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Potential for Nematode Control by Mycofloras Endemic in the Tropics[1]

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Abstract: Results of mycological surveys of root-knot and cyst nematodes from tropical regions indicate that most fungal species associated with females or cysts of species of *Globodera*, *Heterodera*, and *Meloidogyne* are those found with nematodes from temperate areas. Some fungal species, however, were found in higher frequency in tropical regions than in temperate countries; e.g., *Cylindrocarpon destructans* and *Ulocladium atrum* were the most common species associated with *G. pallida* and *G. rostochiensis* cysts in Peru. These fungi are not so frequent in nematodes from temperate areas. Fungi associated with diseased nematodes in the tropics vary greatly in nutritional requirements and include thermophilic species as well as cold-tolerant fungi. Multi-cropping systems possible in most tropical regions may be designed to increase the frequency of occurrence of microbial species antagonistic to phytonematodes.

Key words: amendment, biological control, cyst nematode, microbial ecology, pest management, root-knot nematode.

Soils are reservoirs for microfloras that are highly varied in composition and activity. Through interactions between microorganisms and other components of the soil biota the organic matter is transformed and nutrients are made available to plants. The survival or demise of the latter in soil depends to some extent on the nature and sequence of microbial activities. Agricultural soils in tropical areas of the world are as varied in composition and activities as those elsewhere. Agriculture in the tropics is practiced in a wide range of altitudes and environments; it includes warm weather crops as well as almost all of those grown in temperate and cold regions. Consequently many genera and species of plant-parasitic nematodes that occur in other latitudes are also found in tropical regions of the world, particularly at higher elevations. There is, however, a characteristic of tropical agricultural soils that separates them from others. In the tropics and subtropics, several crops are grown within the course of a year and multicrop systems are common. Therefore, phytonematode populations increase more rapidly in tropical soils than in other regions of the world. Unrestricted development of nematode populations does not, however, occur in tropical soils; there are regulating mechanisms that prevent unlimited development of the populations. The purpose of this paper is to examine current knowledge of egg-destroying fungi as factors that may influence phytonematode population dynamics in tropical soils.

Historical Antecedents

Knowledge of the existence of a microflora associated with eggs, females, or cysts of nematodes belonging to the Heteroderidae is almost as old as the description of the first economically important species, *Heterodera schachtii* Schmidt. In 1877 Kühn observed a fungus pathogenic to females of *H. schachtii* (44,45) and named it *Tarichium auxiliare* Kühn, today known as *Catenaria auxiliaris* (Kühn) Tribe. Because of its devastating effect on sugarbeet production and the importance of the sugar industry to Europe, several investigators in the late 19th and early 20th centuries expanded on the observations of Kühn and reported on other fungal species parasitizing *H. schachtii*. Nine genera of fungi were found to be associated with *Heterodera* spp. by European workers prior to 1940 (Table 1). This indicates that several taxonomically unrelated fungal species were known to parasitize and destroy cyst nematodes. These fungi varied from predacious forms (3) to some, such as *C. destructans* and *C. auxiliaris*,

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TABLE 1. Fungal species isolated from *Heterodera* spp. in Europe from the late 19th century to 1940.

Current name	Synonyms	Reference	
		H. avenae	*H. schachtii*
Arthrobotrys oligospora			43
Catenaria auxiliaris	*Tarichium auxiliare*		43,44,45
Cylindrocarpon destructans	*Cylindrocarpon radicicola*	26	
Endogone sp.	*Protomyces*		43
Entomophthora sp.			4,32
Isaria sp.			43
Metarrhizium anisopliae	*Isaria destructor*		4,32,68
Phialophora malorum	*Phialophora heteroderae*		43,73
Pythium sp.	*Torula heteroderae*		43,82
	Trichosporium populneum		
	Olpidium nematodae		

that have been repeatedly observed in association with root-knot (*Meloidogyne* spp.) and cyst (*Globodera* spp. + *Heterodera* spp.) nematode reproductive stages in more recent surveys.

