FUNGI ASSOCIATED WITH SEVERAL DEVELOPMENTAL STAGES OF *HETERODERA GLYCINES* FROM AN ALABAMA SOYBEAN FIELD SOIL

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ABSTRACT

Gintis, B. Ownley, G. Morgan-Jones, and R. Rodríguez-Kábana. 1983. Fungi associated with several developmental stages of *Heterodera glycines* from an Alabama soybean field soil. Nematropica 13:181-200.

An examination of young, swollen, cream-colored cysts of *Heterodera glycines* Ichinohe attached to root surfaces and of white, newly exposed females partly immersed within roots of Alabama-grown soybeans [*Glycine max* (L.) Merr.] revealed the presence of a number of fungal pathogens. Fungi most frequently isolated from these stages of development were: *Chaetomium cochliodes* Palliser, *Exophiala pisciphila* McGinnis & Ajello, *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Phytophthora cinnamoni* Rands, *Pythium* sp., a sterile mycelium, and *Trichosporon beigelii* (Küchenm. & Rabenh.) Vuill. Fifty percent of the cream-colored cysts were infected as compared to 20 percent of the younger white, lens-shaped females. An examination of sausage-shaped females within roots and brown cysts from soil indicated that two and 70 percent respectively were invaded by fungi. Results indicated that a progressive increase in fungal colonization occurred with nematode development. Some overlapping of the mycoflora from one stage to another was evident. The frequency of occurrence of individual fungi as well as the number of fungal species involved was dependent upon the stage of nematode development.

Several species prevalent in young cysts were reduced in numbers in the older stages. Fungi occurring in significant numbers in the older cysts only included *Cylindrocarpon tonkinense* Bugn., *Neocosmospora vasinfecta* Smith, *Paecilomyces lilacinus* (Thom) Samson, *P. variotii* Bain., *Phoma terrestris* Mont., *Scytalidium fulvum* Morgan-Jones & Gintis, and *Verticillium chlamydosporium* Goddard.

A distinct but restricted mycoflora is associated with *H. glycines* pathology. Modes of action of individual fungi are thought to include enzymatic degradation and/or biosynthesis of toxic metabolites. Some fungi regularly encountered in this study, namely *C. cochliodes, Paecilomyces* spp., *P. terrestris, P. cinnamoni, Pythium* sp., *V. chlamydosporium,* and *Verticillium lecanii* (Zimm.) Viégas, demonstrated chitinase activity when grown on chitin agar plates. Microscopic examination of cysts occupied by these fungi has shown their ability to invade eggs and destroy the larvae within. Endogenous fungal hyphae were also discerned within eggs from cysts invaded by *C. tonkinense, E. pisciphila, Fusarium* spp., and *S. fulvum.* Evidence is accumulating that a measure of biological control of *H. glycines,* exerted by fungi, is in place in soybean field soils.

Additional key words: fungal physiology, nematode control, nematode ecology, pest management, population dynamics, soil enzymes, mucopolysaccharides.

RESUMEN

Gintis, B. Ownley, G. Morgan-Jones, y R. Rodríguez-Kábana. 1983. Hongos asociados con varias etapas del desarrollo de *Heterodera glycines* proveniente de un campo de soya en Alabama. Nematrópica 13:181-200.

Un exámen de quistes de color crema dilatados, de *Heterodera glycines* Ichinohe, adheridos a la superficie de raíces de soya [*Glycine max* (L.) Merr.] y de hembras parcialmente emergidas de las raíces, reveló la presencia de varias especies de hongos patógenos del nematodo. Los hongos aislados con más frecuencia de estos dos estadios del desarrollo de *H. glycines* fueron: *Chaetomium cochliodes* Palliser, *Exophiala pisciphila* McGinnis & Ajello, *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Phytophthora cinnamoni* Rands, *Pythium* sp., dos micelios estériles, y *Trichosporon beigelii* (Küchenm. & Rabenh.) Vuill. Aunque el 50% de los quistes de color crema estuvieron infectados por los hongos sólo lo estuvieron el 20% de las hembras blancas lenticulares examinadas. Otras observaciones con hembras salsiciformes dentro de las raíces y de quistes pardo-oscuros obtenidos directamente del suelo, indicaron que el dos y 70% respectivamente de estas dos etapas del desarrollo del nematodo, estaban invadidas por hongos. Los resultados señalan que existe un aumento progresivo en la colonización por hongos del nematodo a medida que éste se desarrolla. También, que aunque hay una cierta coincidencia con algunas de las especies fungosas que se encuentran en todas las etapas del desarrollo de *H. glycines* estudiadas, la frecuencia con la que se observaron especies individuales de hongos así como la enumeración respectiva, dependieron mucho de la etapa de desarrollo del nematodo. Muchas de las especies prevalentes en quistes nuevos fueron observadas con menor frecuencia en otros más viejos. Las especies fungosas que se encontraron en números significativos en quistes viejos fueron: *Cylindrocarpon tonkinense* Bugn., *Neocosmospora vasinfecta* Smith, *Paecilomyces lilacinus* (Thom) Samson, *P. variotii* Bain., *Phoma terrestris* Mont., *Scytalidium fulvum* Morgan-Jones & Gintis y *Verticillium chlamydosporium* Goddard.

Los resultados de este estudio señalan que existe una micoflora peculiar asociada con la patología de *H. glycines*. Se cree que la manera de actuar de estos hongos contra el nematodo esta basada en la descomposición enzimática y/o la biosíntesis de toxinas que afectan al nematodo. Algunas de las especies fungosas observadas en este estudio, a saber, *C. cochliodes*, *Paecilomyces* spp., *P. terrestris*, *P. cinnamoni*, *Pythium* sp., *V. chlamydosporium* y *Verticillium lecanii* (Zimm.) Viégas, demostraron ser quitinolíticas al desarrollarse en agar con quintina. El exámen microscópico de quistes invadidos por estos hongos demostró sus capacidades para invadir huevos y destruir larvas dentro de los mismos. También se observaron hifas fungosas endógenas dentro de huevos en quistes invadidos. Los resultados de este estudio y otros anteriores apoyan la tesis que existe en los campos de soya ya activo un cierto nivel de combate biológico basado fundamentalmene en el efecto de los hongos sobre *H. glycines*.

Palabras claves adicionales: combate de nematodos, ecología de nematodos, manejo de plagas, dinámica poblacional, fisiologia de hongos, quitinólisis, mucopolisacáridos, enzimas del suelo.

INTRODUCTION

A restricted mycoflora of opportunistic soil fungi associated with cyst nematodes and capable of various degrees of egg pathogenicity has been determined by surveys initially performed only on the hardened, brown cyst stage collected from soil (2,10,11,12,24,26,28,30,32,40). Research conducted on European populations of *Heterodera avenae* Wollenweber and *H. schachtii* Schmidt demonstrated that there were fungi capable of invading younger stages of *Heterodera* spp. associated with host root tissue (17,18,20,37). Three obligate parasites namely *Catenaria auxiliaris* (Kühn) Tribe, *Nematophthora gynophila* Kerry & Crump and an unnamed lagenidiacious fungus were described which are highly destructive of *H. avenae* and *H. schachtii* females (21,36). Less specialized soil fungi including *Cylindrocarpon destructans* (Zinss.) Scholten, *Verticillium chlamydosporium* Goddard, and an unnamed filamentous fungus termed 'contortion fungus' have been isolated from eggs derived from females and young cysts of these two nematode species (3,20,22,37). *Verticillium chlamydosporium* was considered a principal egg pathogen while *C. destructans* was found to destroy only a small proportion of the females and young cysts.

