PHYTOCHEMICAL BASED STRATEGIES FOR NEMATODE CONTROL

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Abstract  This review examines the discovery of naturally occurring phytochemicals antagonistic toward plant-parasitic and other nematodes. Higher plants have yielded a broad spectrum of active compounds, including polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, lipids, terpenoids, sesquiterpenoids, diterpenoids, quassinoids, steroids, triterpenoids, simple and complex phenolics, and several other classes. Many other antinematodal compounds have been isolated from biocontrol and other fungi. Natural products active against mammalian parasites can serve as useful sources of compounds for examination of activity against plant parasites. The agricultural utilization of phytochemicals, although currently uneconomic in many situations, offers tremendous potential.

INTRODUCTION

Phytoparasitic nematodes are among the most notoriously difficult crop pests to control. Historically, management of nematode-induced crop damage has been achieved with the utilization of plant resistance, crop rotation and other cultural practices, or chemical nematicides. Two groups of chemical nematicides predominate: low-molecular-weight soil fumigants and contact carbamates or organophosphates (13, 170).

The development of new nematicides is a difficult task. Because most plant-parasitic nematode species spend their lives in the soil or within plant roots, the target of any chemical nematicide often resides at a fair distance away from the site of application of the chemical. Moreover, the nematode cuticle and other surface structures are impermeable to many organic molecules. Consequently, most nematicides have tended to be rather toxic or volatile, with poor target specificity and less-than-perfect human or environmental safety, such as groundwater contamination or atmospheric ozone depletion (10, 155).
Therefore, only a few chemical nematicides remain, and many of these lack broad-spectrum activity or efficacy of the same magnitude as that of soil fumigants. The economic cost of research and registration of new chemicals is an enormous hurdle for a prospective new chemical nematicide to overcome. Agrochemical companies are more likely to channel research spending into products with a potentially high market value like herbicides and insecticides rather than nematicides. Therefore, many nematologists are not optimistic about the importance of synthetic chemistry in the development of future chemical management tools for nematodes.

Consequently, several groups of researchers are attempting to develop phytochemical-based strategies for nematode control. To some extent, this research has its roots in the complex chemical interactions between plants and nematodes. Compounds involved in plant–nematode interactions include repellents, attractants, hatching stimulants or inhibitors, and nematotoxicants, either constitutive or formed in response to nematode presence. These interactions have received greater attention during the past 20 years because of the growth of the discipline of allelochemistry, the study of chemical-mediated interactions between a plant and other organisms in its environment. In addition, there has been a vast body of work involving the application of green manures to or within soils. Obviously, incorporation of organic residues strongly impacts the physical and biological properties of soils and may promote an environment favorable to nematode-antagonistic microorganisms (149). In some cases, however, a phytochemically mediated toxicity toward nematodes may also occur. Moreover, because members of the plant kingdom are capable of producing an incredible variety of secondary metabolites, many investigators have ventured beyond allelopathic interactions and looked for nematode-antagonistic substances in plant parts unlikely to be involved in nematode-plant interactions, such as leaves (135). Alternatively, they have examined exotic sources of phytochemicals, such as algae.

Several benefits may result from the identification of the specific phytochemicals involved in these interactions, whether they occur in a field or a laboratory. These compounds can be developed for use as nematicides themselves, or they can serve as model compounds for the development of chemically synthesized derivatives with enhanced activity or environmental friendliness. If phytochemicals are involved in the allelopathic suppression of a green manure or rotation crop, then chemical detection of the quantities of the allelochemical present can facilitate the development of nematode-antagonistic cultivars by eliminating the need for time-consuming bioassays. Many, but certainly not all, phytochemicals are safer to the environment or humans than traditional chemical nematicides. Indeed, the U.S. Environmental Protection Agency generally requires much less data to register a phytochemical than to register a conventional pesticide; registration costs are lower.

Therefore, this review focuses on the identification and potential use of compounds in higher plants or fungi with nematode-antagonistic activity. The review is largely organized by phytochemical structure, although such an organization will necessarily be imperfect. Not included is a discussion of the general chemical
ecology of nematodes, an area addressed elsewhere (46, 126). Similarly, this review does not concentrate upon studies in which plants or crude extracts were examined for nematode-antagonistic activity, unless the active compound was identified. Akhtar & Mahmood (2) consolidated much of the voluminous literature involving crude plant extracts into a table of over 100 plant species, including the specific nematode antagonism and type of bioassay employed. Although experiments with crude preparations are valuable for development of biologically based control strategies, these experiments are often difficult to interpret biologically or biochemically. For example, toxicants in such extracts or nutrients or phytohormones may act directly upon plant hosts. Specific compounds within such extracts may act synergistically or antagonistically.

Research with crude plant extracts may have practical application. The deregistration of nematicides has resulted in the appearance in recent years of various plant-based products with putative antinematodal activity. Most of these have not been available long enough to permit satisfactory evaluation by agricultural researchers. One of the better studied products is Sincocin, the trade name of a recently developed product containing a mixture of extracts from the prickly pear (Opuntia engelmannii lindheimeri, Cactaceae), southern red oak (Quercus falcata, Fagaceae), the sumac (Rhus aromatica, Anacardiaceae), and the mangrove (Rhizophora mangle, Rhizophoraceae). In field situations, Sincocin has provided control of Tylenchulus semipenetrans (citrus nematode) on orange, of Rotylenchulus reniformis (reniform nematode) on sunflower (Helianthus annuus), and of Heterodera schachtii (cyst nematode) on sugarbeet (Beta vulgaris) (15, 40, 116). Control of M. incognita on cassava and Radopholus similis on Anthurium was less successful than that provided by other methods (97, 144).

NEMATODE-ANTAGONISTIC COMPOUNDS FROM HIGHER PLANTS

Polythienyls in Tagetes spp.

The best studied case of a nematicidal principle from a higher plant is probably that of polythienyls from marigolds (Tagetes spp.) and other Asteraceae. Perhaps the first report of the resistance of marigolds to nematodes was by Goff (47), who noted that French marigolds (T. patula) and African types (T. erecta) were two of seven plant species devoid of root-knot nematode (Meloidogyne spp.) infection during trials of 80 different ornamental annuals. Steiner (148) noted that few of the many nematodes penetrating marigold roots reached maturity. Research into the active use of marigolds as nematode controls was stimulated by Slootweg’s (145) serendipitous discovery that T. erecta planted into soil subsequently provided control of the lesion nematode Pratylenchus penetrans in Narcissus tazetta. Hundreds of reports have since appeared in the scientific literature of the frequently suppressive effects on nematode populations of marigolds, whether utilized as a cover crop, rotation crop, green manure, or source of nematode-antagonistic
extracts. One of the more successful field trials showed that rotations of *T. patula* or *T. erecta* can provide economic control of *P. penetrans* on tobacco (*Nicotiana tabacum*) for two successive years (131).