CURRENT KNOWLEDGE

Detailed mycological surveys of heteroderid nematodes have been conducted in Australia, Europe, and in North and South America during the past 20 years. Results from these studies reveal that there is considerable variability both in frequency of occurrence and species identity (Table 2). They range from opportunistic fungi commonly found in agricultural soils (e.g., species of *Fusarium, Penicillium, Phytophthora,* and *Pythium*) to others that are seldom isolated from soils (18). Large as it appears, the number of species of fungi isolated from heteroderid nematodes is limited in comparison to the total number of fungal species present in agricultural soils (18). Relatively few genera—*Acremonium, Alternaria, Catenaria, Cylindrocarpon, Exophiala, Fusarium, Gliocladium, Humicola, Nematophthora, Paecilomyces, Penicillium, Phoma, Pythium,* and *Verticillium*—have been associated with more than one species of nematode.

The consistency of the association of these genera with species of heteroderid nematodes suggests that a degree of specialization may be a prerequisite for successful colonization of the ecological niche represented by eggs, females, or cysts. Indeed, several of these fungal genera are known animal parasites. *Exophiala pisciphila,* as its specific epithet indicates, was first isolated from fish (18), and a number of species of *Paecilomyces* and *Verticillium* are known to colonize insects (67). Some isolates of *P. lilacinus* can cause severe dermal and ocular disorders in humans (1,50,78). The species of *Verticillium* isolated from nematodes belong to the section Prostrata which includes *V. lecanii,* an insect parasite (30). The genera *Fusarium* and *Neocosmospora* contain several well-known pathogens that cause wilts and root rots in many plant species (18). The association of *Fusarium* spp. with nematodes in inducing disease complexes of plants is well established (87). Species of these fungal genera may be able to colonize nematodes and also be pathogenic to plants. Recently, *N. vasinfecta* has been shown to cause severe problems in soybean fields (29), and there may be a correlation between the presence of *H. glycines* and severity of *Neocosmospora* wilt.

Although there is a common denominator among opportunistic fungal species invading heteroderid nematode eggs, females, and cysts, the composition of the associated mycoflora in individual fields probably is determined by such factors as plant host, cropping history, soil properties, and climatic conditions. There may even be seasonal variations in the mycoflora associated with nematodes in any given

field (5,8). Monocropping of oats in England has been shown to reduce *H. avenae* population levels through increased parasitism of the nematode by fungi (7,35–42). In the southeastern United States continuous cropping of soybean can lead to significant increases in the level of fungal colonization of females and cysts of *H. glycines* (21,55,58). Cropping systems, other than monocultures, may enhance the level of parasitism of phytonematodes by fungi; however, the ecology and population dynamics of mycofloras associated with phytonematodes are least understood. There is need to understand the effects of cropping sequences, cultural practices, and fungicides and other pesticides on populations of fungi associated with nematodes. Without knowledge of the factors that influence field dynamics of these fungi we cannot hope to assess their importance in regulating nematode populations, much less exploit their antagonistic activities on nematodes. We do know that the level of parasitism of *Meloidogyne* eggs by fungi in the field is influenced by soil fertility (12) and that under greenhouse conditions the use of chitinous organic soil amendments can increase parasitism of eggs by fungi (12,13,52,71). Therefore, we may be able to manipulate antagonistic mycofloras in the soil to increase destruction of nematode eggs.

Data on the mycology of heteroderid nematodes from tropical regions of the world is limited. Surveys conducted in other regions of the world are not readily compared with the few data from tropical studies. There are, in addition, problems in variation in methodology. Each research group develops distinctive methodologies and cultural media that may drastically influence what is isolated from nematodes. Dackman and Nordbring (15) recently showed the importance of methodology in performing mycological surveys of cyst nematodes.

A survey was conducted to discern what fungi were associated with *H. glycines* in soybean fields in the Cauca Valley, Colombia (61), where the nematode was recently introduced (79). The soybean cyst nematode at that locality harbored a mycoflora (Table 3) essentially identical to that reported earlier in *H. glycines* from different parts of the United States. A survey of the mycoflora of *Globodera pallida* and *G. rostochiensis* cysts in potato fields at Arequipa in southern Peru (60) (Table 4) supported the view that a consistent group of fungi occur, not only in *Globodera* spp. but also in other Heteroderidae, in widely scattered geographical locations.

