PARASITISM OF EGGS OF *HETERODERA GLYCINES* AND *MELOIDOGYNE ARENARIA* BY FUNGI ISOLATED FROM CYSTS OF *H. GLYCINES*

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Accepted:
19.V.1982
Aceptado:

ABSTRACT

Godoy, G., R. Rodríguez-Kábana, and G. Morgan-Jones. 1982. Parasitism of eggs of *Heterodera glycines* and *Meloidogyne arenaria* by fungi isolated from cysts of *H. glycines*. Nematropica 12: 111-119.

The degree of parasitism of 14 fungal species isolated from cysts and eggs of *Heterodera glycines* Ichinohe against eggs of *H. glycines*, and of *Meloidogyne arenaria* (Neal) Chitwood was tested *in vitro*. The fungi were: *Chaetomium indicum* Corda, *Codinaea heteroderae* Morgan-Jones, *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Gliocladium catenulatum* Gilman & Abbot, *G. roseum* Bain, *Neocosmospora vasinfecta* E.F. Smith, *Phoma macrostoma* Mont., *P. multirostrata* (Mathur, Menon & Thirum) Dorenb. & Boerema, *Stagonospora heteroderae* Morgan-Jones, *Verticillium lamellicola* (R.E.V. Smith) Gams, *V. leptobactrum* Gams, and *Thielavia terricola* (Gilman & Abbott) Emmons. The fungi were cultured in a chitin-mineral salts agar medium and nematode eggs were added to actively growing cultures to assess the ability of the fungal species to parasitize them. The cultures that showed the highest percent of parasitized eggs were those with *V. lamellicola, V. leptobactrum, P. macrostoma* and *P. multirostrata. C. indicum, N. vasinfecta, F. oxysporum, F. solani* and *T. terricola* did not parasitize the eggs and the remaining species showed some degree of parasitism with either of the two types of eggs. Chitinolytic activity (CA) by the fungi on the chitin medium was evidenced by a clearing zone around or under the growing colonies. CA was observed in cultures with *C. indicum, C. heteroderae, G. roseum, S. heteroderae, T. terricola, V. lamellicola*, and *V. leptobactrum*. Results indicated that ability to degrade chitin by the fungi was not the only process involved in parasitism of the eggs.
Additional key words: biological control, population dynamics, soybean cyst nematodes, root knot nematodes, pest management, pathogenicity of eggs, chitinase, soil fungi.

RESUMEN

Godoy, G., R. Rodríguez-Kábana, y G. Morgan-Jones. 1982. Parasitismo de huevos de *Heterodera glycines* y *Meloidogyne arenaria* por hongos obtenidos de quistes de *H. glycines*. Nematrópica 12: 111-119.

El grado de parasitismo de 14 especies fungosas obtenidas de quistes y huevos de *Heterodera glycines* Ichinohe, contra huevos de *H. glycines* y de *Meloidogyne arenaria* (Neal) Chitwood se estudió a nivel de laborato-

rio. Las especies fungosas estudiadas fueron: *Chaetomium indicum* Corda, *Codinaea heteroderae* Morgan-Jones, *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Gliocladium catenulatum* Gilman & Abbot, *G. roseum* Bain, *Neocosmospora vasinfecta* E.F. Smith, *Phoma macrostoma* Mont., *P. multirostrata* (Mathur, Menon & Thirum) Dorenb. & Boerema, *Stagonospora heteroderae* Morgan-Jones, *Verticillium lamellicola* (R.E.V. Smith) Gams, *V. leptobactrum* Gams, y *Thielavia terricola* (Gilman & Abbott) Emmons. Los hongos fueron cultivados en un medio con agar, quitina, y sales inorgánicas. Los huevos de nematodos se añadieron a cultivos puros de las especies fungosas para determinar la capacidad de parasitismo de éstas sobre los huevos. Los hongos que mostraron el grado más alto de parasitismo fueron: *V. lamellicola, V. leptobactrum, P. macrostoma* y *P. multirostrata.* Otras especies tales como *C. indicum, N. vasinfecta, F. oxysporum, F. solani* y *T. terricola* no mostraron parasitismo sobre los huevos. Las demás especies fungosas manifestaron cierto grado de parasitismo en los huevos de una o ambas especies de los nematodos estudiados La actividad quitinolítica de los hongos fué también estudida evidenciándose al formarse unas zonas de aclaramiento alrededor o debajo de las colonias en el medio con quitina. *C. indicum, C. heteroderae, G. roseum, S. heteroderae, T. terricola, V. lamelicola* y *V. leptobactrum* mostraron ser quitionolíticos. Los resultados indicaron que quitinólisis no es el único proceso utilizado por hongos al parasitar huevos de nematodos.
Palabras claves adicionales: control biológico, dinámica de poblaciones, nematodo enquistador de la soya, nematodos noduladores, manejo de plagas, patogénesis de huevos, quitinasa, hongos del suelo.

