Diseases of nematodes

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Diseases of Nematodes

Volume I

Editors

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The period of time nematodes have existed is not known due to the paucity of fossil records, especially Pre-Tertiary. However, some investigators place the phylum Nematoda as arising during the Cambrian or Pre-Cambrian periods. This would allow at least half a billion years for symbiotic associations to become established between nematodes and other agents in the environment. There is fossil evidence indicating that nematophagous fungi were present some 20 to 30 million years ago.

The present work deals with the diseases of nematodes. Although the term disease implies a pathological condition brought about by an infectious agent, a broader concept is used here. Disease is considered to be a departure from the state of health or normality in which the body, or a cell, tissue, or organ of the body, is disturbed functionally or structurally. This departure can manifest itself as a disruption of the growth, development, function, or adjustment of the organism to its environment. Thus, any agent which either by its presence or absence, causes any destructive process at the cellular or organismic level is a disease-producing agent.

Diseases can be considered infectious or noninfectious. Noninfectious diseases are covered in the first section of this work and arise from aberrations in the genetics, nutrition, metabolism, or physiology of the organism. Although the direct cause of noninfectious diseases are usually abiotic factors, such conditions are often indirectly related to the presence or absence of other organisms and their products (e.g., toxins produced by plants, starvation resulting from the absence of bacteria, injury through the action of predators).

Infectious diseases are caused by parasites or pathogens which develop at the expense of the host. Some parasites which live in intestinal lumen of nematodes (e.g., protozoa) may not cause any noticeable disturbance and therefore would not be considered pathogens (microorganisms capable of producing disease under normal conditions). The known pathogens of nematodes, which are treated in this work, are viruses, bacteria, protozoa, and rickettsia. Although fungi are considered pathogens in relation to insect disease, in the field of nematology they are generally grouped under parasites and predators, depending on their mode of infection and ability to exist saprophytically in the nematode's environment. The complete range of fungal parasites and predators of nematodes are treated here.

A chapter on the invertebrate predators of nematodes is also included, although this heading would not normally fall under disease. However, aside from its importance in relation to nematode population dynamics, invertebrate predation is often initiated through the action of toxins which have an instantaneous effect on the nematode or its tissues.

In some respects, the field of nematode diseases can be considered in its infancy, especially in areas involving viral, protozoal, and bacterial associations. However, it is clear that basic research will reveal many new and interesting types of pathogens and relationships, and it is hoped that this work will stimulate investigations along these lines. The evaluation of diseases in the population dynamics and distribution of nematode species is also worth attention.

Another purpose in creating this work was to focus attention on organisms which have potential for the biological control of nematodes. Millions of dollars are spent each year treating nematode pests of plants and animals. The use of nematicides for the control of plant parasitic nematodes has become a well established practice. However, with new legislation resulting from the potential dangers of these chemicals, the use of nematicides is being restricted, and alternative methods of control are becoming a necessity. Thus the concept of biological control of nematodes has now come out of the laboratory into the field arena. Investigators are attempting to make up for what can be considered a considerable lack of foresight some 20 to 30 years ago, when scientists interested in the diseases of nematodes were given little support.

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Chapter 1

PHYSIOLOGICAL DISEASES INDUCED BY PLANT RESPONSES OR PRODUCTS

F. J. Gommers and J. Bakker

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I. INTRODUCTION

Plant parasitic nematodes feed by piercing plant cells with their stylets and removing the cell contents. Plant parasitism by nematodes is confined to two orders, the Tylenchida and the Dorylaimida, respectively, of the classes Secernentea and Adenophorea. The stylets in the two orders have similar functions but are not homologous.

Most plant parasitic nematodes are free living ectoparasites which move along the roots and feed on more or less specific tissues. Specialization in this group has occurred. The typical browsers, which may or may not kill the root hairs and epidermal cells, are considered the more primitive forms. They generally have a wide host range. In a number of cases, in both the Dorylaimida and the Tylenchida, it has been demonstrated that introduction of saliva in the parasitized cells precedes ingestion of nutrients. This shows that these forms do not just suck up the cytoplasm, and that parasitism is the result of a sequence of complex processes.[1] Whether or not cellulases, pectinases, or other cell wall degrading enzymes found in a number of plant parasitic nematodes are involved in this predigestion process is still an open question. There are also ectoparasites that feed on subepidermal cells and some of these induce temporary feeding sites which are abandoned after parasitism. These uni- or multinucleate feeding sites reflect altered morphogenesis of the plant cells. Nematodes influence these by injecting glandular secretions and by removing host cytoplasm. The susceptible response may therefore consist of a rapid accumulation by the plant and removal of cytoplasm by the parasite. If feeding rates exceed the rate of response, cell death may occur.

