboratory suggested that *D. oviparasitica* may be a useful biocontrol agent of *Meloidogyne* spp. In laboratory studies, the mycelium readily entered young egg masses soon after exposure to fungus-infested soils (Fig. 5). Occasionally, the fungus also entered the developing female, halting early egg production and filling her body with mycelia. Isolates of the fungus varied in virulence to eggs of various root-knot species and also attacked eggs of other plant-parasitic as well as saprozoic nematodes. This ability to parasitize eggs of microbivorous species may be important to survival of the fungus when root-knot eggs are not available, since the fungus apparently does not have a resistant stage to carry it through adverse conditions. Unfortunately, larval stages of root-knot are not parasitized by the fungus, and its efficacy as a biocontrol agent depends on its ability to parasitize a major portion of the eggs before they can hatch. The fungus does have a distinct advantage as a parasite of root-knot nematodes, however. Of the stages in the life cycle of the nematode, the eggs, clumped together on the outside of the root, are the most vulnerable to fungal parasitism. The other stages are hidden within the plant root or are motile.

The Bacterial Parasite

B. penetrans, previously described as *Duboscqia penetrans* Thorne, 1940, is a bacterial parasite of the adult root-knot nematode and several other plant nematode species. The bacterial nature of the organism was only recently recognized. Mankau (12) and Sayre and Wergin (18) described bacterial cell characteristics even though the vegetative growth stage of the bacterium was funguslike in appearance (Fig. 6A). The life cycle of the bacterium and that of its host, the root-knot nematode, *Meloidogyne incognita*, were found to be synchronous with one another (Fig. 7). The stages of the life cycle of the bacterium are: penetration of the larva, vegetative growth, fragmentation, sporogenesis, soil phase, and spore attachment. Embodied in these stages are the desirable characteristics of a potential biocontrol agent.

Bacterial penetration occurs during the juvenile second larval stage (Fig. 6B); parasitism is not fully manifested, however, until two molts later in the adult. At that time, virtually all physiological processes of the nematode are redirected to bacterial spore production. The adult female is literally transformed into a bag containing 2 million spores (Fig. 6C). The parasite prevents any significant nematode reproduction—a desirable characteristic for a control agent. Thus, the bacterium has its greatest impact by eliminating the adult reproductive capacity.

During sporogenesis, thick cortical layers are formed within the sporangial walls (Fig. 6D). These layers confer a resistance to desiccation on the developing spores, as well as an apparent protection from some soil fumigants that may last several months to possibly a few years, depending on soil condition. After the parasitized female ruptures and releases the sporangia into the soil, the wall of the released sporangium degrades to expose a unique fibrous spore layer. This layer, which is highly specialized, attaches to the specific host nematode.

Although these characteristics are promising for a biocontrol agent, the inability to culture the bacterium on any standard bacteriological medium presents a serious problem for its ultimate use as an agent. This may be rectified by determining the bacterium's nutritional requirements.

Why Nematodes Persevere Despite Natural Enemies

A legion of soil antagonists are arrayed against nematodes. A list of all currently known enemies would probably exceed 200 species. About three-quarters would be fungi and the remainder would be soil invertebrates, including many predacious nematodes, or bacterial species. Because interest and effort in biocontrol research