In addition to some of the fungi previously mentioned, more recent studies on *H. avenae* have recorded a number of saprophytic soil myco-organisms. These were *Chrysosporium* sp., *Cladosporium* sp., *Fusarium* sp., *Gliocladium roseum* Bain., *Hormodendron* sp., *Microdochium bolleyi* (Spraque) de Hoog & Hermanides-Nijhof, *Mortierella* sp., *Paecilomyces* sp., *Phialophora* sp., *Penicillium* sp., and a species of *Phoma* (22). With the exception of *M. bolleyi* these fungi were each present in less than 10% of the eggs examined and all were considered weakly parasitic.

In the United States, *Fusarium oxysporum* Schlect and *Acremonium strictum* Gams were found to occur in eggs from females and young cysts of *H. schachtii* from California (29). These fungi are thought to be responsible for control of this nematode under certain field conditions. Species of *Alternaria, Cephalosporium, Chaetomium, Cylindrocarpon, Fusarium, Penicillium* and *Phoma* were recorded with low frequency in the same study. A survey of young cysts of *Heterodera glycines* Ichinohoe from North Carolina soils revealed several opportunistic soil fungi including *Exophiala pisciphila* McGinnis & Ajello, *Fusarium* spp. and species of *Phoma* (7). Endogenous fungal hyphae within eggs from cysts containing these fungi were reported. Despite knowledge acquired from previous studies there is a lack of information on the relation between fungal parasitism and the developmental stages of the nematode. The present paper will present a study on the frequency of occurrence of pathogens in four developmental stages of *H. glycines*.

MATERIALS AND METHODS

A soybean field in Roba, Macon County, Alabama was selected as a cyst-nematode source to conduct a mycoflora survey of four stages in the development of *Heterodera glycines* cysts. The stages examined were mature cysts from soil, young cream-colored cysts, white lens-shaped females attached to root tissue, and sausage-shaped females within the soybean root. The soil was a silt loam with less than 1% organic matter and pH = 6.0.

Soil from the Roba field was apportioned into 1-L capacity 10-cm-diam, cylindrical plastic pots which were planted with 'Ransom' soybeans [*Glycines max* (L.) Merr.] and maintained in the greenhouse in good growing condition. After five weeks the plants were carefully separated from the soil and intact roots were washed with running tap water for 48 h to remove most surface contaminants. The clean roots were incubated at 30°C for 24 h in a solution containing 1% each of cellulase and pectinase (U.S. Biochem. Corp., Cleveland, Ohio) to facilitate removal of young females from within the roots (8). The macerated root tissue was again washed with running tap water and transferred to Petri dishes with sterile demineralized water containing streptomycin sulfate (150 μg/ml). The tissue was dissected with root canal file needles under a 15X stereoscan microscope to release females. Three hundred seventy-four sausage-shaped females, 1,000 white lens-shaped females and 1,000 cream-colored cysts were randomly collected with fine tissue forceps and placed in Petri dishes containing sterile demineralized water. The females and cysts were placed on a Gelman #1 (pore size 5 μm) membrane filter in a filter flask apparatus and washed repeatedly with sterile demineralized water followed by sterile demineralized water containing streptomycin sulfate (150 μg/ml). The aqueous solutions were removed by vacuum aspiration. After washing, females and young cysts were aseptically transferred from the filter onto potato dextrose agar (PDA) supplemented with 0.05% (w/w) streptomycin sulfate.

Mature brown cysts were recovered from moist soil employing a flotation technique. The soil was suspended in tap water, particulate matter was allowed to settle and the aqueous portion poured through a stainless steel sieve (mesh = 150 μm). The older cysts were washed for 48 h with running tap water then placed in Petri dishes containing sterile demineralized water with the antibiotic. One thousand mature cysts were randomly selected, washed in the filter apparatus as described and plated onto PDA in the same manner as for the younger cysts and females. Following incubation for five days at 25°C, the fungal colonies resulting from all cysts were identified. Sporulation was induced in many isolates by transferring to chitin agar plates (9).

Cysts and females with the most frequently occurring fungal species were removed from the agar and enclosed eggs were microscopically examined for the presence of endogenous fungal hyphae as described by Gintis, Morgan-Jones, and Rodríguez-Kábana (7).

RESULTS

Fifty-five fungal species (including nine sterile somatomorphs) were found to be associated with the 3,374 specimens of cysts and females examined; only 19 of these were found to occur more than five times.

Seventy percent of the 1,000 specimens of mature cysts produced fungi (Fig. 1A). The most commonly isolated mycoorganisms were *Fusarium oxysporum, F. solani* (Mart.) Sacc., *Neocosmospora vasinfecta* Smith, *Phoma terrestris* Mont., and *Scytalidium fulvum* Morgan-Jones & Gintis. Other fungi found with frequency in mature cysts included two species of *Paecilomyces, P. lilacinus* (Thom) Samson and *P. variotii* Bain., as well as *Trichosporon beigelii* (Küchenm. & Rabenh.) Vuill. With relatively low frequency of occurrence there were *Cylindrocarpon tonkinense* Bugn., *Exophiala pisciphila,* and two species of *Verticillium: V. chlamydosporium* and *V. lecanii* (Zimm.) Viegas. Other fungi occurring with low frequency were *Acremonium* sp. 2, *Aspergillus flavus* Link, *Chaetomium cochliodes* Palliser, *Humicola fuscoatra* Traaen, *Penicillium decumbens* Thom, *Phytophthora cinnamomi* Rands, and a species of *Pythium. Monacrosporium bembicoides* (Dreschler) Subramanian, a predacious nematode trapping species, was recorded only once as were a number of miscellaneous fungi, including *Acremonium strictum, Chalara hyalina* Morgan-Jones & Gintis, *Cladosporium sphaerospermum* Penz., *Codinaea heteroderae* Morgan-Jones, *Macrophomina phaseoli* (Maubl.) Ashby, *Mariannaea elegans* var. *punicea* (Corda) Samson, *Penicillium restrictum* Gilman & Abbott, *P. simplicissimum* (Oudem.) Thom, *Phoma americana* Morgan-Jones & White, *Sagenomella hanlinii* Morgan-Jones & Gintis in ed., *Trichoderma harzianum* Rifai, and seven sterile somatomorphs.