Endoparasitism is confined to the Tylenchida. The development of modes of parasitism is thought to have evolved from ectoparasites and migratory endoparasites to semiendoparasites and sedentary nematodes. The sedentary habit has arisen several times, independently. The migratory endoparasites are in closer contact with host plants than ectoparasites. They move freely from soil to host and vice versa. This closer contact probably also allows permeation of primary and secondary metabolites from plant tissues into the nematodes since many compounds, especially smaller apolar molecules, easily enter the nematode's body.[2-4]

In some plant parasitic nematodes the female is sedentary and feeds from saliva-induced feeding sites. In these cases there is a unique relationship between the parasite and the host. The feeding sites are under genetic control of both the host and the parasite. Survival of the nematode is fully dependent on the functioning of the feeding site in order to complete its life cycle.

Plants have several mechanisms available to combat parasitic nematodes. These mechanisms may operate separately or concurrently. As emphasized by Dropkin each plant nematode interaction has a unique combination of genes which affect every aspect of the association.[5] In compatible combinations the relevant genes match. Therefore, differences between compatible and incompatible associations may be seen as part of the expression of susceptibility or resistance. Resistance may be defined as the absence or inhibition of disease development in plants upon challenge by the nematode. Factors that hamper the nematode contribute to host resistance.[6] In incompatible or less compatible combinations the plant may, at the level of preinfection, produce repellents or toxicants in the rhizosphere, may lack attractants or hatching agents, or, at the postinfectional level, may not supply the proper nutrients. Plants may also contain nematicidal compounds (allelochemicals), or produce them (phytoalexins), may neutralize the nematode saliva, or may isolate the nematode or its feeding site by a hypersensitive reaction.

A substantial amount of literature exists on attraction and hatching properties of root exudates. Repellent or toxic effects of root exudates are less well documented. Isothiocyanates exuded by roots of certain Cruciferae are thought to be responsible for the inhibition

of emergence of larvae of potato cyst nematodes.[7,8] Exudates of roots of millet *(Pennisetum typhoideum)* inhibit the movements of *Hemicycliophora paradoxa* and *Polygonum hydropiper*, and when cultured in combination with wheat, decreased the number of attacks of *Anguina tritici*.[9,10] This effect was ascribed to released compounds from the roots of waterpepper.[11] Repellent effects are also known from cucumber.[12] Plants carrying the "bitter locus" attracted fewer *Meloidogyne* larvae than those without this locus. The repellent effect may be related to the toxic cucurbitacins.

We will focus attention on postinfectional factors affecting parasitic nematodes. Relevant reviews in this field are those of Kaplan and Keen,[11] Gommers,[14] and Veech.[6]

II. NATURALLY OCCURRING NEMATICIDES

Several naturally occurring nematicides were isolated from various plant species, especially members of the Compositae. However, it is difficult to ascribe the suppressant properties of certain plant species to the presence of these toxic principles as such. First, the toxic compounds need not be evenly distributed throughout the plant tissues and differences in toxicity towards different nematode species may be related to the distribution patterns of the toxic principles.[15] For instance, Van Fleet claims that polyacetylenes, presumed precursors of nematicidal and other thiophenes, are concentrated in the endodermis.[16,17] Secondly, proof of nematicidal activity by plants based on the presence or production of toxic principles requires a thorough knowledge of the mode of action in vitro, as well as evidence that in vivo the same system kills the nematodes. As will be discussed, this is even not the case with the compound α-terthienyl from marigolds, one of the best known naturally occurring nematicides which has been intensively studied in our department.[18]