The efficacy of marigold-based nematode control is a function of the marigold cultivar employed and the biological and environmental parameters in a given agroecosystem. Ploeg’s (121) brief summary of these studies concluded that *Pratylenchus* spp., *M. incognita*, and *M. javanica* were more consistently suppressed than *M. hapla* and *M. arenaria*. His greenhouse examination of nine cultivars of three *Tagetes* species indicated that the four major *Meloidogyne* species reproduce on *T. signata* but not on some varieties of *T. erecta* and *T. patula*. The most conspicuous differences among the four nematode species was in their reproduction on tomato (*Lycopersicon esculentum*) plants transplanted into soil containing marigolds that had had their shoots removed. A few cultivars suppressed all four species but one suppressed only *M. hapla* and one another suppressed all four species except *M. hapla*. Control provided by growing *T. patula* for 8 weeks before transplanting tomatoes was more effective than that provided by amending soil with roots or shoots (122). The suppression of marigolds on *M. incognita* is temperature dependent. Galling occurred on two normally resistant cultivars at 30°C, and most cultivars were ineffective when grown at 15°C or lower (123).

Uhlenbroek & Bijloo (158) identified an active phytochemical in *Tagetes erecta plena* as the thiophene α-terthienyl (Figure 1); it was nematicidal in vitro against the potato cyst nematode *Globodera rostochiensis* at 0.1–0.2 µg/ml, the wheat seed gall nematode (*Anguina tritici*) at 0.5 µg/ml, and the stem and bulb nematode (*Ditylenchus dipsaci*) at 5 µg/ml. The nematotoxicity of another isolated *Tagetes* polythienyl, 5-(3-buten-1-ynyl)-2,2'-bithienyl, was not determined, although the hydrogenated derivative 5-butyl-2,2'-bithienyl displayed activity against the same three nematode species (159). In a survey of 110 different Asteraceae for suppressive effects on *P. penetrans* in greenhouse experiments, over 40 species suppressed nematode population densities and at least 15 contained one of the thienyls (51). In vitro tests against the microbivorous nematode *Caenorhabditis elegans* and *P. penetrans* indicated that hairy root cultures of marigolds also produce α-terthienyl and other nematicidal phytochemicals (79); *C. elegans* was ten times more sensitive than *P. penetrans*. Interestingly, greater quantities of polythienyls are produced and secreted into culture medium when hairy root cultures are treated with cell-free extracts of the fungal root pathogen *Fusarium oxysporum* (11). The effect of nematode feeding upon polythienyl levels in such cultures is unknown. Among other nematodes, α-terthienyl inhibited host finding and induced mortality in the entomopathogenic nematode *Steinernema glaseri* at concentrations of 20 and 40 µg/ml (63).

A substantial body of work has examined the likely, complex mode of action of these compounds and synthetic analogs. The marigold polythienyls are only weakly nematicidal when incorporated into soil and require light or the action of peroxidase or other activators to release the singlet oxygen that kills nematodes (49, 50). The details of the specific activators and processes involved in the insecticidal,
Figure 1  Chemical structures of selected phytochemicals with nematotoxic activity. The structures of most of the compounds mentioned in this review are located on the Internet at http://www.annualreviews.org/.
nematicidal, or other toxic activities of these compounds continue to be examined (21). Tetrachlorothiophene, a simple analog, was once a registered nematicide in the United States (43).

Isothiocyanates and Glucosinolates from Brassicaceae

One of the first studied chemically mediated interactions between plants and nematodes was that between mustard plants (Brassica and Sinapsis spp.) and G. rostochiensis. Although initial research was undoubtedly confounded by the fact that nematologists initially believed the potato cyst nematode to be the same species as the sugarbeet cyst nematode, a series of investigations led to the discovery that allyl isothiocyanate (Figure 1), the major component of black mustard (Brassica nigra) seed oil, inhibited hatching of G. rostochiensis eggs at concentrations as little as 1 µg/ml (37, 103). A related compound, 2-phenylethyl isothiocyanate, occurs in S. alba roots; like allyl isothiocyanate, it inhibited egg hatch in laboratory experiments at 50 µg/ml and improved yield of potatoes in field experiments (38). Chemical investigations of isothiocyanates as nematicides were initiated at least as early as 1935 (146); one of the most successful nematicides is metam sodium, which degrades in soil to yield methyl isothiocyanate (61). Although as toxic in nematode bioassays as the once successful nematicide DD (dichloropropene-dichloropropane), allyl isothiocyanate is not as volatile and does not move as well within soil (99). Modified injection equipment and use of tarpaulins could increase its efficacy (99).

Rapeseed or canola (Brassica napus) has been receiving increasing attention as a rotation or green manure crop to provide nematode control (56), and isothiocyanates are also involved in this toxicity. Virtually all members of the Brassicaceae produce thioglucose conjugates called glucosinolates, which are hydrolyzed either in the soil or by mammals to form isothiocyanates, which react with the sulfhydryl groups of proteins (22).

In soil, the isothiocyanates often have activity against a variety of crop pests including nematodes (22). Evidence for the involvement of glucosinolate-derived isothiocyanates in toxicity of brassicaceous soil amendments was provided by experiments demonstrating that rapeseed leaf or seed extracts or specific glucosinolates were toxic to C. elegans, Xiphinema americanum, H. schachtii, or G. rostochiensis only when the extracts or compounds were enzymatically hydrolyzed (36, 60, 80, 120). Additional evidence for the involvement of a specific glucosinolate as the determining factor for nematode resistance results from the observation that the ability of Pratylenchus neglectus to reproduce on B. napus cultivars is correlated with their lower percentages of 2-phenylethyl glucosinolate, but not total glucosinolates (125). 2-Phenylethyl isothiocyanate applied to soil at 16.2 µg/g soil suppressed reproduction of P. neglectus (125). In contrast, egg production of M. javanica was not correlated with root glucosinolate concentrations or composition in most of the 11 Brassica cultivars examined, with one or two exceptions (96). The conclusion was that although glucosinolates are involved in nematode suppression, other chemical and biological factors may also be involved.
The complex chemistry and compartmentalization of the glucosinolates and other sulfur-containing compounds in the Brassicaceae and the complexity of nematode feeding may make interspecies comparisons difficult.

**Cyanogenic Glycosides**

Some plants contain other glycosides that release other nematicidal compounds. For example, green manures of Sudangrass (*Sorghum sudanense*, Poaceae) are also being examined as nematode suppressants; Sudangrass, like common sorghum (*Sorghum bicolor*), contains the glycoside dhurrin (Figure 1), which can be hydrolyzed to yield cyanide. Because activity of fractionated Sudangrass extracts against *M. hapla* is associated with the presence of cyanide in the fractions, it seems likely that dhurrin is involved in the mode of action of Sudangrass on *M. hapla* (171).