Many fungal species detected in mature, brown cysts were also associated with younger stages but the frequency of occurrence was lower. Roughly half of the 1,000 cream-colored cysts attached to soybean roots contained fungal pathogens (Fig. 1B). The predominant fungi were *F. oxysporum, F. solani,* and *T. beigelii.* Five fungi present in significant but smaller numbers were *C. cochliodes, E. pisciphila, P. cinnamomi,* a species of *Pythium,* and an unidentified fungus designated Sterile Mycelium 1. Fungi found with lower frequency were *Myrothecium verrucaria* (Alb. & Schw.) Ditm., *N. vasinfecta, Paraphoma radicina* (McAlp.) Morgan-Jones & White, *P. terrestris, S. fulvum,* Sterile Mycelium 3, and *V. lecanii.* Incidental fungi recorded only once or twice included *Acre-*

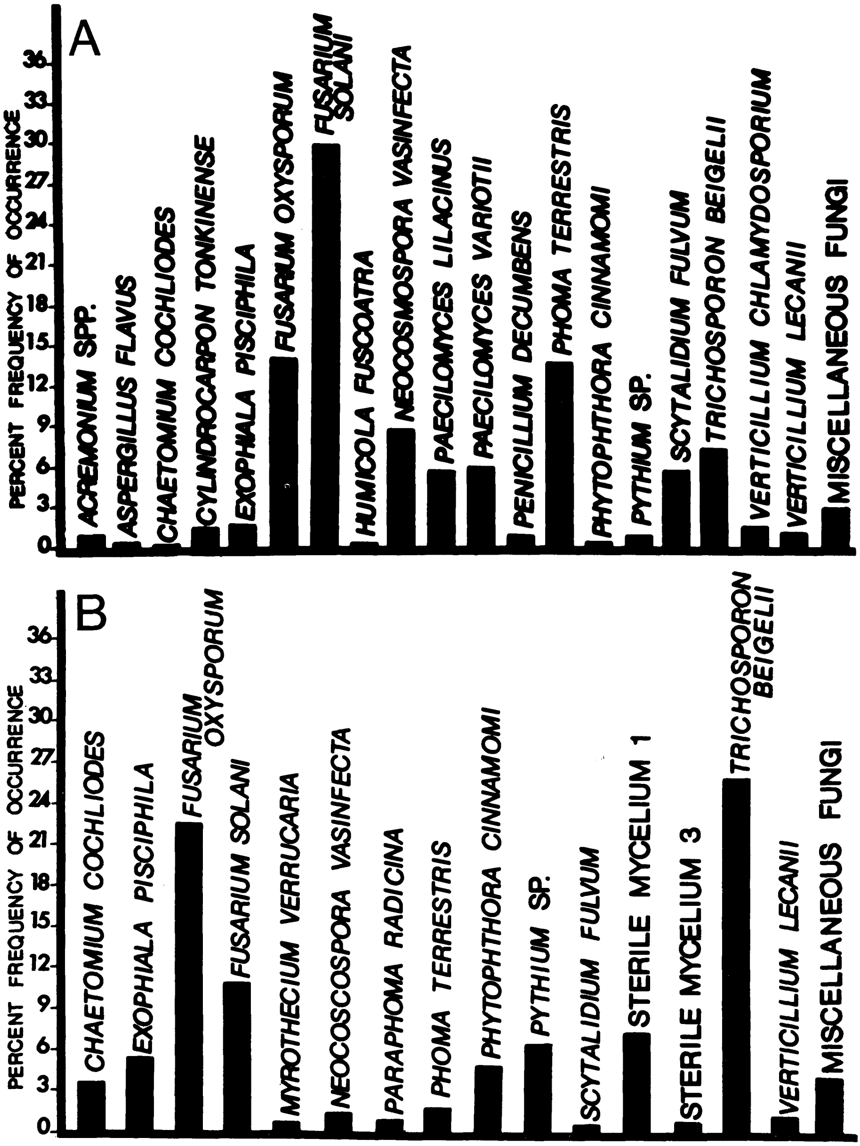
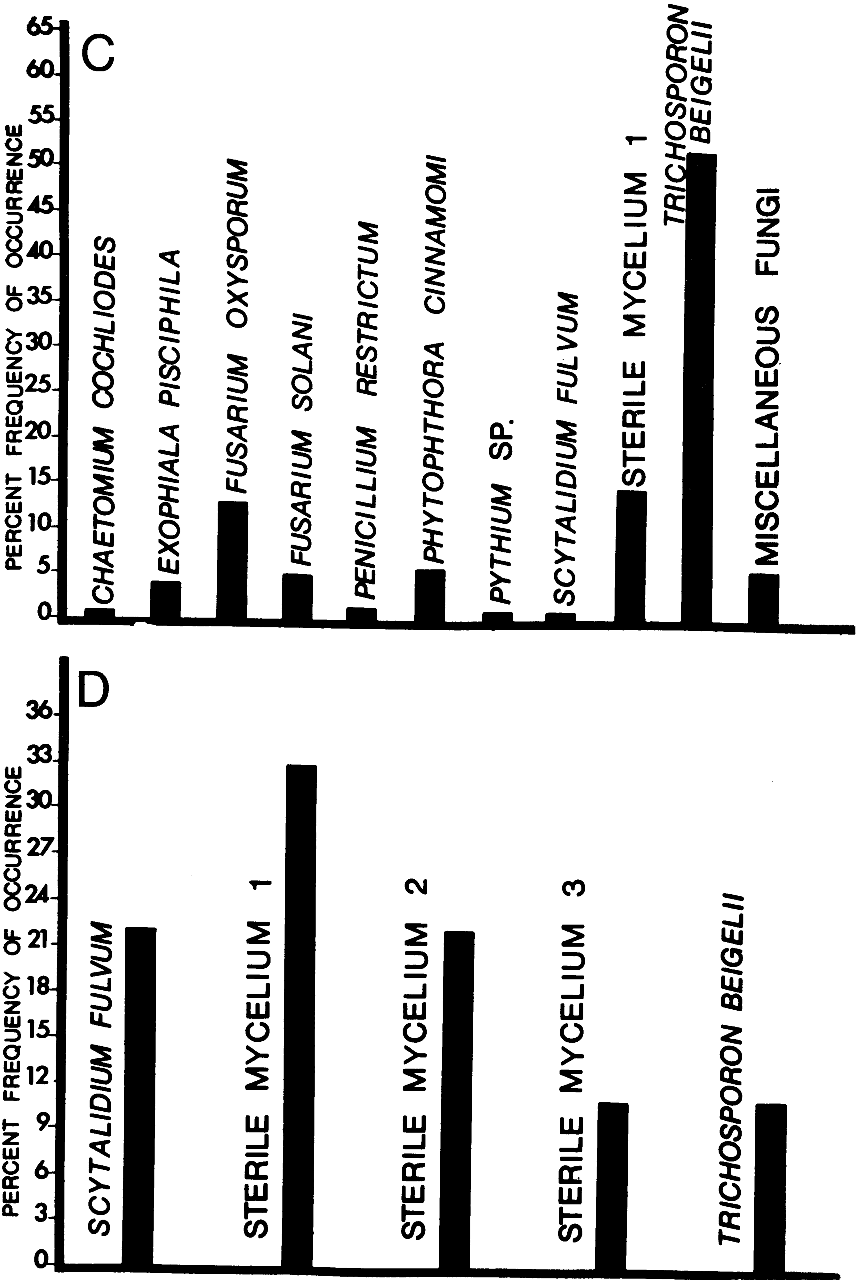


Fig. 1. Percent frequency of occurrence of fungal species isolated from cysts and females of *Heterodera glycines*. Fungi from: A. Old cysts from soil. B. Cream-colored cysts on root surface. C. White lens-shaped females on root surface. D. Sausage-shaped females from inside roots.