A. Terthienyl and Related Compounds, Photodynamic Modes of Action

The earliest reports on resistance of *Tagetes* to nematode infection by Tyler[19] and Steiner[20] pertain to the *Meloidogyne* species. Slootweg[21] and Oostenbrink et al.[22] reported that a crop of *Tagetes* markedly suppressed populations of *Praty lonchus* and that the supression approximated the effect of soil disinfection with a nematicide. Uhlenbroek and Bijloo isolated the nematicidal compound terthienyl (2,2'-5,2"-terthienyl) from the roots of *T. erecta*, together with the biogenetically related 5-(3-buten-1-ynyl)-2,2'-dithienyl.[18,23] The terthienyl was isolated earlier from the achenes of marigolds by Zechmeister and Sease, without mentioning any biological activity.[24] Screening of Compositae for the ability to suppress populations of *P. penetrans* in soil demonstrated this feature in about 70 of the 150 Compositae tested, mainly because the screening program was guided by chemotaxonomical relationships in the Compositae.[25-29] (Table 1) A correlation was found between Compositae with suppressant effects on *P. penetrans* and the presence of α-terthienyl and related dithiophenes in the roots. All tested species of the genera *Eclipta, Gaillardia, Didelta, Berkheya,* and *Echinops*, which contain these compounds, suppressed populations of *P. penetrans* in pot and field experiments equally effectively as did marigolds.[26,27] Therefore, a chemical basis for the explanation of the nematicidal effects of these Compositae, and even those which do not contain the thiophene derivatives, seems attractive.

However, terthienyl, as well as a number of synthetic dithiophene analogues and a number of 1,2-di(2-thienyl)-ethenes with excellent nematicidal properties in vitro, were completely devoid of nematicidal activity when mixed with soil.[30,31] Daulton and Curtiss obtained no reduction of *Meloidogyne* after incorporation of 200 ppm terthienyl into soil, whereas in laboratory experiments with aqueous emulsions of 1 ppm or less, nematodes were rapidly killed.[32]

We discovered that irradiation with sunlight considerably increased the nematicidal activity of terthienyl, whereas in the dark, nematodes remained unaffected.[33] Near UV light appeared

Table 1
COMPOSITAE SUPPRESSING POPULATIONS OF
PRATYLENCHUS PENETRANS

Astereae	Solidagininae	*Grindelia robusta* Nutt.
		Grindelia squarosa Dunal
		Solidago virgaurea L.
Heliantheae	Coreopsidinae	*Coreopsis grandiflora* Nutt.
		Coreopsis lanceolata L.
	Helianthinae	*Eclipta prostrata* L.
		Rudbeckia bicolor Nutt.
		Rudbeckia laciniata L.
		Rudbeckia serotina Nutt.
	Melampodiinae	*Melampodium divaricatum* DC
		Silphium asteriscus L.
	Ambrosiinae	*Iva xanthiifolia* Nutt.
		Ambrosia artemisiifolia L.
		Ambrosia chamissonis Greene
		Ambrosia maritima L.
		Ambrosia trifida L.
		Franseria artemisioides Willd.
		Franseria chenopodiifolia Benth.
	Milleriinae	*Milleria quinqueflora* L.
Helenieae	Heleniinae	*Baeria californica* Chamb.
		Baeria chrysostoma Fisch. et Mey
		Baeria minor Ferris
		Lasthenia glabrata Lindl.
		Schkuhria pinnata Kuntze
		Schkuhria senecioides Nees
		Eriophyllum caespitosum Dougl.
		Eriophyllum confertiflorum Gray
		Eriophyllum lanatum Forb.
		Chaenactis douglasii Hook. et Arn.
		Helenium autumnale L.
		Helenium bolanderi Gray
		Helenium flexuosum Rafin.
		Helenium nudiflorum Nutt.
		Helenium hybrid 'Moerheim Beauty'
		Helenium hybrid 'Pumilum'
		Helenium hybrid 'Riverton Gem'
		Gaillardia amblyodon Gray
		Gaillardia aristata Pursh
		Gaillardia arzonica Gray
		Gaillardia lanceolata Michx
		Gaillardia lutea Greene
		Gaillardia pulchella Fouger.
		Gaillardia hybrid 'Burgunder'
	Tagetinae	*Tagetes erecta* L.
		Tagetes patula L.
		Tagetes tenuifolia Cav.
Arctoteae	Arctotinae	*Arctotis acaulis* L.
		Arctotis fastuosa Jacq.
		Arctotis grandis Thunb.
		Arctotis stoechadifolia Berg.
	Gorteriinae	*Gazania lichtensteinii* Less.
		Gazania splendens Hort. Angl.
		Berkheya adlamii Hook.
		Berkheya macrocephala Wood
		Didelta carnosa Ait.