Cassava roots (*Manihot esculenta*, Euphorbiaceae) contain various quantities of cyanogenic glucosides, particularly linamarin. When cells are damaged, enzymes hydrolyze these glucosides, releasing cyanide via cyanohydrin intermediates. Manipueira, a liquid formed during processing of cassava roots, has been utilized for nematode control for decades in Brazil (124, 142). Magalhães et al. (82) discovered that manipueira linamarin rapidly degrades during storage, but the acetone cyanohydrin formed is stable. Antinematodal activity was not measured.

**Polyacetylenes from Asteraceae**

A broad spectrum of polyacetylenes with broad biological activity occur in the Asteraceae, and many of these are nematicidal. The first of these was identified by Gommers (48, as described in 49) from *Helenium* sp. as tridec-1-en-3,5,7,9,11-pentayne, with activity against *P. penetrans*. In the previously mentioned survey of 110 Asteraceae, suppression of *P. penetrans* in greenhouse experiments was often associated with presence of red acetylenic dithio compounds called thiarubrines (51). These compounds have broad-spectrum toxicity and often require light for maximum activity, similar to polythienyls. One such compound, thiarubrine C (Figure 1), was isolated from roots of the black-eyed Susan (*Rudbeckia hirta*); the LC$_{50}$ in a motility and viability bioassay was 12.4 µg/ml for *M. incognita* and 23.5 µg/ml for *P. penetrans* (137); light enhanced the nematicidal activity. Treatment of soil with 50 µg/ml thiarubrine C decreased *M. incognita* infection of tomato seedlings by nearly 95%.

Several other investigations of primarily members of the Asteraceae have revealed acetylenes with nematotoxicity as low as 1.0 µg/ml; compounds include 3-cis,11-trans- and 3-trans,11-trans-trideca-1,3,11-triene-5,7,9-triyne from flowers of *Cardhamus tinctorius* (72, 74), tridec-1-en-3,5,7,9,11-pentayne and 9,10-epoxyheptadec-16-en-4,6-diyn-8-ol) from the roots of *Cirsium japonicum* (66), 1-phenylhepta-1,3,5-triyne and 2-phenyl-5-(1-propynyl)-thiophene from *Coreopsis lanceolata* (66), cis-dehydromatricaria ester from *Solidago canadensis* (66), methyl 2-trans,8-cis-deca-2,8-diene-4,6-diynoate (2-trans,8-cis-matricaria ester) and 2-cis,8-cis-deca-2,8-diene-4,6-diynoate (2-cis,8-cis-matricaria ester) from...
roots of *Erigeron philadelphicus* (70), and heptadeca-1,9-diene-4,6-diyne-3,8-diol from roots of *Angelica pubescens* (Apiaceae) (105).

In an examination of 28 synthetic acetylenes for activity against *Pratylenchus coffeae*, several compounds with only one acetylenic bond were active at less than 1.0 µg/ml (104). Activity was greatest when an aryl, ester, or ketone functional group was conjugated to the triple bond. The nematotoxicity of polyacetylenes against *C. elegans* is increased by UV irradiation (168).

**Alkaloids**

Probably the first report of an alkaloid with activity against nematodes was that of the reversible acetylcholinesterase inhibitor physostigmine, a tricyclic carbamate originally isolated from the Calabar bean (*Physostigma venenosum*, Fabaceae), which reversibly immobilized *D. dipsaci* at 1000 µg/ml (19). Interestingly, treatment of pea seedlings with much smaller quantities (e.g., 30 µg/ml) of physostigmine sulfate significantly protected them against subsequent infection. Three tetracyclic alkaloids from *Bocconia cordata* (Papaveraceae)—chelerythrine, sanguinarine, and bocconine—were nematotoxic at 50–100 µg/ml against the free-living nematodes *Rhabditis* sp. and *Panagrolaimus* sp. (114, 115). The pyrrolizidine alkaloid monocrotaline (from *Crotalaria spectabilis*, Fabaceae; Figure 1) inhibited movement of *M. incognita* juveniles at 10 µg/ml (41). Exposure of juveniles to monocrotaline solutions did not prevent infection, however, nor was there any correlation between monocrotaline content of various species of *Crotalaria* and resistance to *M. incognita*.

Matsuda et al. (91, 92) isolated from *Sophora flavescens* (Fabaceae) five alkaloids (N-methylcytisine, anagyrine, matrine, sophocarpine, and sophoramide) with nematicidal activity against *Bursaphelenchus xylophilus*. Two other naturally occurring alkaloids, nicotine and cytisine, had slightly greater activity. Matrine, cytisine, N-methylcytisine, and aloperine were similarly isolated from *S. alopecuroides* as active compounds against *B. xylophilus* (175).

High concentrations (0.45%) of colchicine were nematicidal to *M. incognita* juveniles (106). The activity was insufficient to account for the toxicity of the source of the compound, seeds of *Gloriosa superba* (Liliaceae). Roots of the Madagascar periwinkle (*Catharanthus roseus*, Apocynaceae), a medicinal plant with anthelmintic activity, contain the pentacyclic alkaloid serpentine; this compound induced death and inhibited hatching of *M. incognita* at 0.2% (25); treatment of tomato seeds with serpentine inhibited subsequent infection of seedlings by *M. incognita* (129). Other alkaloids with nematicidal activity include steroidal alkaloids, discussed later.

**Fatty Acids and Derivatives**

Fatty acids are common plant and animal constituents and usually exist in vivo as esters. Tarjan & Cheo (154) discovered nematicidal activity in most of the 41 fatty acids or their salts evaluated at 1000 µg/ml against the microbivorous nematode
Panagrellus redivivus, as well as most of the 13 compounds tested against the
tobacco cyst nematode Globodera tabacum. Sayre et al. (139) identified butyric
acid in decomposing rye (Secale cereale, Poaceae) and timothy (Phleum pratense,
Poaceae) and demonstrated its activity at 880 µg/ml in immersion bioassays against
M. incognita and P. penetrans. The compound was inactive against the free-living
nematodes Rhabditis, Cephalobus, and Plectus. In contrast, McBride et al. (95)
failed to detect butyric acid in decomposing rye; only very low, non-nematicidal
concentrations of acetic and formic acids were detected. Moreover, because the
amounts of exogenously applied low-molecular-weight acids rapidly declined,
they concluded that other chemical or biological factors must be responsible for
the suppression of M. incognita by rye plants incorporated into soils.