Fungi associated with heteroderid nematodes differ in physiological properties. Our studies have shown that taxa associated with *H. glycines* and *Meloidogyne* spp. vary greatly in temperature requirements (Fig. 1). Optimal temperatures for mycelial development on potato dextrose agar and chitin agar varied among fungal isolates obtained from *H. glycines* or *M. arenaria* (Table 5). Whereas some species (*P. terrestris, V. chlamydosporium*) grow best at relatively low temperatures (15–20 C), other (*C. tonkinense, M. aurantiaca, P. variotii*) have temperature optima of 30 C or above. Also, some species, such as *P. kendrickii,* have a broad optimal temperature range, whereas in others (*V. chlamydosporium*) the range is very narrow. The temperature range of 15–40 C was divided in increments of 5 C, and the number of species that fit into each interval was expressed as a percentage of the total number of species in the study (Fig. 2). The greatest number of fungi had an optimal temperature range of 21–25 C, followed in decreasing frequency by species with optima of 26–30 C and 31–35 C. All the isolates in the study were obtained from eggs, females, or cysts of *H. glycines* from fields in Alabama and other southeastern states or from *M. arenaria* eggs from peanut fields in the same region. These findings indicate that fungi present in these fields have optimal temperature ranges for growth that reflect, for the most part, the annual temperature prevalent in the southeastern United States. The mycoflora associated with phytonematodes, when con-

TABLE 2. Fungal species isolated from root-knot (*Meloidogyne* spp.) and cyst (*Globodera* spp. and *Heterodera* spp.) nematodes from Australia, Europe, and North and South America.

	Reference				
	Globodera spp.	*H. avenae*	*H. glycines*	*H. schachtii*	*Meloidogyne* spp.
Acremonium sp.				66	
Acremonium strictum			21,22	65	
Alternaria alternata			21,59		64
Arthrobotrys oligospora				43	
Aureobasidium pullulans					64
Catenaria auriliaris			39,83	39,44,45	
Chaetomium cochliodes			21		
Chaetomium gracile			57		
Chaetomium indicum			57		
Chalara hyalina			21,56		
Cladosporium cladosporioides			21,57		
Cladosporium sphaerospermum			21		
Codinea heteroderae			21,57		
Colletotrichum coccodes	85,86				
Cylindrocarpon sp.		15			
Cylindrocarpon destructans	60	11,40,41,84		11,26,40,81,84	
Cylindrocarpon didymum	60				
Cylindrocarpon gracile	60				
Dactylella oviparasitica					76,77
Endogone sp.		88			
Exophiala sp.	27				
Exophiala jeanselmei	85,86				
Exophiala mansonii				7	
Exophiala pisciphila		81	21,22,57	80,81,84	
Fusarium spp.		41			
Fusarium equiseti			22,57		
Fusarium lateritium			22,57		
Fusarium oxysporum	27		22,57	65,66	23,64
Fusarium solani	27		21,22,57		
Fusarium tabacinum				7	
Geotrichum candidum			21		
Gliocladium catenulatum			57		
Gliocladium roseum		41	21,57		64
Humicola fuscoatra			21		
Humicola grisea	85,86		22		
Isaria sp.				43	
Macrophomina phaseoli			21		
Margarinomyces heteromorpha	85,86				
Mariannaea elegans			21		
Melanospora zamiae			21		
Metarrhizium anisopliae				32,68	
Microsphaeropsis olivaceum			21		
Microdochium bolleyi		15,41			
Monoacrosporium bembicoides			21		
Mycocentrospora acerina			21		
Myrothecium verrucaria			21,57		
Nematophthora gynophila		36,37,84		37,84	
Neocosmospora vasinfecta			21,22,57		
Nigrospora sphaerica					64
Paecilomyces sp.		41			
Paecilomyces lilacinus		15	21		20,23,33,64
Paecilomyces nostocoides†					
Paecilomyces variotii			21		
Paraphoma radicina			21		
Penicillium aurantiogriseum			21		
Penicillium dangeardii			21		

TABLE 2. Continued.

	Reference				
	Globodera spp.	*H. avenae*	*H. glycines*	*H. schachtii*	*Meloidogyne* spp.
Penicillium decumbens			21		
Penicillium restrictum			21		
Penicillium simplicissimum			21		
Penicillium steckii			57		
Periconia macrospinosa			22		
Pestalotiopsis sp.			21		
Phialophora sp.		41			
Phialophora malorum				7,28,43	
Phoma spp.		41		7	
Phoma americana			21		
Phoma eupyrena			22		
Phoma exigua	85,86				
Phoma leveillei			22		
Phoma macrostoma			22,57		
Phoma medicaginis			22		
Phoma multirostrata			57		
Phoma pomorum			22		
Phoma terrestris			21		
Phytophthora cinnamomi			21		
Pseudopapulospora kendrickii					23
Pseudeurotium ovale	85,86		21		
Pythium sp.			21	43	
Sagenomella hanlinii			21		
Scolecobasidium terreum			21		
Scopulariopsis sp.	85,86				
Scytalidium fulvum			21		
Stagonospora heteroderae			21		
Tarichium auxiliare		37			
Thielavia basicola			57,59		
Thielavia terricola			21		
Trichoderma harzianum			21,57		
Trichosporium populneum				73	
Trichosporon beigelii			21		
Verticillium sp.		15			
Verticillium chlamydosporium		11,15,28,37 40,41,74	21	7,11,40,81,88	23,55
Verticillium lamellicola			57		24
Verticillium lecanii			21		
Verticillium leptobactrum			21		24
Verticillium psalliotae‡					
Xanthothecium peruvianum	85,86				
Xenokylindria obovata					54