C
PERCENT FREQUENCY OF OCCURRENCE
0
5
10
15
20
25
30
35
40
45
50
55
60
65
CHAETOMIUM COCHLIODES
EXOPHIALA PISCIPHILA
FUSARIUM OXYSPORUM
FUSARIUM SOLANI
PENICILLIUM RESTRICTUM
PHYTOPHTHORA CINNAMOMI
PYTHIUM SP.
SCYTALIDIUM FULVUM
STERILE MYCELIUM 1
TRICHOSPORON BEIGELII
MISCELLANEOUS FUNGI
D
PERCENT FREQUENCY OF OCCURRENCE
0
3
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SCYTALIDIUM FULVUM
STERILE MYCELIUM 1
STERILE MYCELIUM 2
STERILE MYCELIUM 3
TRICHOSPORON BEIGELII

monium sp.1, *Alternaria alternata* (Fr.) Keissler, *Geotrichum candidum* Link, *Gliocladium roseum* Bain., *H. fuscoatra, Melanospora zamiae* Corda, *Microsphaeropsis olivaceum* (Bonorden) Hohnel, *Mucor* sp., *Mycocentrospora acerina* (Hartig) Deighton, *P. lilacinus, P. decumbens, P. restrictum, P. americana, Pseudeurotium ovale* Stolk, *Thielavia terricola* (Gilman & Abbott) Emmons, and two sterile somatomorphs.

Fungi were recovered from 20% percent of the 1,000 white lens-shaped females newly exposed on the root surface. *Fusarium oxysporum* and Sterile Mycelium 1 were again regularly encountered in significant numbers (Fig. 1C). *T. beigelii* occurred in substantial numbers accounting for 50% of the fungal species isolated from females. *Exophiala pisciphila, F. solani,* and *P. cinnamomi* were recorded less often. Additional fungi present in low numbers were *C. cochliodes, P. restrictum, Pythium* sp., and *S. fulvum*. Miscellaneous fungi isolated only once were *A. alternata, Cladosporium cladosporiodes* (Fres.) de Vries, *G. candidum, M. verrucaria, N. vasinfecta, P. variotii, P. radicina, Penicillium aurantiogriseum* Dierckx, *Pestulotiopsis* sp., and one sterile somatomorph.

Very few fungi, in both species and number, were recovered from sausage-shaped females lying within the root tissue (Fig. 1D). Of 374 specimens examined, only nine contained fungi. The five fungi isolated were *S. fulvum,* three sterile somatomorphs, and *T. beigelii.*

The increase in the incidence of fungal colonization with nematode development is illustrated in Fig. 2. In addition, some fungal species were encountered more frequently in immature cysts than in brown cysts. (Fig. 3).

Prior to plating on culture media, the outward appearance of the fungus-invaded females and young cysts was indistinguishable from non-infected specimens (Fig. 4A,B) except in some instances involving darkly pigmented fungi. Generally the first sign of colonization was an outward protrusion of fungal hyphae through natural openings (Fig. 4D).

Cysts invaded by a number of these fungi revealed eggs containing endogenous fungal hyphae. Eggs infected with *E. pisciphila* were filled with ellipsoid to globose, pale brown hyphal elements (Fig. 4C). Cysts from which *S. fulvum* was recovered contained the torulose, heavily pigmented hyphae and chlamydospore-like arthroconida characteristic of this fungus. The tough hardened cuticle of cysts occupied by this organism was often badly damaged or completely deteriorated after a few days incubation on agar plates (Fig. 4E). Infected eggs were discolored, inflated, and disordered, with granular contents (Fig. 4F). *Phoma terrestris* was isolated from cysts which showed dark patches and were densely filled with mycelium. Eggs invaded by this fungus contained rows of pig-

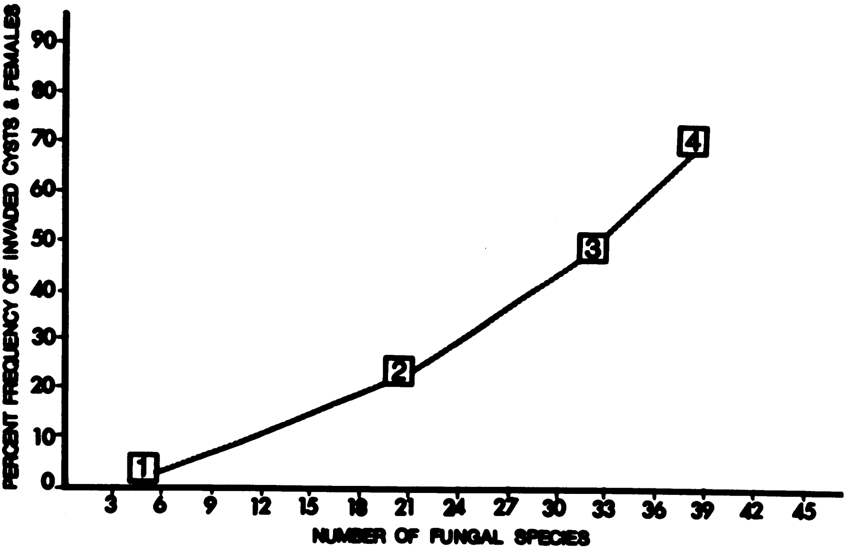


Fig. 2. Relation between developmental stages of *Heterodera glycines*, frequency of invasion of cysts and females, and the number of fungal species isolated. [1] = sausage-shaped females. [2] = white lens-shaped females. [3] = cream-colored cysts. [4] = old cysts from soil.

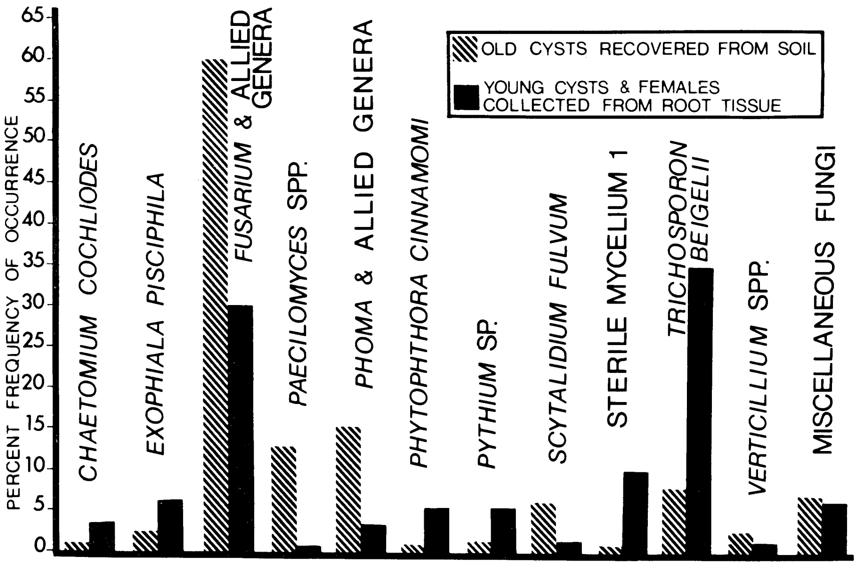


Fig. 3. Comparison of percent frequency of occurrence of fungal species in old cysts from soil and in younger stages attached to or within root tissue.