Myristic, palmitic, and oleic acids were identified as the nematicidal princi-
ples in benzene extracts of roots of Iris japonica (Iridaceae) (105). The activity of
other aliphatic acids was investigated; 2-undecylenic acid had the strongest acti-
vity (70–80% mortality at 10 µg/ml). Free fatty acids have also been identified as
nematotoxic compounds produced by nematode-antagonistic fungi, as described
in a later section. Fatty acid esters are also nematicidal; methyl pelargonate (i.e., the
methyl ester of a C₉ fatty acid; Figure 1) and ethylene glycol pelargonate reduced
galling of tomato roots in laboratory experiments with M. javanica (30). In green-
house tests, activity of methyl pelargonate was demonstrated against H. glycines
and M. incognita on soybean; concentrations as low as 1.6 µl/liter were effective.

While isolating compounds from peanut (Arachis hypogaea, Fabaceae), Kimura
et al. (69) artifactually produced di-n-butyl succinate, a compound active against
P. coffeae at 100 µg/ml. Eleven of 17 other dialkyl succinates were also nematicidal.
A nematicidal triglyceride (sn-glycerol-1-eicosa-9,12-dienoate-2-palmitoleate-3-
linoleate) was reported in seeds of Argemone mexicana (Papaveraceae) (136). Its
ED₅₀ in vitro was 90 µg/ml; treatment of M. incognita juveniles with 100 µg/ml
concentrations prevented subsequent infection of tomato plants.

One of four compounds isolated from methanolic extracts of entire plants of
Allium grayi (Liliaceae) via a bioassay against M. incognita was the aliphatic
alcohol 1-octanol (151). Alcohols may also be involved in the nematode sup-
pression of velvetbeans (Mucuna, Fabaceae), which are gaining increasing usage
as cover or green manure crops. Chromatographic fractionation of a velvetbean
extract yielded 1-triacontanol and triacontanyl tetracosanoate as two compounds
inhibiting hatching of M. incognita at 1.0% (111, 112).

Terpenoids

A large number of plant compounds called isoprenoids are formed by the con-
densation of five-carbon isoprene units. Among the simplest are 10-carbon com-
pounds called terpenoids, which often are major components of fragrant or essen-
tial plant oils. These frequently possess activity against predators and pathogens.
Malik et al. (87) and Sangwan et al. (138) examined steam-distilled essential
oils of three Lamiaceae (basil, Ocimum basilicum; tulsi, O. sanctum; peppermint,
Mentha piperatum), two Myrtaceae (bottle brush, Callistemon lanceolatus; and
clove, Eugenia caryophyllata), and kachi grass (Cymbopogon caesius, Poaceae), as well as five synthetic terpenoid constituents within the oils: linalool (Figure 1), eugenol, menthol, cineole, and geraniol, for activity against juveniles of A. tritici, M. javanica, T. semipenetrans, and Heterodera cajani. Most of the oils and all of the terpenoids possessed some nematicidal activity, with clove oil, geraniol, linalool, and eugenol having the broadest activity. Soil applications of citral, geraniol, and limonene (a component of citrus oil) inhibited reproduction of M. javanica, M. incognita, M. arenaria, and H. schachtii (17, 117, 163).

Oka et al. (113) found that 12 of the essential oils extracted from 25 plant species inhibited mobility and hatching of M. javanica at 1000 µl/liter. Hatching was also inhibited by 125 µl/liter concentrations of four components of the oils: carvacrol and thymol from oregano (Origanum spp., Lamiaceae), trans-anethole from fennel (Foeniculum vulgare, Umbelliferae), and (+)-carvone from caraway (Carum carvi, Umbelliferae). When incorporated into soil at 150 mg/kg, all but thymol completely inhibited gall formation. Linalool, geraniol, and citronellol were isolated from the essential oil of Pelargonium graveolens (Geraniaceae) and induced mortality in M. incognita juveniles (81); seed treatment with the latter compound reduced M. incognita infection of tomato seedlings (129). A synergistically acting combination of linoolol and methyl chavicol inhibited the motility of H. cajani and M. incognita, whereas individual compounds were without effect (54). Menthol and α-terpineol inhibited M. incognita galling on cotton in greenhouse studies (17). In contrast, linalool, eugenol, cineole, and geraniol were poorly active against M. arenaria in greenhouse experiments on tomato plants; geraniol and linalool were phytotoxic (166).

Sesquiterpenoids

Sesquiterpenoids are C₁₅ compounds formed by the condensation of three isoprene units. The first sesquiterpenoids discovered to be nematotoxic were the aldehydes hemigossypol (Figure 1) and 6-methoxyhemigossypol and the C₃₀ dimers gossypol and 6-methoxygossypol. Postinfection production of these compounds in a resistant variety of cotton (Gossypium hirsutum, Malvaceae) was associated with resistance to M. incognita (162). A crude terpenoid aldehyde extract from cotton inhibited movement of M. incognita juveniles at 50 µg/ml, as did gossypol at 125 µg/ml (161). In contrast, a more comprehensive analysis of roots of six cotton cultivars failed to reveal correlation between nematode resistance and root (or leaf) terpenoid aldehyde content or composition (68).

Other studies involving sesquiterpenoids have included a demonstration of the discovered nematicidal activity at 1100 µg/ml in vitro against M. incognita in most of 18 synthetic sesquiterpenoid lactones, including alantolactone, a mammalian anthelmintic from Inula helenium (Asteraceae) (84). Although not examined for nematotoxicity, genetic analysis indicates that the production of the sesquiterpenoid phytoalexin solavetivone is genetically linked to resistance in potato (Solanum tuberosum, Solanaceae) against G. rostochiensis (32). Another sesquiterpenoid phytoalexin, rishitin, was identified in potato tuber discs infected with the
potato rot nematode *Ditylenchus destructor* or *D. dipsaci* (176). The ED$_{50}$ of the compound in a motility assay against *D. dipsaci* was 100 µg/ml.

One of the few organic compounds repelling phytoparasitic nematodes is the sesquiterpene α-humulene, which repelled *B. xylophilus* and occurred in a heartwood extract of *Pinus massoniana* (Pinaceae), resistant to pinewood nematode (150).

**Diterpenoids**

Nematicidal activity against *Aphelenchoïdes besseyi* was discovered in benzene extracts of roots of *Daphne odora* (Thymelaeaceae) (73). A complex analysis of 2 kg of dried roots yielded 100 mg of an active analogue of the mammalian poison daphnetoxin. The compound, named odoracin, was nematotoxic to *A. besseyi* at 5.0 µg/ml and consists of a diterpenoid (C$_{20}$) skeleton esterified to benzoic acid as well as a fatty acid. The related odoratrin was later isolated and possessed greater activity (105).

**Quassinoids, Steroids, and Triterpenoids**

Quassinoids are C$_{18-25}$ tetracyclic or pentacyclic degraded triterpenoids; the first ones were bitter principles isolated from the wood of *Quassia amara* (Simaroubaceae). Three quassinoids from the seeds of *Hannoa undulata* (Simaroubaceae)—chaparrinone (Figure 1), klaineanone, and glaucarubolone—inhibited penetration of tomato roots by *M. incognita* juveniles at 1–5 µg/ml (127). Because the 5 µg/ml concentration disrupted movement without 100 µg/ml being nematotoxic in vitro, the primary effect of the compounds seemed to be on motility.