† Isolated from *H. zeae* cysts (19).
‡ Isolated by authors from cysts of *H. mothi* (unpubl.)

sidered in toto, shows a wide capacity for adaptation to seasonal changes in temperature. Presumably, one can expect that some fungal species will be active on nematodes in southeastern soils at all times of the year. This is speculative, however, because no data exist concerning the effect of seasonal changes on the level of colonization of phytonematodes by fungi.

Fungi associated with heteroderid nematodes are also diverse in their nutritional requirements and ability to utilize substrates of different compositions (Table 6). The fungi varied in their growth on five different solid media, potato dextrose agar (PDA) and four other media containing Czapek's mineral salts (34) with a carbon source-chitin (0.2% [w/w]), cellulose

TABLE 3. Fungal colonization of cysts of *Heterodera glycines*, from the Cauca Valley, Colombia.

Location and species	Colonized cysts (N)	Frequency (%)
La Floresta and Villa Fatima, Palmira		
Aspergillus niger	4	0.67
Curvularia lunata	8	1.33
Fusarium equiseti	10	1.67
Fusarium lateritium	5	0.83
Fusarium moniliforme	11	1.83
Fusarium oxysporum	42	7.00
Fusarium solani	59	9.83
Geotrichum candidum	13	2.17
Gliocladium catenulatum	9	1.50
Gliocladium roseum	17	2.83
Memnoniella echinata	7	1.17
Paecilomyces lilacinus	13	2.17
Phoma medicaginis var. *pinodella*	4	0.67
Sagenomella levispora	6	1.00
Stagonospora heteroderae	9	1.50
Trichocladium asperum	4	0.67
ICA, Molina		
Aspergillus fumigatus	6	2.00
Fusarium oxysporum	46	15.33
Fusarium solani	27	9.00
Geotrichum candidum	11	3.67
Gliocladium roseum	14	4.67
Penicillium verrucosum	3	1.00
Scopulariopsis brevicaulis	5	1.67
Stagonospora heteroderae	7	2.33

TABLE 4. Fungal colonization of cysts of potato-cyst nematodes, Arequipa, Peru.

Species	Colonized cysts (N)	Frequency† (%)
Aspergillus sydowii	1	0.33
Cladosporium cladosporioides	8	2.6
Cylindrocarpon destructans	67	22.2
Cylindrocarpon didymum	3	0.99
Cylindrocarpon gracile	11	3.6
Drechslera australiensis	3	0.99
Exophiala pisciphila	7	2.2
Fusarium moniliforme	10	3.3
Fusarium oxysporum	27	8.9
Fusarium semitectum	46	15.2
Fusarium solani	32	10.6
Gliocladium catenulatum	4	1.3
Gliocladium roseum	16	5.3
Humicola grisea	5	1.7
Paecilomyces lilacinus	6	1.98
Paecilomyces variotii	2	0.7
Penicillium chrysogenum	2	0.7
Penicillium fellutatum	6	1.98
Penicillium restrictum	3	0.99
Penicillium rubrum	3	0.99
Phoma americana	4	1.3
Ramichloridium schulzeri	3	0.99
Scolecobasidium tschawytschae	4	1.3
Stachybotrys chartarum	2	0.7
Trichocladium asperum	8	2.6
Trichoderma harzianum	4	1.3
Trichoderma longibrachiatum	2	0.7
Ulocladium atrum	13	4.3

† Occurrence in terms of the total number (302) of cysts colonized.