Table 1 (continued)
COMPOSITAE SUPPRESSING POPULATIONS OF
PRATYLENCHUS PENETRANS

Cardueae	Echinopinae	*Echinops bannaticus* Rochel
		Echinops exaltatus Schrad.
		Echinops horridus Desf.
		Echinops macrophyllus Boiss. et Hausk.
		Echinops ritro L.
		Echinops sphaerocephalus L.
Cichorieae	Crepidinae	*Urospermum dalechampii* Schmidt

to be the effective part of the sunlight spectrum and this is exactly the region where α-terthienyl possesses an absorption maximum. Irradiation of nematodes in an emulsion of terthienyl resulted in dosage response curves with respect to the time of irradiation. Later reports demonstrated that other organisms, or parts of them, were also killed or affected by the photoactivated compound. Among these were Gram positive and Gram negative bacteria, yeasts, fungi, marine and fresh water algae, *Paramecium*, insects, plants, tadpoles, and also human erythrocytes, human skin, and a number of enzymes.[34]

Bakker et al.[35] demonstrated that enzymes from *Ditylenchus dipsaci* and also purified enzymes were inactivated in the presence of terthienyl and irradiation with near UV light. Details were studied with glucose-6-phosphate dehydrogenase. The inactivation was blocked by the singlet oxygen quenchers azide, histidine, methionine, and tryptophan. The enhanced enzyme inactivation in deuterated water relative to H_2O due to the longer lifetime of singlet oxygen in D_2O, confirmed the production of singlet oxygen by photoactivated α-terthienyl.[36-38] Direct evidence for the production of singlet oxygen was obtained by irradiation of the compound in CH_2Cl_2 in the presence of the olefin adamantylideneadamantane (I). The dioxetane (II) formed could gas-chromatographically be detected via its decompositon by heat to adamantanone (III) (Figure 1). Clearly, terthienyl acts as a sensitizer in the conversion of triplet oxygen to singlet oxygen, Type II photodynamic action.[39,40] A number of nematicidal synthetic dithiophenes and chlorosubstituted dithienylethenes were also singlet oxygen sensitizers.[30,31,41] These results were confirmed for the first time by Arnason et al.[42] in part with independent methods. Singlet oxygen is an excited form of molecular oxygen. Molecular oxygen in the ground state (triplet oxygen, $^3O_2$) has two unpaired electrons with parallel spin. Spin inversion by excitation results in two different singlet states ($^1O_2$). The first singlet state ($^1\Delta_g$) has an excitation energy of 0.98 eV and is involved in photodynamic and biological processes because of its relative long lifetime. The lifetime in water approximates 4 μsec. The second singlet state is, due to its short lifetime, of no importance in biology. Singlet oxygen may be produced by certain enzyme systems but the major mechanism of singlet oxygen formation in biological systems is by energy transfer from photoexcited compounds, the photosensitizers. The absorption of photons by a sensitizer brings the compound in a singlet state,

$$S \longrightarrow {}^{\cdot\cdot 1}S$$

which by intercrossing is converted to the triplet state.