Steroids and triterpenoids are C$_{27-30}$ compounds containing six isoprene units. Glycosides of steroids and triterpenoids are called saponins. Two solanaceous steroidal glycoalkaloids, α-tomatine and α-chaconine (Figure 1), were toxic to *P. redivivus* (5, 6). As would be expected for many nitrogenous compounds, the ED$_{50}$ varied with pH and were as low as 50 µg/ml and 85 µg/ml, respectively. In both cases, maximal activity was associated with a nonprotonated nitrogen atom. Meher et al. (98) examined the nematicidal activity in motility bioassays with *M. incognita* of eight glycosides primarily from plants related to garden asparagus. Four compounds were active at concentrations as low as 200 µg/ml: the steroidal glycosides asparanin I and asparanin B, from *Asparagus adscendens* seed, Liliaceae; and two triterpenoid glycosides from the Fabaceae, albichinin II from *Albizia chinensis* and sonunin III from *Acacia concinna*. Two other triterpenoid saponins, acaciasides A and B from *Acacia auriculiformis*, inhibited root galling by *M. incognita* when the compounds were applied as soil drenches at 10,000 µg/ml or foliar sprays at 4000 µg/ml (134). A mixture two furastanol glycosides (protodioscin and deltoside from *Dioscorea deltoidea*, Dioscoreaceae) inhibited *M. incognita* motility at 5000 µg/ml but decreased nematode infection of tomato roots at much lower concentrations via a host-mediated effect (177). Other nematotoxic triterpenoid saponins were isolated from *Bacopa monniera* (Scrophulariaceae), a plant with a history of medicinal use in India. One compound active
against *C. elegans* was identified as 3-β-\((O-β-D\text{-glucopyranosyl-(1→3)}-O-[α-L-arabinofuranosyl-(1→2)]-O-β-D-arabinopyranosyl)oxy\)] jujubogenin (130).

The triterpenoid camarinic acid from *Lantana camara* (Verbenaceae) killed *M. incognita* juveniles at 1.0% (18). Njoku et al. (110) isolated another triterpenoid, oleanolic acid, from the leaves of *Ocimum gratissimum* (Lamiaceae), a plant used as an anthelmintic in Nigeria. The compound killed *C. elegans* at concentrations as low as 1.0 μg/ml.

The neem tree, *Azadirachta indica* (Meliaceae), has been utilized by growers in India for insect control for centuries; several insecticidal formulations are commercially available. Analysis of the complex chemical constituents of the seed oil in particular and associated bioactivity has been the focus of much research (58, 77). One neem compound, the limonoid triterpenoid azadirachtin, is an insect antifeedant and molt inhibitor (102). Dozens of studies have demonstrated the toxicity of neem oils, oil cakes, extracts, leaves, roots, or root exudates against many species of nematodes (1). The specific chemical basis for the antinematodal activity of neem remains obscure, although fractions containing steroids and terpenoid glycosides appear to be toxic in vitro to *M. incognita* (89). Because azadirachtin at 10 μg/ml inhibits microfilarial release in the animal-parasitic nematode *Brugia pahangi* (16), it also probably is at least one of the components in neem active against phytoparasitic nematodes, possibly by a mode of action similar to that in insects.

Cucurbitacins are triterpenoids responsible for bitterness in cucumber plants (*Cucumis sativus*, Cucurbitaceae); they display antifeedant activity toward many insects but phagostimulatory activity toward others (153). Cucurbitacin-containing cucumber roots attracted *M. incognita* juveniles less effectively than roots without the ability to produce cucurbitacins (57). Nematodes were repelled by partially purified cucurbitacin extracts. Although direct toxicity of specific cucurbitacins to nematodes is unknown, cucurbitacins do have antinematodal activity when fed to mice (3).

Although hatching stimulants are not nematicidal, they could theoretically be used in field situations to induce hatch in the absence of host plants. Slightly more than 1 mg of a hatching stimulant for the soybean cyst nematode *Heterodera glycines* was isolated from 1058 kg (from 10 ha) of dried roots of kidney bean (*Phaseolus vulgaris*, Fabaceae). The compound was named glycinoclepin A and is a triterpenoid derivative (45, 90). Miwa et al. (100) designed two simpler analogs that stimulated hatch, although at higher concentrations than that required for glycinoclepin A. Two simpler analogs were also designed by Kraus et al. (76); one inhibited hatch but the other stimulated it. The hatching factors secreted by the potato for the potato cyst nematodes are a complex mixture of at least 10 distinct compounds; the naturally occurring steroid alkaloids solanine and chaconine also induce hatching at rather high concentrations (ca. 80 μg/ml) (33).

**Phenolics**

Most plant phenolics are produced via the phenylpropanoid and phenylpropanoid acetate pathways, which begin with the action of phenylalanine ammonia lyase
upon the amino acids phenylalanine or tyrosine. Because phenolics and lignification have been associated with plant resistance to a variety of pests and pathogens, investigations have sometimes revealed a correlation of elevated levels of phenolics with resistance or response of plants to nematode infection. However, few of these compounds have been isolated from plant roots, identified, and then examined for nematicidal activity. Among compounds with a single ring, pyrocatechol isolated from *Eragrostis curvula* (Poaceae) was toxic to root-knot nematode juveniles (141). Evans et al. (39) isolated five propenylphenols from leaves of *Piper betle* (Piperaceae) that induced 100% mortality of *C. elegans*; the compounds were chavicol (Figure 1), chavibetol and its acetate, and allylpyrocatechol and its acetate. Two of the four compounds isolated from *A. grayi* active against *M. incognita* were phenolics: methyl 4-hydroxybenzoate and methyl 4′-hydroxycinnamate (151). Salicylic acid, although better known as a mediator of resistance in plants, exhibited toxicity toward *M. incognita* at 50 µg/ml and provided some degree of control of galling when applied to tomato plants at the time of inoculation (85). Eleven of 55 phenolic compounds exhibited toxicity towards *M. incognita* juveniles at 1100 µg/ml (83). Immersion of tomato roots in solutions of five phenolics (pyrocatechol, hydroquinone, phloroglucinol, pyrogallol, and orcinol) inhibited infection by *R. reniformis* (86).