(0.2%), tannic acid (0.05%), or glucose (2.0%). Some species (e.g., *F. solani, F. oxysporum*) developed equally well on all media, but others (*P. lilacinus, V. lecanii*) grew best only on some media. The data also indicate that these fungi can utilize substrates such as polysaccharides (chitin, cellulose) and phenols (tannic acid) that are of the type found in agricultural soils especially in the rhizosphere of plants (14,51). These results suggest that each fungus possesses some of the nutritional abilities necessary to survive saprophytically in soil in competition with other members of the soil microflora.

The ability of fungi to tolerate toxicants is another important aspect of their capacity to survive and compete in agricultural soils, but data on the effect of toxicants on fungi associated with heteroderid nematodes are very limited. We have studied the tolerance of a single isolate of *P. lilacinus* to phenols and food additives. The fungus was grown in flasks containing rice grains that had been submerged in boiling water or appropriate aqueous solution for 2 minutes and then drained and placed in the flasks. Twelve days after inoculation of the flasks with a suspension of *P. lilacinus* conidia, production in flasks with rice grains boiled in water only was identical to that in flasks that had rice grains boiled in aqueous solutions containing 1 mg/ml of the following chemicals: coumarin, catechol, t-cinnamic acid, gallic acid, p-hydroxybenzoic acid, phenyl benzoate, n-propyl gallate, sodium benzoate, sodium propionate, or sorbic acid. The study indicates that this fungus can tolerate phenols and food additives (Na propionate, Na ben-

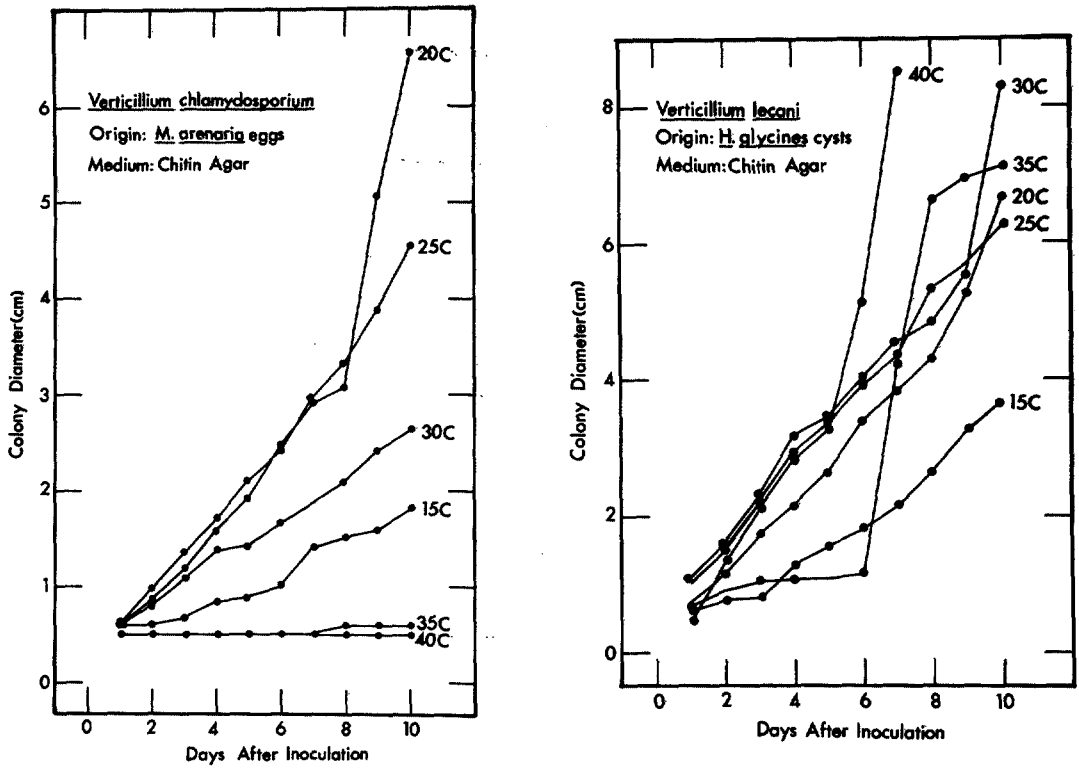

FIG. 1. Effect of temperature on mycelial growth of two species of *Verticillium* isolated from *H. glycines* eggs.

zoate, and sorbic acid). Several of these compounds (e.g., coumarin and t-cinnamic acid) or their analogs or metabolic precursors are common constituents of the soil organic matter (51) or rhizosphere chemicals (14).