INTRODUCTION

A number of fungal species have been found parasitizing eggs of cyst nematodes in the genera *Globodera* Mulvey & Stone, and *Heterodera* Schmidt (6, 7, 8, 9, 10, 13, 15). Results from a recent survey of fungi in cysts of *H. glycines* Ichinohe from several soybean [*Glycine max* (L) Merr.] producing states in the United States indicated that a specific mycoflora is associated with the nematode (11, 12); a number of fungal species were observed parasitizing eggs of the nematode. Although the mechanism involved in parasitism of nematode eggs by fungi is not clearly understood, Stirling and Mankau (14) and Morgan-Jones and Rodríguez-Kábana (12) have suggested enzymatic degradation of the eggs' walls by the fungi as one of the steps involved in the process.

Chitin is present in a protein complex in the middle layer of nematode egg walls; the chitinous layer is thought to be responsible for rigidity of the egg shell (2). The present paper reports on the ability of fungi isolated from cysts and eggs of *H. glycines* to degrade chitin and parasitize eggs of *H. glycines* and *Meloidogyne arenaria* (Neal) Chitwood.

MATERIALS AND METHODS

Fungal species for the study were isolated from cysts and eggs of *H. glycines*

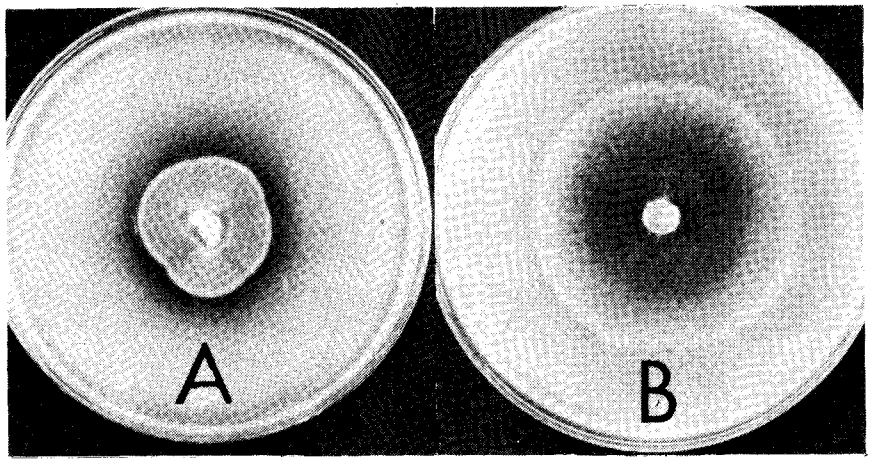

Figure 1. Clearing zones in cultures of fungi isolated from cysts and eggs of *Heterodera glycines* growing in chitin-mineral salts agar. A. *Verticillium leptobactrum* colony showing peripheral clearing; B. *Stagonospora heteroderae* colony with clearing zone within the colony.