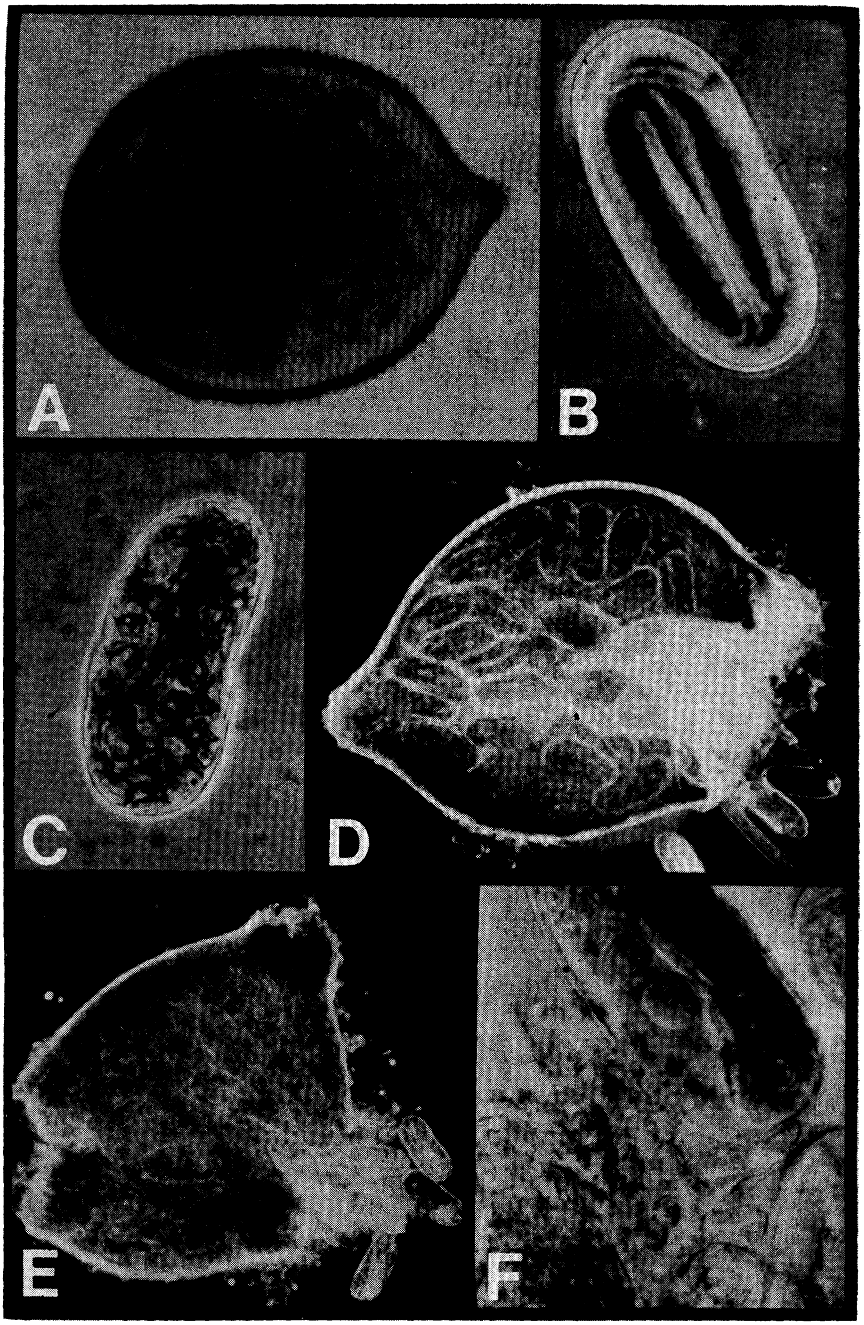
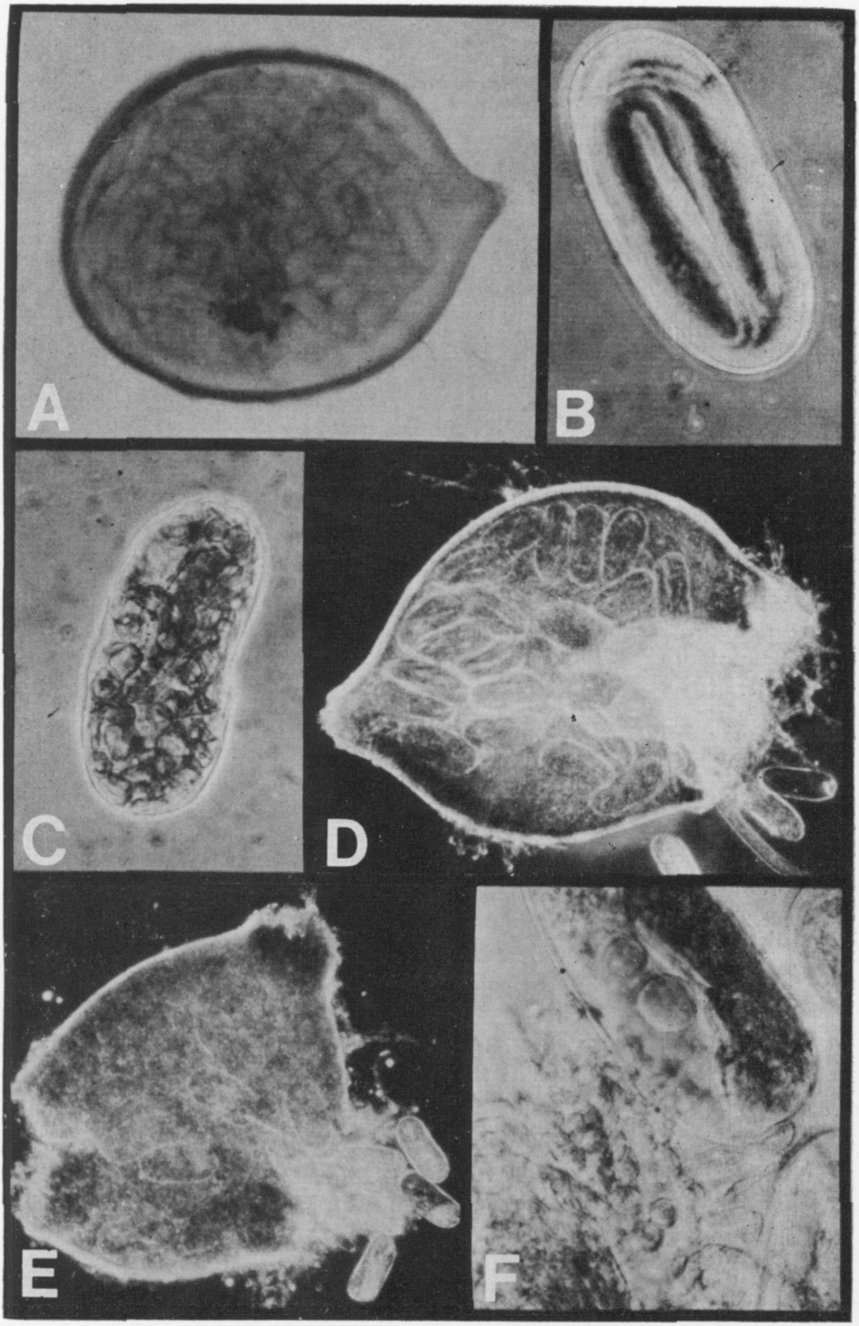


Fig. 4. Parasitism of cysts and eggs of *Heterodera glycines* by fungi. A. Healthy cyst. B. Healthy egg. C. Egg invaded by *Exophiala pisciphila*. D. Cyst parasitized by *Paraphoma radicina*. E. Cyst containing *Scytalidium fulvum*. F. Eggs invaded by *S. fulvum*.