PATHOGENICITY AND PARASITISM

The presence of a particular fungus within a cyst or female or among egg masses of a nematode does not necessarily mean that it is a parasite. As indicated previously, numerous fungi have been isolated from heteroderid nematodes but relatively few occur consistently in association with the nematodes. Several species of this latter group are capable of destroying nematodes. Species of *Cylindrocarpon*, notably *C. destructans*, penetrate and destroy eggs of *H. schachtii* (7,37,40). Not all isolates of the species, however, have this capacity. Several workers have reported that *C. destructans* is incapable of parasitizing eggs of species of *Globodera* or *Heterodera* (31,86). Variability in virulence of isolates of a single fungal species against nematodes appears to be the rule rather than the exception. In California, Nigh et al. found some strains of *F. oxysporum* and *Acremonium strictum* capable of destroying eggs of *H. schachtii*, whereas others were nonpathogenic (65,66). Results from in vitro tests at Auburn University indicated that some isolates of *F. oxysporum* and *F. solani* will not parasitize *H. glycines* or *M. arenaria* eggs (23). Differences in virulence of individual isolates of *P. lilacinus* to nematodes are well documented (24,63,72). Isolates of this species differ widely in their ability to become established in soil and in their pathogenicity to phytonematodes. The same is true for biotypes of *Gliocladium roseum* (72). Recent data obtained in our laboratory indicate a correlation between ability of *G. roseum* isolates to produce a diffusible yellow pigment in PDA cultures and their bio-

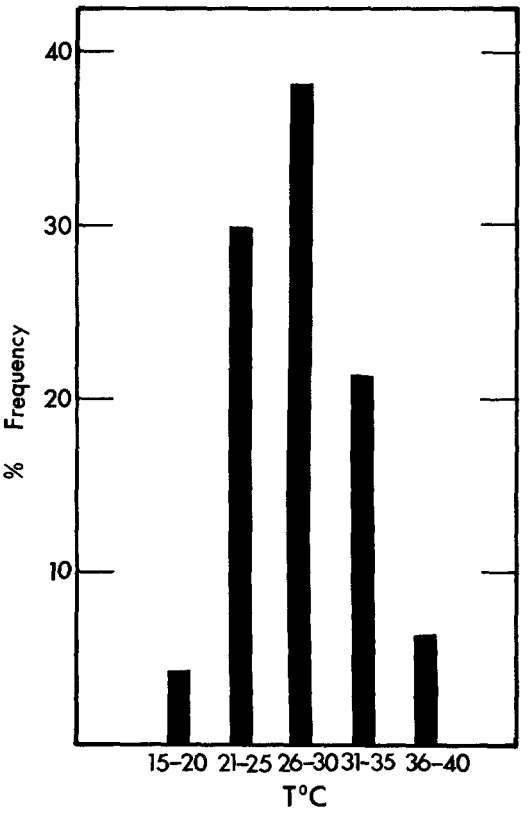

FIG. 2. Frequency distribution of fungal species isolated from eggs, females or cysts of heteroderid nematodes in relation to their optimal temperature for colony development.

control efficacy. Species of *Phoma* and allied genera, although unable to physically disrupt and destroy eggs of heteroderid nematodes, produce pigmented diffusible metabolites that penetrate egg shells. Affected eggs become pigmented and fail to hatch (unpubl.).

Verticillium species of the section Prostrata constitute another group of fungi that can parasitize and destroy nematode eggs and juveniles. *Verticillium chlamydosporium, V. lamellicola, V. lecanii, V. leptobactrum,* and *V. psalliotae* occur in cysts and (or) eggs of *Globodera, Heterodera,* and *Meloidogyne* (7,22,24,37,41,57,58,62,75,80), and *V. bulbilosum* is capable of penetrating egg shells of *Ascaris* (47–49).

Lack of pathogenicity to nematodes does not preclude a fungus from influencing nematode survival. Gintis et al. (22) showed that fungi occur singly in *H. glycines* females or cysts. Rarely were more than one species found together in these structures. Whereas the method of isolation and medium may affect what is isolated, their results suggest that in each colonized cyst or female there is usually a predominant fungus. By occupying the ecological niche afforded by the cyst or female, a nonpathogenic fungus may prevent invasion by other, possibly egg-destroying, species. In effect a nonpathogenic entity results in a form of protection for the eggs. Unfortunately, because information on egg–fungus interactions within cysts of heteroderid nematodes is not available, we can only speculate. The possibility that some fungi occupying cysts or females may produce compounds that stimulate egg hatch (9) has also been suggested (59).