following the procedures described before (11, 12). The fungal species selected for the study were: *Chaetomium indicum* Corda, *Codinaea heteroderae* Morgan-Jones, *Fusarium oxysporum* Schlecht, *F. solani* (Mart.) Sacc., *Gliocladium catenulatum* Gilman & Abbot, *G. roseum* Bain *Neocosmospora vasinfecta* E.F. Smith, *Phoma macrostoma* Mont., *P. multirostrata* (Mathur, Menon & Thirum) Dorenb. & Boerema, *Stagonospora heteroderae* Morgan-Jones, *Verticillium lamellicola* (R.E.V. Smith) Gams, *V. leptobactrum* Gams, and *Thielavia terricola* (Gilman & Abbott) Emmons.

The isolates were kept on potato-dextrose-agar (PDA) at 29C. Petri plates with sterile chitin agar were inoculated with 0.5 cm-diam discs taken from the periphery of actively growing PDA cultures. After growth in the chitin medium had occupied approximately one-half of the agar surface the fungus was again inoculated as described into fresh chitin medium where determinations of radial mycelial growth and chitinolytic activity were performed. There were four plates (replications) per fungal isolate. All inoculated plates were maintained at 29C for one week and measurements of growth were performed daily by determining the extent of growth along two vertical diameters in each plate.

In addition to measurements of mycelial growth the ability of fungi to decompose chitin was also determined by observing the presence or absence of a clearing zone in the medium under or around the developing colonies (Fig. 1). Fungal colonies evidencing this clearing effect were the only ones considered to have chitinolytic properties.