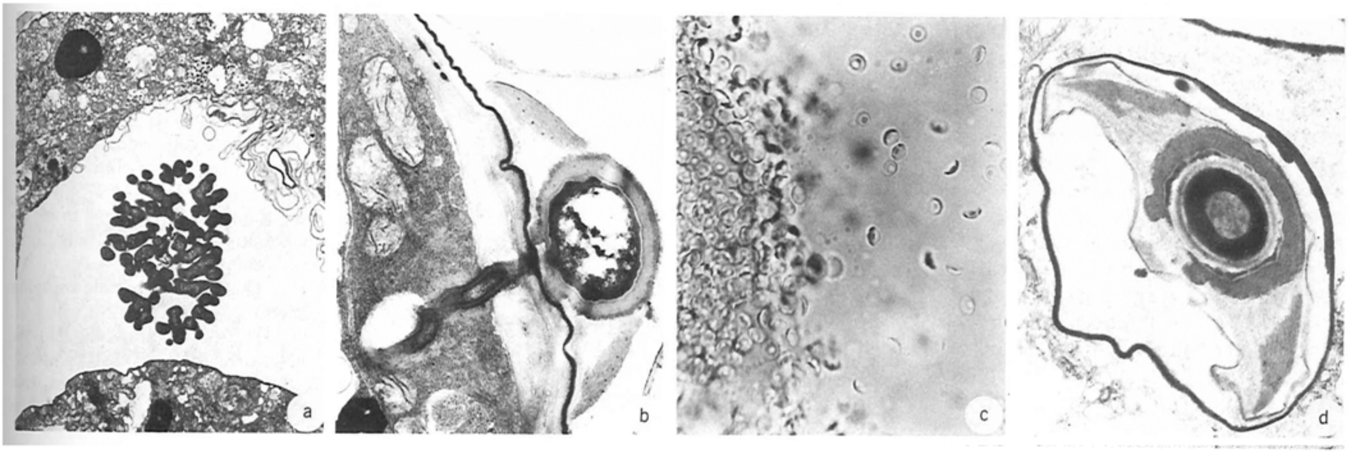

Fig. 6. Life stages of *Bacillus penetrans*: (A) Vegetative microcolony of bacterium within pseudocoelom of developing female nematode. (B) Germinating spore with its penetration peg passing through cuticle and hypodermis of root-knot larva. (C) Numerous spores of bacterium floating free from ruptured, parasitized female. (D) Mature sporangium, with old sporangial wall and very thin exosporium surrounding the spore.