BIOCONTROL POTENTIAL

Recognition of the existence of a mycoflora capable of destroying or deleteriously affecting phytonematode eggs stimulated enthusiasm for utilizing these fungi in the management of nematode populations. Studies on the feasibility of exploiting fungi as biocontrol agents have been focused on 1) direct delivery of the agents to the soil and 2) utilization of the fungi in the preparation of organic amendments to soil.

Ability to parasitize or deleteriously affect eggs in vitro does not mean that a given antagonist will do so in soil. Successful introduction of antagonistic micro-organisms into soil is dependent on several prerequisites: 1) there must not be any serious undesirable effects resulting from the introduction of the agent; 2) the organism(s) must be able to survive, reproduce, and compete successfully with the indigenous microflora; and 3) the agent(s) must have a stable and high level of virulence against the target nematode. Consideration of these minimal requirements eliminates many of the fungi isolated from nematodes. Some, such as *P. lilacinus,* have biotypes known to be pathogenic to man (1,50,78). *N. vasinfecta, F. solani, F. oxysporum,* and other *Fusarium* spp. are well-

TABLE 5. Optimal temperature range for colony development of selected nematode egg destroying fungi in dish cultures with potato dextrose agar (PDA) and chitin agar (CA).

Fungal species	Origin	Temperature range C	
		PDA	CA
Acremonium strictum I	*M. arenaria* eggs	20–30	25–30
Acremonium strictum II	*H. glycines* cysts	25–35	25–35
Cylindrocarpon tonkinense	*H. glycines* cysts	30–35	20–35
Exophiala pisciphila	*H. glycines* cysts	20–25	20–25
Fusarium oxysporum I	*M. arenaria* eggs	20–30	20–30
Fusarium oxysporum II	*M. arenaria* eggs	20–30	20–30
Fusarium solani	*H. glycines* cysts	25–30	25–40
Humicola fuscoatra	*H. glycines* cysts	30	30–35
Myrothecium verrucaria	*H. glycines* cysts	25–30	25–35
Paecilomyces lilacinus I	Insects	25–30	20–30
Paecilomyces lilacinus II	*M. arenaria* eggs	25–30	25–30
Paecilomyces lilacinus III	*H. glycines* cysts	25–30	25–30
Paecilomyces variotii	*H. glycines* cysts	25–35	20–35
Paraphoma radicina	*H. glycines* cysts	20–30	20–30
Penicillium decumbens	*H. glycines* cysts	25–30	20–35
Penicillium restrictum	*H. glycines* cysts	25–30	25–30
Phoma terrestris I	*H. glycines* cysts	15–25	20–25
Phoma terrestris II	*H. glycines* cysts	20–25	20–30
Pseudopapulospora kendrickii	*M. arenaria* eggs	15–35	15–35
Thielavia terrestris	*H. glycines* cysts	35–40	25–40
Verticillium chlamydosporium	*M. arenaria* eggs	20–25	20–25
Verticillium lecanii	*H. glycines* cysts	30	30–40

known pathogens of plants (18). Some biotypes of these fungi may be usable, but preliminary studies should be conducted to determine lack of pathogenicity to man, plants, or other valuable nontarget entities. The establishment of an organism in soil depends on its intrinsic competitive saprophytic ability (10), i.e., its capacity to overcome competition from an indigenous microflora. The organisms must displace other microbial forms from existing ecological niches in soil and predominate or adopt an unexploited niche. To effect either of these, the organism must be provided with suitable initial substrate, i.e., a food base (2,10), from which it can grow. Without such an energy base, the introduction of an organism in soil invariably fails. Addition of organisms to soil without a food base succeeds only in cases where the introduced organism is an obligate parasite that remains inactive, hence not subject to competition, until it comes in contact with a vulnerable host, e.g., *Pasteuria penetrans.*

In recent years many greenhouse and laboratory experiments have been conducted throughout the world with isolates of *P. lilacinus* (16,17,33). In these trials the fungus was typically added to soil using cereal grains (rice, oats, wheat) or other organic matter as the food base on which the fungus had been previously established. Typically the formulations were added to soils at rates exceeding several tons per hectare. Results on the relative efficacy of these preparations against root-knot and cyst nematodes (*Globodera, Heterodera*) varied from outright failure (17) to outstanding successes (16,33). Review of the literature available on successful experiments reveals that, for most of these trials, no proper substrate controls were included. Therefore the degree of nematode control achieved by fungal colonization cannot be separated from that brought about merely by the addition of organic matter to soil. When organic amendments are added to soil at rates comparable to those used in these *P. lilacinus* trials, a degree of nematode control is obtained (46,53,69,70). The organic matter used as a food base contains

TABLE 6. Relative radial growth (cm) of fungi isolated from *H. glycines* or *Meloidogyne* spp. on five different agar media measured 10 days after inoculation of culture dishes.