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mented hyphal elements (Fig. 5A). The wide hyphal filaments of *P. cinnamomi* were also quite visible within parasitized eggs (Fig. 5B).

Dissected cysts from which *F. oxysporum* and *F. solani* were isolated usually contained a profusion of fungal hyphae and hyaline hyphal strands were evident within eggs (Fig. 5C,D). Remnants of larval cuticle were apparent in some of these invaded eggs suggesting that normal larval morphogenesis preceded fungal penetration. No obvious egg destruction was observed for *N. vasinfecta* but the fungus frequently formed ascocarp fruiting structures on the outer wall of mature brown cysts. Microscopic examination indicated that *C. tonkinense* was capable of some degree of egg parasitism.

Four additional fungi: *P. lilacinus, P. variotii, V. chlamydosporium,* and *V. lecanii,* demonstrated capacity to adversely affect cyst-nematode eggs. Eggs colonized by *P. variotii* were often swollen and discolored, some appeared lysed, and others contained large lipid globules (Fig. 5F). The contents of eggs with *P. lilacinus* (Fig. 5E) and *Verticillium* spp. were often completely consumed and replaced by hyphal filaments.

DISCUSSION

The mycoflora associated with Alabama cysts was relatively consistent with that of previous surveys in the southeastern U.S. (7,26,28). This study also revealed an increase in colonization with age of *H. glycines* both in percentage of cysts invaded and in diversity of fungal species. Although some fungi occurred in more than one stage of the developing nematode, some change in the composition of the mycoflora was discernible from one developmental stage of the nematode to another. Factors which might influence fungal associations with specific stages of the nematode cyst may include natural habitat of the fungus (particularly proximity of the organism to plant roots) and fungal antagonisms.

The very low incidence of colonization of the earliest stage of *H. glycines* surveyed, sausage-shaped females within root tissue, indicates that the root provides a degree of protection. Studies on the female parasites of *H. avenae* have shown that juveniles within roots are not parasitized unless the nematode is developing semiendoparasitically (19,20). The occurrence of fungal pathogens at the sausage-shaped stage suggests that these fungi are in close association with the soybean root; fungi may have entered through undetectable breaks in the root tissue such as those left by migrating larvae as they penetrate the root cortex.

Chaetomium cochliodes, E. pisciphila, P. cinnamomi, Pythium sp., Sterile Mycelium 1, and *T. beigelii* were recovered primarily from newly emerged females and young cysts attached to root tissue. The susceptibility of these stages to *Pythium* sp. and *P. cinnamomi* was probably a result of

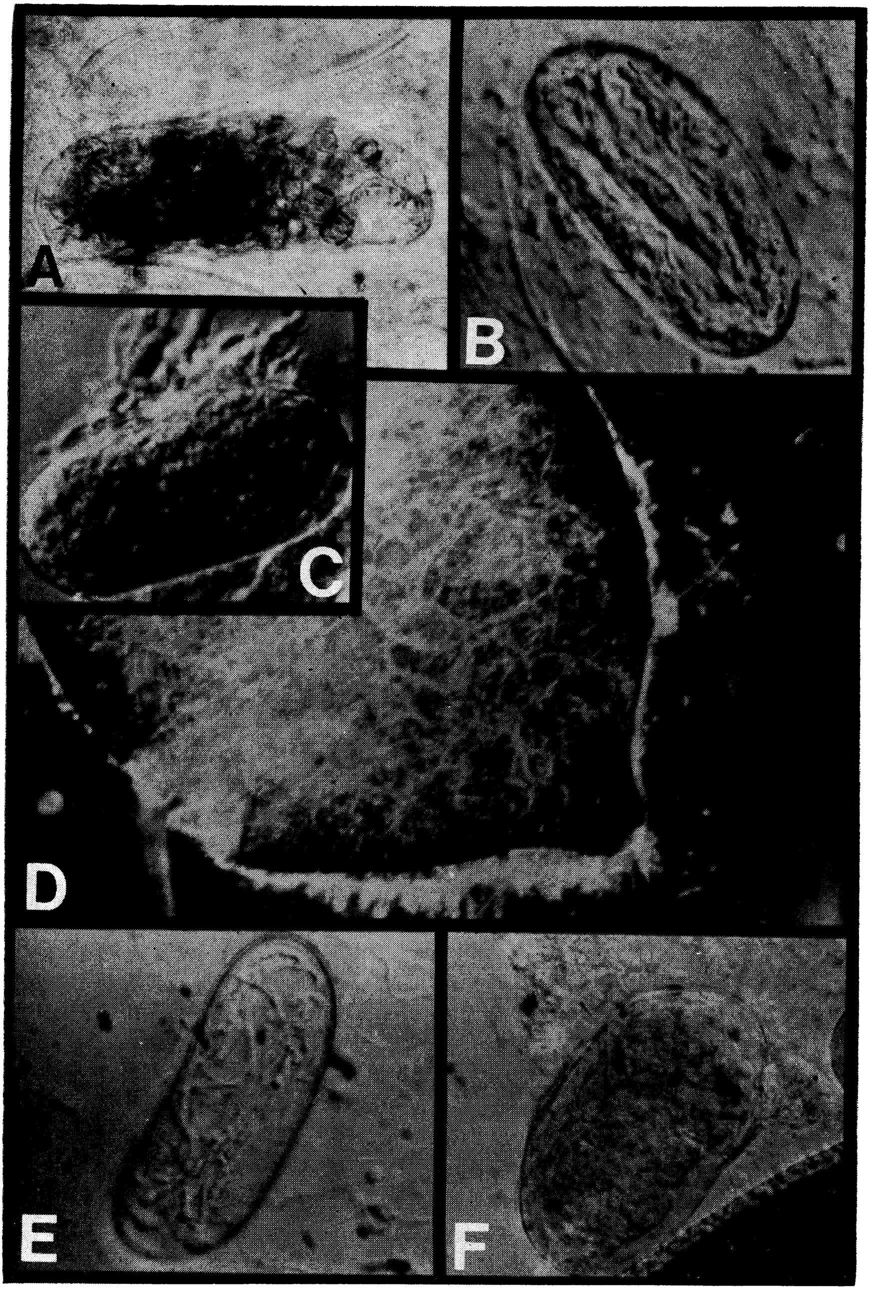

Fig. 5. Parasitism of cysts and eggs of *Heterodera glycines* by fungi. A. Egg containing *Phoma terrestris*. B. Egg parasitized by *Phytophthora cinnamomi*. C. Egg invaded by *Fusarium solani*. D. Cyst containing *Fusarium oxysporum*. E. Egg invaded by *Paecilomyces lilacinus*. F. Egg parasitized by *Paecilomyces variotii*.