$$^1S \longrightarrow {}^{\cdot\cdot 3}S$$
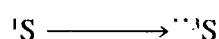
The triplet excited sensitizer may be quenched by triplet oxygen resulting by energy transfer in singlet oxygen and the sensitizer in the ground state.
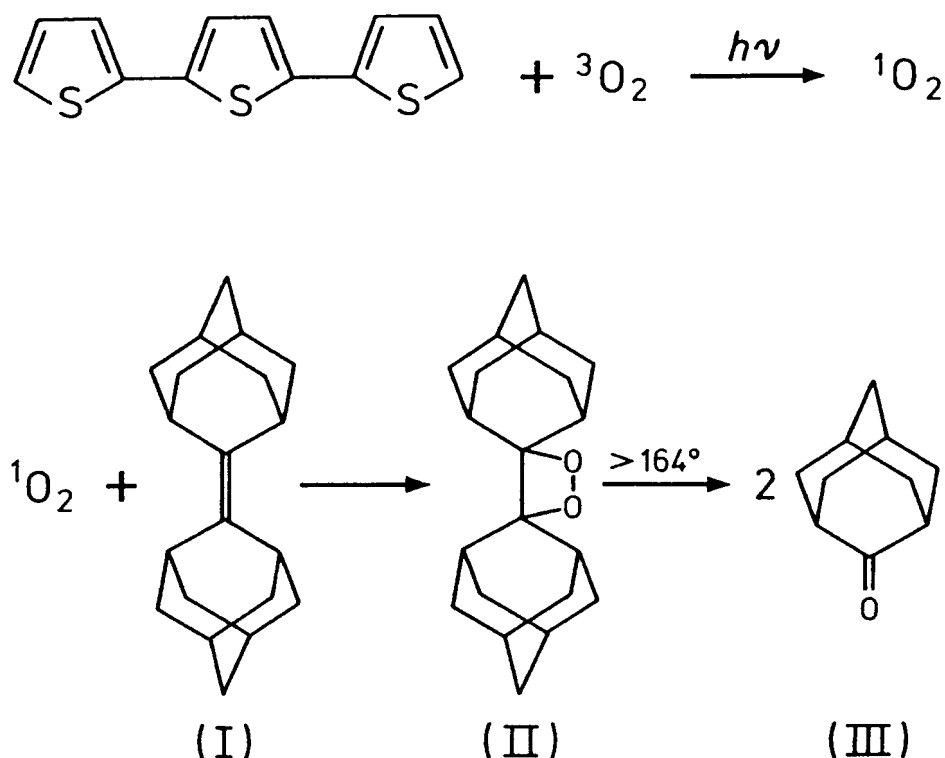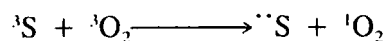
$$^1O_2 + \quad\longrightarrow\quad \overset{>164°}{\longrightarrow} 2$$

(I)   (II)   (III)

FIGURE 1.   Oxydation of the olefin adamantylideneadamantane by singlet oxygen generated by photoactivated α-terthienyl.

$$^3S + \, ^3O_2 \longrightarrow \, ^{\cdot\cdot}S + \, ^1O_2$$

Singlet oxygen is toxic to organisms because it oxidizes the amino acids histidine, tryptophan, and methionine, and proteins containing these acids.[36-43] This results in inactivation of enzymes. Membranes also may be distorted because unsaturated fatty acids are oxidized.

The generation of singlet oxygen by photoactivated α-terthienyl is probably the sole mechanism that kills nematodes. Enzymes, and also *Aphelenchus avenae* in an anaerobic state, remained unaffected when irradiated in the presence of terthienyl.[35,44] There are however, reports which are challenged, that claim that photoactivated terthienyl interacts with DNA and that this interaction substantially contributes to the toxicity of the compound.[45,46] The problem which remains to be solved is the way in which the nematodes that penetrate the roots of marigolds are killed. There is evidence that mainly polyphagous endoparasites such as *Pratylenchus* and *Meloidogyne* species can be suppressed by growing *Tagetes*.[26,47,48] Populations of saprozoic soil-inhabiting nematodes are not affected and there are ectoparasites that use *Tagetes* as a host plant.[49-51] This agrees with the findings that root diffusates of marigolds do not themselves affect plant-parasitic nematodes.[47,52-54] Moreover, in soil in the absence of light, photoactivation of thiophenes, which is necessary for nematicidal activity, does not occur. This also explains why α-terthienyl and analogues are completely devoid of activity when mixed with soil.[31,32] The ability of certain ectoparasites to breed on marigolds indicates that in the plant, nematicidal thiophenes are not evenly distributed throughout the roots. Also, *P. penetrans* that had been in roots of marigolds for up to 10 days were rapidly killed in near UV light, whereas *P. penetrans* that had been in the roots of oats (the control) remained alive, a clear indication that terthienyl and related compounds (or both) are involved in killing these nematodes.[56]