Species	Radial growth (cm)				
	PDA	Chitin 0.2%	Cellulose 0.2%	Tannic acid 0.05%	Glucose
Acremonium strictum	3.7	4.7	4.7	3.9	4.0
Cylindrocarpon tonkinense	6.7	8.5	8.5	8.5	8.5
Fusarium solani	8.5	8.5	8.5	8.5	8.5
Fusarium oxysporum	8.5	8.5	8.5	8.5	8.5
Phoma terrestris	6.8	7.4	7.9	7.0	8.0
Paecilomyces lilacinus	8.5	8.5	8.5	8.5	8.5
Paecilomyces lilacinus (*M. arenaria* eggs)	6.7	5.6	8.5	6.3	5.9
Paecilomyces nostocoides	5.9	6.8	4.0	4.6	6.7
Paraphoma radicina	2.8	4.6	5.9	4.5	6.2
Paecilomyces variotii	7.4	8.5	8.5	8.5	8.5
Verticillium chlamydosporium	3.6	4.2	4.5	3.8	5.2
Verticillium lecanii	5.4	5.4	8.0	7.0	6.4

Chitin, cellulose, glucose, and tannic acid media contained 2% agar and mineral salts (72) and initial pH of the media = 6.0.

nutrients that benefit crops planted in the treated soil. Therefore, yield responses obtained in the experiments cannot be attributed solely to nematode control. We have shown repeatedly (13,24,72) that a major portion of the growth response obtained with *P. lilacinus* and other egg-destroying species delivered to soil in boiled oat or rice grains can be obtained with the addition to the soil of the uninoculated oat or rice substrate.

The cause–effect relation between an organism added to soil in the form of a colonized substrate and the degree of nematode control obtained may be difficult to establish, but some of them do work. We have shown that organic matter can be modified so that when inoculated with appropriate antagonistic microbial species its nematode control can be enhanced (13).

Species of egg-destroying fungi are ubiquitous in soils. Soils can be amended with organic matter of composition so designed as to stimulate the activities of this mycoflora (70). The addition of chitin to soil results in increased parasitism of nematode eggs by fungi (13,25,52,71) and in increased numbers of fungal species associated with heteroderid nematodes, many of which are capable of destroying eggs. Chitin is a component of the egg shell of tylenchoid nematodes (6), and selection for chitinolytic organisms in soil may stimulate increased destruction of the eggs by members of this microflora (12).

Tropical areas of the world offer opportunities for development and use of nematicidal amendments. Large quantities of waste materials (e.g., sugarcane bagasse, oil cakes, and crustacean, fish, and other animal wastes) are available in the tropics and can be utilized either directly or with some modification for the purpose of controlling nematodes (69,70). The relatively low manpower costs in much of the tropics makes possible the incorporation into soil of the large amounts of material necessary for nematode control. Soil microbial activities in tropical soils are high most of the year; this, coupled with intensive production of crops, makes tropical areas most suitable for the use of amendments for phytonematode management.

RESEARCH NEEDS

In spite of recent advances in understanding the mode of action of nematicidal amendments and the characterization of the mycofloras associated with heteroderid nematodes, there is a lack of basic information on the interactions between phytonematodes and soil micro-organisms. The

effects of cropping sequences, cultural practices, and crop production systems on microfloras associated with nematodes must be defined. Bernard and Self (5) recently showed that cultural practices can affect the population dynamics of several egg-destroying fungi. Field studies by Culbreath et al. (12) showed that soil fertility can affect significantly the level of *M. arenaria* egg parasitism by fungi. These limited data suggest that soil mycofloras may be manipulated through cultural practices or with appropriate crop production systems, to stimulate development of species antagonistic to phytonematodes. The requirements for stimulation of antagonistic micro-organisms are needed in order to attain biological control of nematodes, not only in tropical areas but also in other agricultural regions of the world.

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