A *C. elegans* bioassay-directed fractionation led to the identification of chavicol and demethyleugenol from wounded leaves of *Viburnum furcatum* (Caprifoliaceae) and 4-vinylphenol from wounded leaves of three Rosaceae: *Sorbaria sorbifolia*, *Spiraea salicifolia*, and *Malus baccata* (174). Minimum effective concentrations ranged from 300 to 600 µg/ml; several synthetic analogs were more active. Other phenolics possessed activity in bioassays with *M. javanica* on tomato and were detected in wastewater generated during olive oil processing (164). The compounds included vanillic acid, caffeic acid, syringic acid, and o-coumaric acid and were active at 15 µg/ml. The sap of *Knema hookeriana* (Myristicaceae) was particularly active against *B. xylophilus* in a cotton ball bioassay; the activity resulted from the presence of 3-undecylphenol and 3-((8Z)-tridecenyl)phenol (4). Finally, although not truly phenolics, benzaldehyde and furfural inhibited *M. incognita*–induced gall formation in greenhouse or microplot studies in cotton, in a search for biologically based fumigants (17).

Other phenylpropanoid derivatives include two chromenes from the aquatic tropical plant *Rhyncholacis penicillata* (Podostemaceae) with activity against *C. elegans* (23). The seeds of celery (*Apium graveolens*, Umbelliferae) yielded 3-n-butyl-4,5-dihydropthalide, which induced 100% mortality at concentrations of 12.5 µg/ml and 50 µg/ml in *P. redivivus* and *C. elegans* (101). Finally, five diphenylethanoans with EC₉₅s as low as 0.7 µg/ml in motility assays against *C. elegans* were isolated from the roots of turmeric (*Curcuma comosa*, Zingiberaceae) (62).

Lignans are phenylpropanoid dimers. In an examination of heartwood and bark from three pine species (*P. massoniana*, *P. strobus*, and *P. palustris*) resistant to *B. xylophilus*, Suga et al. (150) discovered four nematicidial compounds: the lignans (−)-nortrachelogenin and (+)-piroresinol, the phenylpropanoid methyl ferulate,
and the stilbene pinosylvin monomethyl ether. The LD$_{50}$ of the latter compound was 4 µg/ml. Although not displaying nematidical activity against G. solanacearum and Globodera pallida, two lignans (bursehernin and matairesinol) from leaves of Bupleurum salicifolium inhibited hatching at concentrations of 50 µg/ml (53). Because of the activity of these lignans, over 20 relatively simple, synthetic compounds with a phenyl group were evaluated for toxicity against juveniles of the same two species; nearly half were active, with the most toxic compound (4-phenyl-3-butyn-2-one) having an LC$_{50}$ of 0.3 µg/ml (52). Last, nematidical activity of Pycnanthus angolensis (Myristaceae) against C. elegans was fractionated to yield the lignan dihydroguaiaretic acid, which had an LD$_{50}$ of 10 µg/ml (109).

The flavonoids constitute a broad class of products of the phenylpropanoid acetate pathway. Examples of nematotoxic isoflavonoids include the following: the widely distributed flavonoid quercetin, which inhibited reproduction of M. javanica as a soil drench at 400 µg/ml (117); the flavone glycosides linaroside and lantanoside from L. camara, which were lethal to M. incognita juveniles at 1.0% (18); and rotenone, which displayed nematidical activity towards C. elegans (147).

Some phytoalexins are flavonoids. Rich et al. (132) identified coumestrol and psoralidin as compounds produced by lima beans in response to infection by Pratylenchus scribneri. Coumestrol inhibited the motility of P. scribneri at 5–25 µg/ml but did not inhibit motility of M. javanica. Accumulation of the phytoalexin glyceollin in soybean roots was associated with an incompatible response to root-knot nematodes; at 15 µg/ml, the compound strongly but reversibly inhibited movement of M. incognita in vitro (63, 64). Medicarpin, the major phytoalexin of alfalfa, is constitutively expressed in alfalfa, occurs in higher concentrations in resistant alfalfa cultivars, and inhibits motility of P. penetrans in vitro (14). Medicarpin and 4-hydroxymedicarpin were isolated as nematicidial principles in roots of the Ethiopian medicinal plant Taverniera abyssinica (Fabaceae) by virtue of their activity in a C. elegans bioassay (147).

Tannins are phenolic oligomers. Condensed tannins or proanthocyanadins are one group of tannin, formed by the polymerization of flavonoids. The types and amounts of pre- and postinfectional proanthocyanadins were higher in a resistant banana cultivar than in two susceptible ones (28). König et al. (75) found that complex tannins from Quercus petraea bark and a fraction containing a mixture of ellagitanins (i.e., tannins formed by esterification of glucose with mono- or oligomers of ellagic acid) inhibited the reproduction of C. elegans with an IC$_{50}$ of 500 µg/ml. Finally, tannic acid, a mixture of galloyl esters of glucose, stimulated hatching of H. glycines at concentrations up to 39 µg/ml; higher concentrations were inhibitory (26).

Miscellaneous

One of the first efforts to identify a phytochemical active against a nematode was in the case of the chemical factor in roots and exudates of asparagus (Asparagus officinalis, Liliaceae) that conveyed resistance to the stubby-root nematode
Paratrichodorus christiei (133). Unfortunately, the compound was incompletely identified as a glycoside with an unknown, low-molecular-weight aglycone. Much later, the sulfur-containing compound asparagusic acid was identified in asparagus roots as a nematicide; at 50 µg/ml it inhibited hatching in H. glycines and G. rostochiensis and induced mortality in three other nematode species (152). Other sulfur-containing nematicidal substances are allygrin from A. grayi, active against M. incognita (151), and five thiosulfonates isolated from an ether extract of Allium fistulosum var. caespiitosum, each with greater activity than the four compounds from A. grayi. Several related thiosulphonates and thiosulfonates were synthesized and possessed similar nematicidal (and antibacterial) activity. Additionally, allicin (Figure 1), a major component of garlic (Allium sativum), inhibited hatching of M. incognita at concentrations as low as 0.5 µg/ml and was toxic to juveniles at 2.5 µg/ml (55). Immersion of tomato roots in allicin solutions as a prophylactic measure was beset by problems of phytotoxicity and lack of nematicidal toxicity, but a 5-minute immersion in 25 µg/ml allicin inhibited penetration of roots by juveniles by 50% and was not phytotoxic.

Attraction or repellency of host plants to nematodes has been the subject of several investigations (46, 119), but only a few host- or nonhost-specific compounds mediating this attraction are known. Maize roots exude cyclic hydroxamic acids, one of which (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) attracts Pratylenchus zeae at concentrations in host exudates (44).

Several investigators have conducted major research programs on the biological relevance of plant lectins in plant-nematode interactions and the expression of lectin genes in transgenic plants (24). Application of Concanavalin A, a lectin from the jackbean, Canavalia ensiformis (Fabaceae), resulted in substantial control of M. incognita on tomato in growth chamber, greenhouse, and microplot experiments, possibly by binding to nematode chemoreceptors (88). In another experiment, increased hypersensitivity of soybean to infection by M. incognita resulted from treatment of infective juveniles with Concanavalin A, wheat germ agglutinin, or soybean agglutinin (29). In contrast, a lectin from Canavalaria brasiliensis was not very effective in controlling reproduction of Meloidogyne incognita on tomato (124). Expression of snowdrop lectin (Gala nthus nivalis agglutinin, Liliaceae) in transgenic potato or rapeseed plants conferred partial resistance to G. pallida, H. schachtii, and P. neglectus (24).