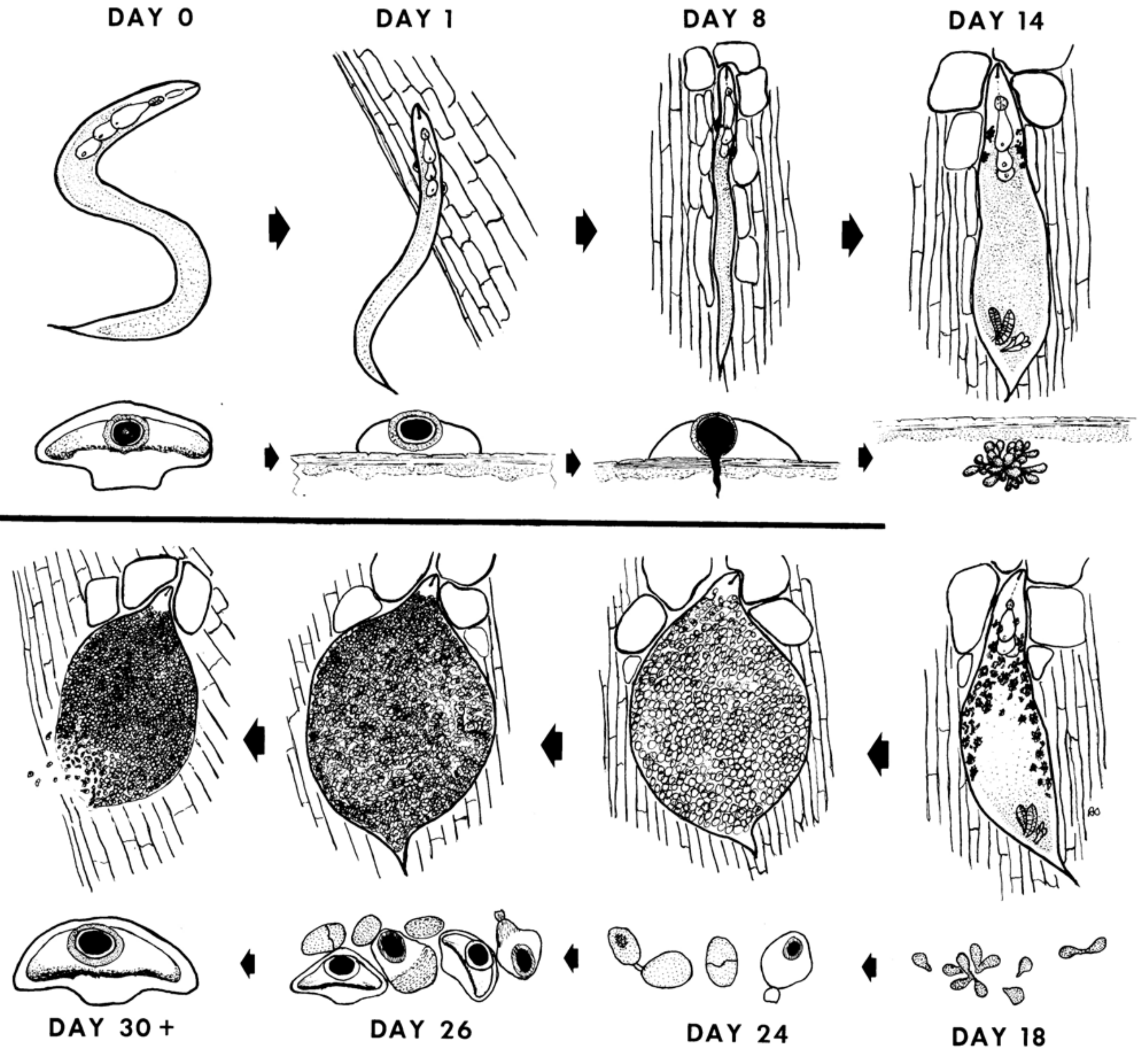

Fig. 7. Life cycles of the root-knot nematode and *Bacillus penetrans*; enlarged life stage of *B. penetrans* is shown below each developmental stage of the nematode.

are increasing, many more species are likely to be discovered, possibly including viral agents.

With so many enemies, how can nematodes be successful parasites on crop plants? Simply because their reproductive capacity is prodigious. A few nematode generations every growing season result in enough eggs and juveniles to sustain a very heavy mortality. Doubtlessly, antagonists kill many larvae; but many might be eliminated anyway by the competition for the limited plant invasion sites. Agents controlling larval forms seem to be inefficient. Parasites and predators attacking adult nematodes are more efficient because they prevent reproduction and replenishment of larvae. The three apparently successful biocontrol agents described in this paper are parasites of adults.

Some Entomological Approaches

The importance of the stage of the life cycle in which parasitism occurs and its impact on nematode populations would be more obvious if the data were presented in a life table. For years, entomologists have been using life tables to predict outbreaks of pest populations. Application of this technique to nematode field studies would lead to recognition of impending nematode crashes and to correlation with possible parasites and predators.

One way to measure the efficacy of nematode antagonists is through bioassays. A few bioassay methods have been developed to determine the level of parasitism occurring in some soils to suppress nematodes. Entomologists have long been aware of the need for standard bioassay procedures. The use of standard techniques among several nematology laboratories could lead to the selection of more virulent or aggressive antagonists of nematodes.

Another entomological approach that could be applied to nematology is the introduction of antagonists that have been separated from hosts, which were then introduced into new geographic areas. This may have occurred with some cyst nematodes because the cysts were resistant to many adverse situations to which the antagonists succumbed. For example, *H. schachtii* cysts from North Carolina and Michigan do not contain any of the parasites isolated from a European strain of the cyst. This discrepancy could be exploited by importing and testing exotic species as possible control agents.

No Insuperable Obstacles

Cobb (3) addressed the problem of introducing exotic nematode antagonists. It is fitting that this paper, which began with his words, should end on his hopeful note about the feasibility of biological control of plant-parasitic nematodes:

"... There can be no doubt, however, that the enormous losses due to plant-infesting nemas fully justify the expenditure of even large sums of money in an effort to apply this remedy, more particularly because the remedy, when successful, bids fair to be permanent and self-sustaining.

"After long-continued and intensive studies I am thoroughly convinced that many of the practises evolved in the transfer of beneficial insects can, with appropriate modification, be applied to the nemas. At the present time the greatest drawback in the case of the nemas is the small number of people who are technically competent to make the necessary biological examinations. It is in this respect principally that their introduction will differ from that of the introduction of useful insects, for the nema problem is essentially a microscopic one. Though the collection of the nemas from the soil differs entirely from the collection of beneficial insects, the methods have already been brought to such a state that there are no insuperable obstacles."

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