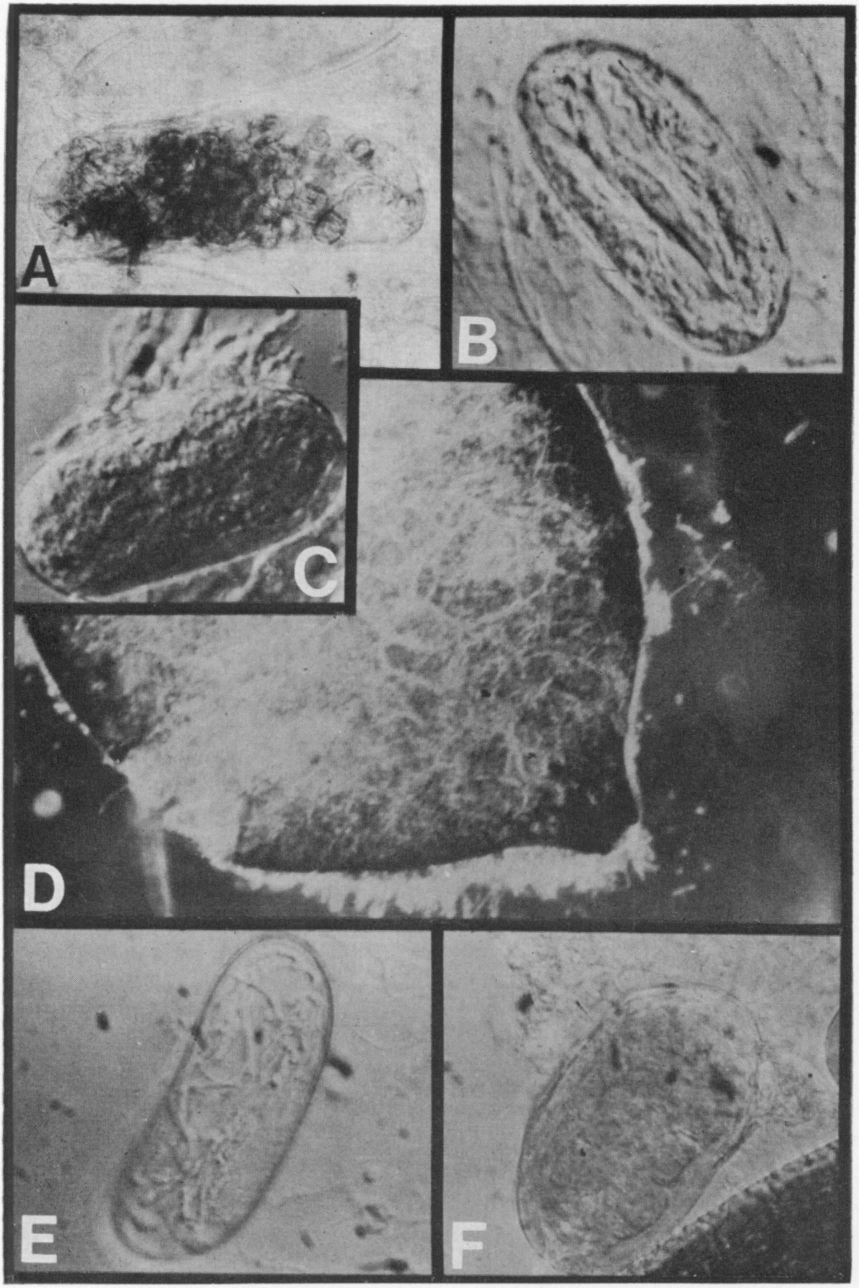

Fig. 5. Parasitism of cysts and eggs of *Heterodera glycines* by fungi. A. Egg containing *Phoma terrestris*. B. Egg parasitized by *Phytophthora cinnamomi*. C. Egg invaded by *Fusarium solani*. D. Cyst containing *Fusarium oxysporum*. E. Egg invaded by *Paecilomyces lilacinus*. F. Egg parasitized by *Paecilomyces variotii*.

the intimate association of these chitinolytic, plant pathogenic fungi with root tissue. The rare occurrence of these organisms in older cysts from soil is indicative of their pronounced sensitivity to antagonisms by other soil microorganisms (4). This soil fungistatic effect may be somewhat reduced in the area of the root where plant exudates are present. In addition these zoosporic fungi do not develop well in the relatively drier conditions found in soil away from the root. Rhizosphere moisture is more favorable for production of zoospores. More study is needed on the role of *T. beigelii* and Sterile Mycelium 1. The repeated isolation of the latter fungus predominantly from younger stages suggests an association with the plant. *Trichosporon beigelii* is a common saprophyte of decaying plant material found in fresh and saltwater as well as soil environments (23). This organism is also well-known as the causal agent of white piedra hair infections (6) and skin infections (25). Although more serious diseases are rare, *T. beigelii* has been implicated in pulmonary and systemic infections in man and dog (31) as well as in bovine mastitis (16). This suggests involvement on substrates high in proteinaceous keratin-like compounds and possibly mucopolysaccharides, i.e., the type of substances that constitute the nematode egg and body. The relative infrequency of *C. cochliodes* and *E. pisciphila* may reflect lower amounts of inoculum and/or a reduced competitive ability. *Exophiala pisciphila* in particular grows slowly in culture.

Our results indicate that following exposure to the soil, developing cysts are increasingly vulnerable to fungi. *Fusarium* spp. and allied genera (*Neocosmospora* and *Cylindrocarpon*), species of *Paecilomyces, Phoma,* and *Verticillium,* as well as *Scytalidium fulvum,* were recovered more often from older cysts in soil. The mature cysts, released into soil from dead roots, would be found in an area rich in decaying organic matter favoring the establishment of saprophytic soil fungi. The highest percent frequency of occurrence (60%) in this stage was recorded for *Fusarium* spp. and allied genera (Fig. 3). These fungi were also frequent (30%) in the younger stages. *Fusarium* spp. have been reported from cyst-nematodes in Europe (2,11,22), Alabama (28), North Carolina (7) and other southeastern states (26), as well as California (29), while *N. vasinfecta* has been found in *H. glycines* cysts from the southeastern U.S. (7, 26,28). *Neocosmospora vasinfecta* is a known pathogen of soybeans (38) in tropical and subtropical regions and in Alabama (13). *Fusarium oxysporum* is characteristically found in warm, humid climates (34) and is the predominant fungal species associated with soybean monoculture (39). Although less frequent in occurrence *F. solani* has also been associated with continuous soybean cropping (39). *Fusarium* spp. are soil fungi with cosmopolitan distribution. They are active in plant residue de-composition and many species possess both saprophytic and plant pathogenic strains. This is also true for *Phoma* species while the species of *Paecilomyces* and *Verticillium* isolated in this study are not known as plant pathogens (4).