Other proteins with known toxicity to nematodes are the cysteine protease inhibitors called cystatins. The expression of cystatins in genetically transformed plants is another strategy for developing plants with resistance to a variety of pests and pathogens. Transfer of first-to-third juvenile stages of C. elegans to oryzacystatin-supplemented medium (2.5 µg/ml) resulted in cessation of development and death (160). Moreover, females of G. pallida cultured upon oryzacystatin-expressing Agrobacterium rhizogenes–transformed tomato roots were smaller than those cultured upon expressing oryzacystatin, particularly one variant with a single amino acid deletion. Expression of the same oryzacystatin variant in rice halved egg production in M. incognita (160).
NEMATODE-ANTAGONISTIC COMPOUNDS FROM ALGAE

Several reports have indicated that many species of algae or algal extracts inhibit nematode reproduction; methods of application have included foliar sprays, incorporation into soil, or incubation in vitro (31, 42, 118, 169). Substantial discussion has centered on the issue of whether nematode suppression results from direct toxicological action or a host-mediated suppression caused by phytohormones in the algal materials. The effects of *Ascophyllum nodosum* (Phaeophyceae) extracts in suppressing *M. incognita* and *M. javanica* infection appear to result from the betaines δ-aminovaleric acid betaine, γ-aminobutyric acid betaine, and glycine-betaine (59, 172, 173).

NEMATODE-ANTAGONISTIC COMPOUNDS FROM FUNGI

In some respects, fungi are preferable to vascular plants as sources of naturally occurring antinematodal compounds because of the less complex anatomical structure of fungi and their adaptability to being grown in large fermentors. Obviously, a logical place to initiate the isolation of nematode-antagonistic fungal compounds would be with nematode-antagonistic fungi. One crude fungal product is DiTera™, a recently introduced commercial nematicide produced by the nematode-parasitic fungus *Myrothecium verrucaria*. Developed with motility bioassays with *M. javanica*, DiTera is effective against a broad range of phytoparasitic nematodes (167). Its toxicity appears limited to plant-parasitic nematodes, as effects were not observed on the rat intestinal parasite *Nippostrongylus brasiliensis*, *P. redivivus*, or *C. elegans*. Toxicity is due to synergistically acting low-molecular-weight, water-soluble compounds. The material interfered with the hatching of *G. rostochiensis* but not *M. incognita* (157).

A thorough analysis of fungi as a source of nematicidal substances has been conducted by Anke & Sterner and colleagues (8, 9). In initial tests, extracts of half of 12 nematophagous fungi were toxic to *C. elegans*; interestingly, secretion of nematode-antagonistic compounds into culture medium was a function of the chemical composition of the medium. Extracts of nine of ten nematophagous fungi were toxic to *M. incognita* (93). Linoleic acid was identified as the primary nematotoxic compound in mycelia of *Arthrobotrys conoides* and *A. oligospora*; the possible subcellular localization of this fatty acid in trap cells and its biological role in capture of prey have been discussed (8). The LD50s of linoleic acid vs *C. elegans* and *M. incognita* were 5 and 50 µg/ml, respectively; other fatty acids had a similar difference in relative toxicities (9).

Other compounds possessing nematicidal activity against *C. elegans* or *M. incognita* and largely isolated by Anke & Sterner and colleagues from fungal culture filtrates include the following (8, 9, 94): two chlorinated compounds (lachnumol A and lachnumon), two related mycorrhizins (mycorrhizin A and...
dechloromycorrhizin A), six papryracons, lachnumfuran, and lachnumlactone A from *Lachnum papyraceum*; the chlorinated compound mycenone (Figure 1) from *Mycena* sp.; the lactone lethaloxin from *Mycosphaerella lethalis*; floccolactone from *Strobilomyces floccopus*; the carcinogenic lactone patulin from *Penicillium* spp.; 1-methoxy-8-hydroxynaphthylene and 1,8-dimethoxynaphthylene from *Daldinia concentrica*; 14-epicochlioquinone B from *Neobulgaria pura*; the anthraquinone emodin from various fungi; the related marasmic acid from *Marasmius conigeus*; phenoxazone from *Calocybe gambosa*; p-anisaldehyde, coriolic acid and linoleic acid from *Pleurotus pulmonarius*, linoleic acid from *Chlorosplenium* sp.; the cyclic depsipeptide beauvericin from *Beauveria bassiana*; the cyclic depsihexapeptide ennatin A from *Fusarium*; the cyclic undecapeptide cyclosporin A from *Tolypocladium inflatum*; and the cyclic dodecapeptide omphalotin A (Figure 1) from *Omphalotis olearius*. Because of its high activity (LD50 of 0.75 µg/ml) against *M. incognita*, omphalotin A was examined against other plant parasites: LD50s against *P. penetrans*, *H. schachtii*, and *R. similis* were 25, 30, and >75 µg/ml (94). Soil drenches provided complete control of *M. incognita* infection of cucumber and lettuce. Unfortunately, progress in development of the compound as a nematicide is impeded by its low yield. Other nematicidal cyclic depsipeptides termed bursaphelocides A and B occur in a member of the *Mycelia sterilia* (65).

Other workers have isolated many fungal compounds active against nematodes. Fungi under investigation as biocontrol agents have often provided logical sources for some of these compounds. Acetic acid was identified as the nematicidal principle in culture filtrates of *Paecilomyces lilacinus* and *Trichoderma longibrachiatum* (34); activity was as low as 20 µg/ml against *G. rostochiensis*. This finding led to an investigation of the toxicity of a large number of fatty and other acids and their derivatives against 20 nematode species (35). Activity was largely specific against phytoparasitic nematodes, and esters of dicarboxylic acids possessed the greatest activity. Similarly, 2-decenedioic acid was identified as the nematode-paralyzing substance secreted by the wood-rotting basidiomycete *Pleurotus ostreatus* (78). The compound was identified via a motility assay against *P. redivivus*, *C8–12* monoenoic fatty acids had even greater activity. Citric acid and oxalic acid were weakly nematicidal (4000 µg/ml) components from culture filtrates of a nematode suppressive strain of *Aspergillus niger* (178).