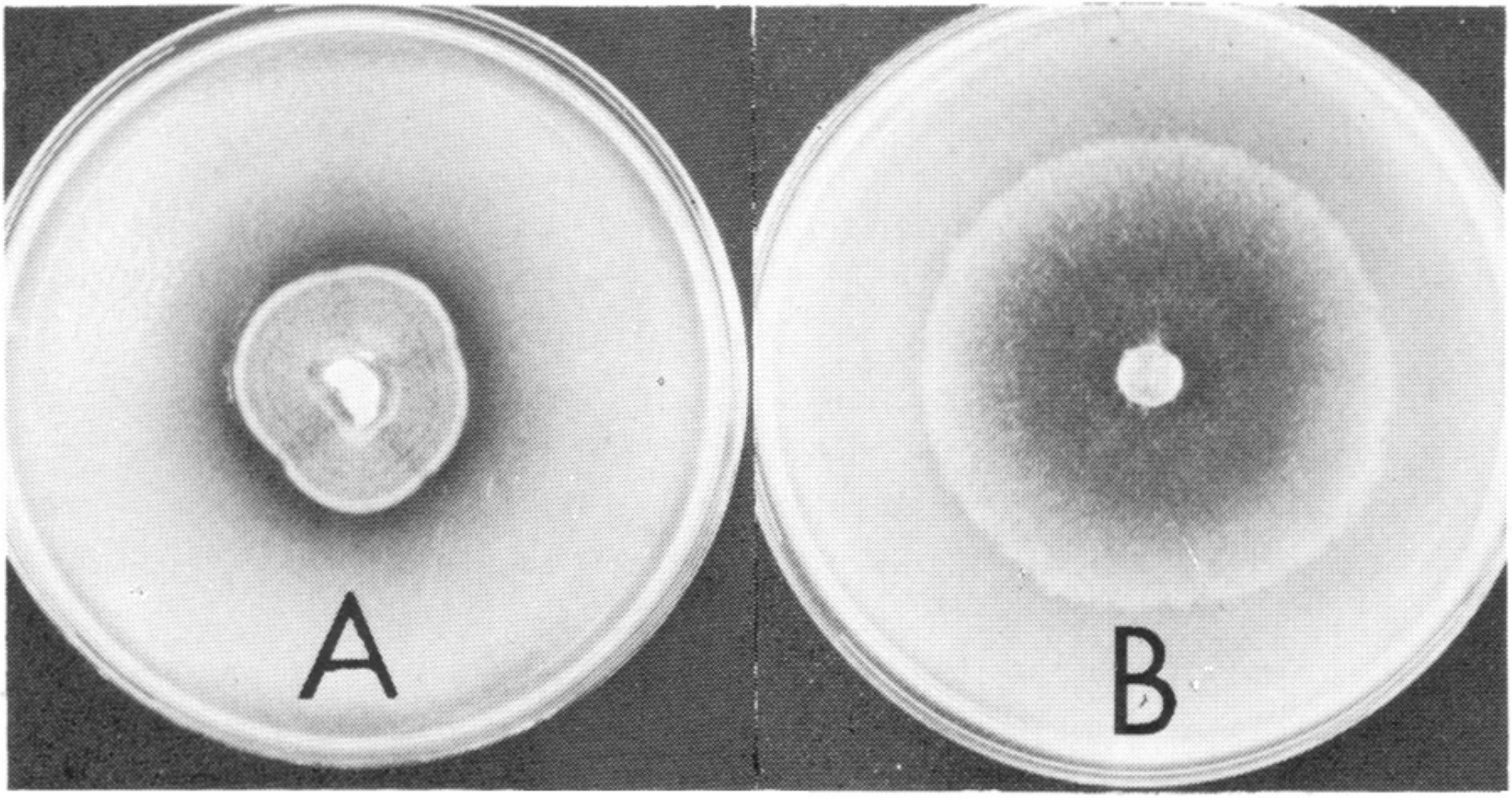

Figure 1. Clearing zones in cultures of fungi isolated from cysts and eggs of *Heterodera glycines* growing in chitin-mineral salts agar. A. *Verticillium leptobactrum* colony showing peripheral clearing; B. *Stagonospora heteroderae* colony with clearing zone within the colony.

Preparation of chitin medium. A colloidal suspension of chitin was prepared by dissolving 100 gm of ground (0.25 mm) crustacean chitin (U.S.B. Corporation, Cleveland, OH) in 1.2 l of 85% (w/w) phosphoric acid; the chitin in the acid was stirred intermittently for 2 hr at 25C until dissolved. The solution was passed through a dacron gauze (0.5 mm mesh) into a five liter glass bottle and four liters of tap water were added slowly with stirring to form a colloidal suspension. The suspension was allowed to stand overnight to settle the chitin suspension and permit siphoning of excess supernatant fluid. The remaining chitin suspension was then transferred to several 1 m long dialysis tubes (4.8 cm diam; molecular weight cut off = 12000) which were then subjected to dialysis against running tap water (two l/min) for 96 hr followed by dialysis against demineralized water for 24 hr. Overlaying excess water in the tubes was removed at intervals during the dialysis process. The resulting chitin suspension had a pH of 5.5-6.0. The amount of chitin in the suspension was determined gravimetrically by drying four 10 ml aliquots at 70C for 24 hrs. The suspension was maintained at 4C in the dark until used.

The chitin medium for the study contained 0.2% (w/w) chitin, and (per liter) 1.0 gm K_2HPO_4, 0.5 gm $MgSO_4.7H_20$, 10 ml of trace element solution, and 20 gm agar. Final pH of the medium was adjusted to 5.0 using 0.1 N H_2SO_4. The trace element solution contained 1.0 gm each of $FeSO_4.7H_20$, $MnS0_4$, and $ZnSO_4$ dissolved in one liter of demineralized water to which were added two drops of conc. H_2SO_4.

The medium was delivered into 400 ml prescription bottles which were then sterilized by autoclaving and kept in the dark at room temperature (*ca.* 26C) until used.

Pathogenicity of fungi on nematode eggs. - The ability of fungal species to parasitize eggs of *H. glycines* and *M. arenaria* was studied *in vitro* by introducing eggs of each nematode into cultures of fungi growing on chitin medium.

Eggs of *H. glycines* (race 3) were extracted from young and maturing cysts on roots of greenhouse-grown Ransom soybean. The isolate of *H. glycines* was obtained from a Mississippi soybean field and was essentially free of egg parasites; soybean plants were grown in soil from the field.