It was therefore postulated that these compounds are activated through mechanisms other than light.[35] Although plants do pipe light, it is very unlikely that in branched rootsystems such as those of *Tagetes*, sufficient light of proper wavelengths would be piped over 10 cm
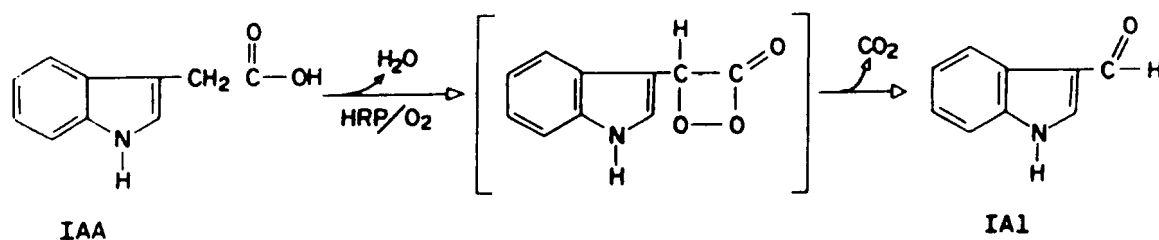
IAA

IAl

FIGURE 2.   The generation of triplet excited indolealdehyde by peroxidase oxydation of indole acetic acid.

and more to the infection sites.[57,58] We therefore investigated other activation systems. One such system involves 1,2-dioxetanes, which are among the best chemical generators of excited species.[59-62] Upon cleavage, one of the carbonyl fragments is formed in an electronically excited state, principally the triplet state.

Many photochemical processes have been induced in the dark using dioxetanes as generators of excited species. The energy of these chemiexcited species may perform work on other systems as shown by White et al.[62] and be called "photochemistry in the dark". As pointed out by White et al.,[62] chemically generated excited states may also play a role in biological processes.

Several well known enzymatic reactions are of interest since they yield products from the cleavage of a hypothetical 1,2-dioxetane intermediate capable of transferring energy to acceptors such as flavins, eosin, rose bengal, or methylene blue by nonradiative mechanisms, and the acceptor being present in very low concentrations, we have "photobiochemistry in the dark".[63,64] It has been established that indole-3-aldehyde formed in the peroxidase catalized oxidation of indole-3-acetic acid (IAA) is generated in the electronically excited triplet state and is capable of energy transfer to biological-like acceptors such as eosin (Figure 2).[65-68]

Preliminary experiments showed that upon penetration by *P. penetrans*, the overall activities of peroxidases in roots of *T. patula* increased about six times compared with the uninfected control plants. These activities dropped to the levels of the controls in approximately 14 days.[157] This is about the time needed by marigolds to kill the invaded *P. penetrans* (Figure 3).[47] Furthermore, again in preliminary experiments, it was shown to be possible to excite α-terthienyl with horseradish peroxidase and IAA under aerobic conditions and in an appropriate buffered system.[158] The excitation was measured by the increased amount of emitted light compared to the control. Spectral analysis of the light emitted from the enzymatically excited terthienyl has not yet been performed.

Although these experiments were carried out under conditions quite foreign to those in the roots of marigolds these results offer a model that may explain how *P. penetrans* is killed inside the roots of *Tagetes* species.

1. *P. penetrans* accumulates terthienyl and/or related compounds by permeation or ingestion during its stay in the roots.[56]
2. Activities of peroxidases in the roots increase after the nematodes invade.[159]
3. Nematodes do ingest peroxidases from their hosts as shown by Starr in the case of *Meloidogyne*.[69]
4. These three components are able, at least in vitro, to excite terthienyl which is capable of producing the poisonous singlet oxygen in or near the nematode's body. The generation of singlet oxygen may also contribute to the lesion formation in the roots near the nematode.