A variety of other antinematodal compounds have been isolated from other fungi. Seven distinct ophiobolins, tricyclic sesterpenoids (i.e., C25) from *Aspergillus ustus* and *Cochliobolus heterosporus*, inhibited the movement of *C. elegans* and noncompetitively inhibited binding of ivermectin (143, 156). Cochlioquinone A from *Bipolaris sorokiniana* inhibited *C. elegans* movement but competitively inhibited ivermectin binding (140). Cladobotrin I, isolated from culture filtrates of *Cladobotryum rubrobrunnescens*, displayed weak (LD50 of 100 µg/ml) activity against *M. incognita* (165).
A strain of *Verticillium chlamydosporium* originally isolated from *M. incognita* eggs was fractionated with in vitro toxicity and root invasion bioassays (67). The nematicidal compound, phomalactone (C$_8$), was active at 500 $\mu$g/ml. Two polyketides, cannabiorcichromenic acid and 8-chlorocannabiorcichromenic acid, were isolated from a *Cylindrocarpon olidum*, originally isolated from *M. incognita*; a mixture of the acids was toxic to *Heterorhabditis* sp. (128). Another polyketide, aspyrone, was isolated from the culture filtrate of *Aspergillus melleus* and was toxic to *P. penetrans* at 100 $\mu$g/ml (71).

Classical mycotoxins often exhibit activity against nematodes. The macrocyclic lactone Brefeldin A (from *Penicillium* spp.) inhibited motility of the vinegar eelworm *Turbatrix aceti* at 25 $\mu$g/ml; fumagillin (from *Aspergillus fumigatus*) was active at 50 $\mu$g/ml, and trichothecin (from *Trichothecium roseum*) was active at 50 $\mu$g/ml (12). Six other mycotoxins were reported to possess toxicity to *M. javanica*, *M. hapla*, or *P. neglectus* at concentrations as low as 0.2 $\mu$g/ml (27). Bioassay-driven fractionation of culture filtrates of a strain of *Fusarium equiseti* isolated from *H. glycines* led to the detection of two trichothecene mycotoxins (4,15-diacetynivalenol and diacetoxyscirpenol) as inhibitors of hatching and motility in *M. incognita* (107). Similar research with a strain of *Chaetomium globosum* yielded the dialdehydic simple phenol flavipin (Figure 1) as the major active component (108).

**OUTLOOK**

The development of nematicidal phytochemicals has not yet reached maturity; efforts have largely consisted of basic, descriptive research. One conspicuous aspect of this descriptive research is the small number of botanical families examined. Obviously, a broader range of plant taxa needs to be included in the future. In addition, most plant nematological studies have begun with plants or plant compounds known to be active against other pests and pathogens. Nonetheless, the few investigations of phytochemicals with biological activity against nematodes have yielded a wide variety of structurally diverse compounds. Although it may be tempting to speculate that such compounds are functionally active in higher plants as antinematodal substances, most investigators have pointed out that inadequate attention has been given in most cases to the concentrations of many of these compounds within plants, or their cellular or subcellular locations. The mode of action of most nematicidal phytochemicals is largely unknown. Indeed, in some cases the concentrations evaluated in vitro have been so large that physiological activity would be contraindicated. In such experiments, osmotic, pH, and other nonspecific effects cannot be excluded.

There is less interest among the public at large in mode of action than in the development of environmentally safe, inexpensive, agronomically useful compounds. Are nematode-antagonistic phytochemicals likely to be safe, effective, and useful? Generalizations addressing this question are difficult to formulate; any response would be a function of the specific compounds and plants described in
the major sections of this review. With respect to safety, the popular perception that natural compounds are more benign than synthetic compounds is not necessarily based upon science (7). Although an argument can be made that naturally occurring compounds are often more readily degraded in the environment than synthetic compounds, the modes of action of the two groups of compounds and the presence of immediate, nontarget effects are often similar. Rapid biodegradation of a phytochemical may not be an asset if the target nematode must be exposed to it for a long duration of time, as sometimes is necessary during laboratory experiments (137). The bioassays used to guide phytochemical fractionations often select poorly against general toxicants.

With respect to efficacy, a few of the compounds have exhibited activity as low as commercial nematicides. Synthetic chemists have sometimes, albeit rarely, successfully developed structures with greater activity than plant-based model compounds. Although efficacy of specific compounds may be high in vitro against some developmental stages of some nematodes, the behavior of the compounds in soil or other considerations have limited the agricultural use of specific phytochemicals.

The usefulness of phytochemicals as control tools is a function of economics, which in turn is a function of the alternative chemical and nonchemical methods for nematode management available to a specific grower. Specific phytochemicals may be expensive to purify or synthesize chemically. Probably the most important mammalian anthelmintics developed in recent decades are the avermectins, natural products secreted by \textit{Streptomyces avermitilis}. Avermectins are effective as soil nematicides in some cases, but their use is not usually economically feasible (20). The use of a crude phytochemical extract instead of a purified or synthetic compound may result in beneficial effects beyond mere nematode control and thus may convey additional economic benefit. A crude extract may involve the extra expense of application of larger volumes of material, however. The cost of crude versus synthetic materials is a function of the complexity involved in the manufacture of each. Crude extracts also have the potential for synergism or antagonism among individual components.

Utilization of green manures or nematotoxic rotation crops is another method of incorporating phytochemicals into management practices. The benefits of green manuring often include weed suppression and lack of substantial chemical inputs. However, such crops often provide no short-term farm income and often involve additional equipment and thus expense. One example of a situation in which green manuring may be suited would be in orchard replanting, in which rotation is often done to increase soil organic matter and decrease compaction (56). If the effects of rotation with a nematotoxic crop extend for more than a single growing season, its adoption may be practicable, as in the case with marigolds and tobacco in Ontario (131). Expression of a phytochemical in genetically transformed plants is another method of phytochemical utilization but will be difficult because of the complex biosynthesis of some (but not all) phytochemicals. Moreover, because many phytochemicals can be unpalatable or poisonous to mammals, root or feeding site-specificity of expression would be desirable.
The bioassays selected in phytochemical research are critical. The least time-consuming bioassays are those involving microbivorous, rapidly cultured nematode species. However, activity against *C. elegans* and other microbivorous species is often poorly correlated with activity against phytoparasitic species (e.g., 9); use of *C. elegans* may result in the identification a higher percentage of broad-spectrum toxins instead of phytoparasitic nematode-specific compounds. Indeed, few investigators have utilized more than one nematode species in bioassays; 50-fold differences in activity have been reported (35). Direct in vitro tests must be complemented by in vivo, soil-based experiments in order to examine phytotoxicity or binding to the soil. In order to determine if a purified compound is actually the nematode-antagonistic compound, results should be duplicated with a chemically synthesized compound, a replication that is usually not practical.

Although possible, phytochemical research will probably not yield the tremendously successful biocides used for soil nematode control in the previous century. Future nematode control will depend upon a set of judiciously used, site-specific practices, and phytochemicals could prove to be a major component of this set. The multibillion dollar losses inflicted by phytoparasitic nematodes and the paucity of other chemical management tools warrants an increased research effort in this area.

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