Phoma terrestris appears to be quite widespread in the southeastern U.S. as a cyst pathogen ([as *P. macrostoma* Mont.] 7,26). Boerema (personal communication) indicated that this fungus is commonly found in the U.S. but rarely in Europe, suggesting that it is an organism of North American origin. Although this is the first recorded account of *C. tonkinense* from *H. glycines,* isolates of this genus have been reported from *Heterodera* cysts, eggs, and females (2,3,10,18,22,29).

The present study reports for the first time the occurrence of *Verticillium chlamydosporium* in cyst nematodes from the U.S. and is the first record of its association with *H. glycines* anywhere. There are several reports of this fungus from *H. avenae* (12,20,22,23) and from *H. schachtii* (2,3,37,40) as well as from *Meloidogyne arenaria* (27). Given the known association of *V. chlamydosporium* with cyst nematode pathology, it was somewhat puzzling that the organism was isolated but rarely in this survey. Microscopic examination of cysts revealed that the fungus was present more often than recovered as colonies on agar plates. The distinctive dictyochlamydospores of the fungus which had apparently failed to germinate were observed in some cysts from which the organism had not been isolated. Although this information was not quantified, it strengthens the evidence that *V. chlamydosporium* may play an important role in the cyst and egg disease of soybean cyst nematodes.

Verticillium lecanii has not been previously reported from cyst nematodes but it is well known as an entomopathogen, primarily of scale insects and aphids. Recently Hall & Burges (14) reported effective greenhouse control of aphids using aqueous sprays of conidia. *Paecilomyces lilacinus* and *P. variotii* are also reported herein from cyst nematodes for the first time although the former has been described from the root-knot species, *Meloidogyne incognita acrita* (15). *Paecilomyces nostocoides* Dunn, a species closely related to P. lilacinus, has been recovered from *H. zeae* cysts (5). An unidentified species of *Paecilomyces* has also been isolated from *H. avenae* eggs (22).

Among the fungi regularly recovered and considered to be implicated in cyst disease, different fungi have various pathogenic capacities. Both enzymatic activity and/or toxic metabolite biosynthesis are thought to be involved in cyst nematode pathology. Fungi capable of invading cysts and rapidly penetrating and destroying egg contents in significant numbers probably invade eggs primarily through enzymatic degradation of chitinous egg shell membranes (1). This group would include chitinolytic species. Fungi which lack chitinase activity may enter cysts, initially growing as saprophytes on mucilage but eventually affecting egg and larval viability or be inhibitory of hatching through production of diffusible toxins. This is likely where there is no discernible degradation of egg shell membranes but where there is clear evidence of discoloration and physiological egg disorder as well as larval degeneration as were observed in our studies for *Phoma* spp. Once an egg is physiologically disordered it becomes predisposed to further decay and fungal entry. It is also possible that a number of fungi may operate through a combination of enzymatic activity and toxin biosynthesis. For example, chitinolytic fungi such as *C. cochliodes* and *P. variotii* are known to produce cytotoxic agents and toxic metabolites in feedstuffs respectively (4).

In addition to the enzymatic capacity of the fungus and the physiological condition of cyst contents, the degree to which a fungus may invade and subsequently degrade a cyst and the eggs within may depend on the presence of other microorganisms within the cyst. Organisms may be antagonistic or may provide a secondary invader with a food source of breakdown products resulting from the degradation of cyst contents. Approximately 94% of the cysts and females examined gave rise to pure fungal colonies indicating that the presence of one fungus generally precludes entrance by another regardless of whether the first invader parasitized the eggs or saprophytically fed on cyst mucilage. A small proportion of the fungal isolates demonstrated apparent synergistic activity, particularly within older cysts recovered from soil (Table 1). In these instances two fungal species were recovered from one *H. glycines* cyst. Over half of these cases involved a fungus exhibiting chitinolytic activity [as evidenced by clearing of chitin agar plates (9)] and a nonchitinolytic species. The greatest relative involvement in these associations was demonstrated by *P. variotii*. About one-third of the total number of isolations of this fungus also entailed recovery of another fungal species. Although these relationships are as yet poorly understood they serve to emphasize the complexity of interactions among fungi and between fungi and phytonematodes as well as other members of the soil microenvironment.

Accumulated evidence based on frequency of occurrence, microscopic observation of egg penetration and known enzymatic capacity of the implicated organisms suggests that a measure of biological control may already be in place in many cultivated soils. The details of its operation and the extent of its occurrence is likely to vary from soil to soil but there is every reason to believe that measures exist that may permit us to enhance natural suppression of cyst nematode populations.

Table 1. Frequency of occurrence of combinations of fungi in *Heterodera glycines* cysts and females which contained two fungal species. Chitinase activity (as evidenced by clearing of chitin agar plates) is denoted by (*).

Fungal combination	Frequency
Older Cysts Recovered from Soil	
Fusarium solani & *Humicola fuscoatra**	1
" " & *Neocosmospora vasinfecta*	3
" " & *Paecilomyces variotii**	9
" " & *Phoma americana*	1
" " & *Phoma terrestris**	2
" " & *Phytophthora cinnamomi**	1
" " & *Trichosporon beigelii*	9
" " & *Verticillium lecanii**	1
Fusarium oxysporum & *Chaetomium cochliodes**	1
" " & *Cylindrocarpon tonkinense*	1
" " & *Humicola fuscoatra**	1
" " & *Neocosmospora vasinfecta*	4
" " & *Paecilomyces variotii**	1
" " & *Phoma terrestris**	2
" " & *Trichosporon beigelii*	5
" " & *Verticillium lecanii**	1
Neocosmospora vasinfecta & *Paecilomyces lilacinus**	2
" " & *Penicillium decumbens**	1
" " & *Trichosporon beigelii*	1
" " & *Verticillium chlamydosporium**	2
*Paecilomyces lilacinus** & *Aspergillus flavus*	1
" " & *Cylindrocarpon tonkinense*	1
*Paecilomyces variotii** & *Penicillium decumbens**	1
" " & *Trichosporon beigelii*	1
*Phoma terrestris** & *Neocosmospora vasinfecta*	2
" " & *Paecilomyces lilacinus**	4
" " & *Paecilomyces variotii**	3
" " & *Trichosporon beigelii*	1
" " & *Verticillium chlamydosporium**	1
Pythium sp.* & *Trichosporon beigelii*	1
Scytalidium fulvum & *Pythium* sp.*	3
" " & *Trichosporon beigelii*	2
Cream-colored Cysts Attached to Root Tissue	
Exophiala pisciphila & *Phoma americana*	1
Fusarium solani & *Pythium* sp.*	1

Neocosmospora vasinfecta & *Thielavia terricola**	1
*Phoma terrestris** & Sterile Mycelium 1	1
Trichosporon beigelii & *Exophiala pisciphila*	1
" " & *Fusarium oxysporum*	1
" " & *Geotrichum candidum*	1
" " & *Neocosmospora vasinfecta*	1
" " & *Phoma terrestris**	1
White Lens-shaped Females Attached to Root Tissue	
*Phytophthora cinnamomi** & *Fusarium oxysporum*	2
" " & *Neocosmospora vasinfecta*	1
Trichosporon beigelii & *Fusarium oxysporum*	2

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