There are more nematicidal compounds known from Compositae. Their mode of action is, in most cases, unknown. Gommers isolated and identified 2,3-dihydro-2-hydroxy-3-methylen-6 methylbenzofuran as a nematicidal principle from roots of the *Helenium* hybrid
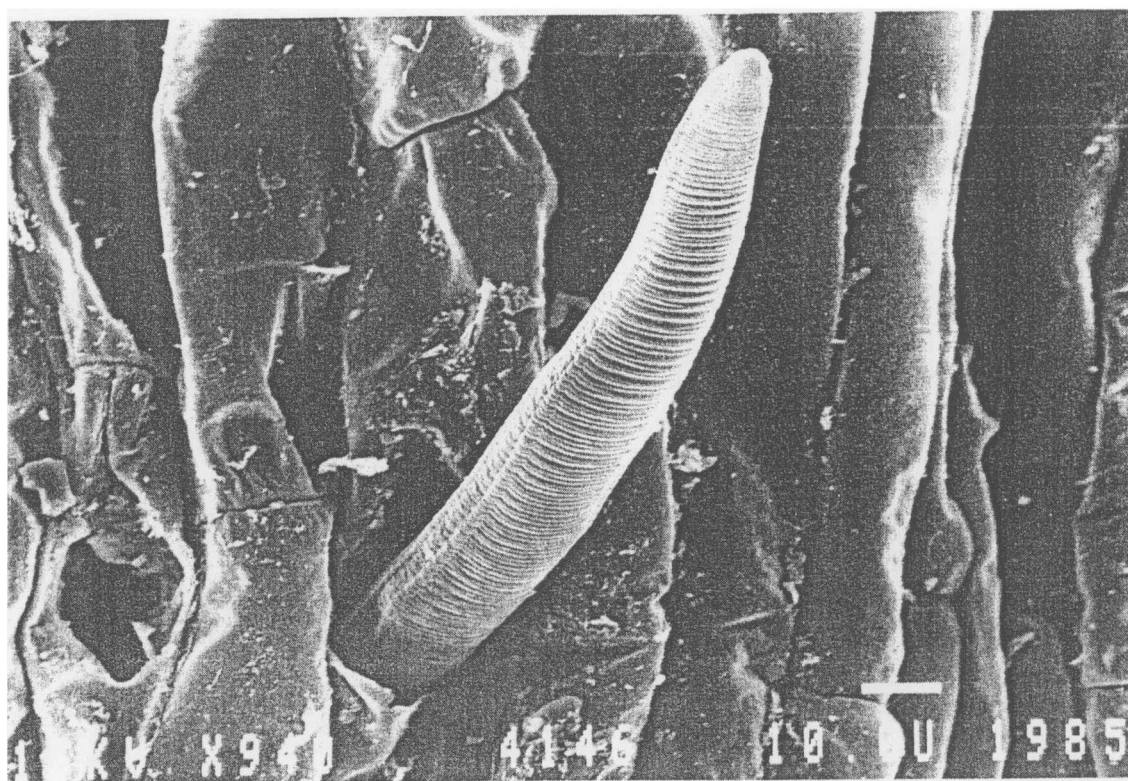
FIGURE 3. A larvae of *Pratylenchus penetrans* entering a root of *Tagetes patula*.

"Moerheim Beauty".[70] Two C_{13} triyne-enes from *Carthamus tinctorius* turned out to be highly toxic to the nematode *Aphelenchoides besseyi*, and also photoactive.[71,72] 1-Tridecaene-3,5,7,11-pentayne, 9,10-epoxy-heptadeca-16-ene-4,6-enediyne-8-ol from *Cirsium japonicum*, 1-phenyl-5-(1-propyl)-thiophene and 1-phenylhepta-1,3,5-triyne (phenylheptatriyne) from *Solidago altissima*, and *cis*-dehydromatricariaester from *Coreopsis lanceolata* were recorded as naturally occurring nematicides.[26,72,74] Gommers and voor in 't Holt found strong evidence for nematicidal activity of a number of red colored dithioacetylenes.[27] It is questionable whether or not these compounds exert nematicidal activity in vivo since many of these compounds are often abundantly present in Compositae that serve as excellent host plants for *P. penetrans*. For instance, *Xanthium strumarum* is a good host plant for *P. penetrans*, but nevertheless contains large quantities of tridecaene-3,5,7,11-pentayne.

Phenylheptatriyne, and a number of related polyacetylenes, are also phototoxic compounds, but unlike terthienyl they mainly exert phototoxicity under both aerobic and anaerobic conditions as was shown with the bacterium *Escherichia coli*, selected marine and freshwater algae, yeasts, and with hemolysis of red blood cells.[75-78] Apparently, photoactivated phenylheptatriyne and other naturally occurring polyacetylenes, possess both photodynamic and nonphotodynamic modes of action. The type II photodynamic action results in the production of singlet oxygen. Because rates of photodegradation of polyacetylenes are higher than those for thiophenes it was suggested that bond breaking/formation processes (nonphotodynamic mechanism) are more favorable relative to energy transfer to oxygen for polyacetylenes than thiophenes.[77] The mode of action of these polyacetylenes on plant parasitic nematodes has not yet been investigated.

B. Naturally Occurring Nematicides With Unknown Biological Significance in Host-Parasite Relationships

As summarized, sap from a number of plants or their parts, and extracts with organic