To extract eggs from cysts, infected roots were cut into pieces 0.5-1.0 cm long and blended with 150 ml of 10% (v/v) Clorox® [5.25% (w/w) NaClO] solution in a Virtis® 45 homogenizer at the "medium" speed setting. The homogenate was passed successively through four eight-cm diam nested stainless steel sieves with opening of 250, 150, 75 and 30 μm (500 mesh). The eggs retained in the 30 μm sieve were washed several times with sterile demineralized water and washed into a 100 ml beaker. The number of eggs per ml was then determined (4) and the concentration was adjusted to have a suspension containing approximately 250 eggs per ml. Aliquots of 0.5 ml of the egg suspension were pipetted into Petri plates with ten-day-old cultures of the fungi growing on chitin agar. There were four plates for each fungal species and four plates with eggs on uninoculated sterile chitin medium. All

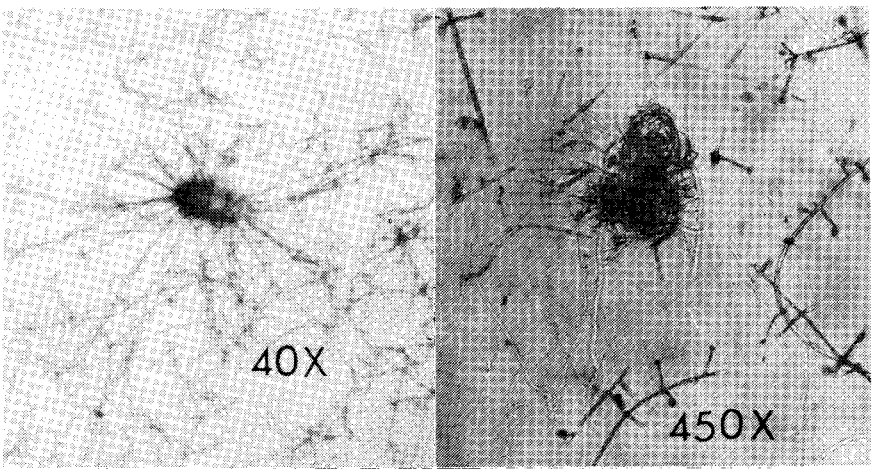

Figure 2. Eggs of *Meloidogyne arenaria* parasitized by *Verticillium leptobactrum.*

the plates were incubated at 29C for seven days when the percentage of parasitized eggs was determined. To determine the number of eggs parasitized each plate was divided into four fields and 25 eggs were examined in each field. Successful parasitism was recorded when there was mycelial invasion of the internal parts of an egg and its consequent destruction (Fig. 2).

M. arenaria test. Eggs of *M. arenaria* were extracted from galled roots of greenhouse Rutgers tomatoes (*Lycopersicon esculentum* L.) grown in an infested soil collected from a peanut (*Arachis hypogaea* L.) field near Headland, AL. The extraction of eggs from roots and the procedures and criteria used to determine parasitism of eggs by the fungi were the same as described for *H. glycines* except that 500 eggs were introduced into each plate.

RESULTS

Clearing of the chitin medium was observed 48 hrs after inoculation in cultures of *C. indicum, C. heteroderae, G. roseum, S. heteroderae, T. terricola, V. leptobactrum,* and *V. lamellicola* (Table 1). Clearing of the medium differed among the species. Cultures with *C. heteroderae* and *V. leptobactrum* evidenced a clearing zone on the periphery of their colonies while those of *C. indicum, G. roseum, S. heteroderae* and *T. terricola* showed the zone beneath their colonies. The remaining species in the study did not show any clearing of the medium.

Radial mycelial growth on chitin medium seven days after inoculation differed significantly between the species (Table 1). *G. roseum* and *P. multirostrata* were the fastest growing species covering the entire surface of the plates four and five days after inoculation, respectively. Mycelial growth of

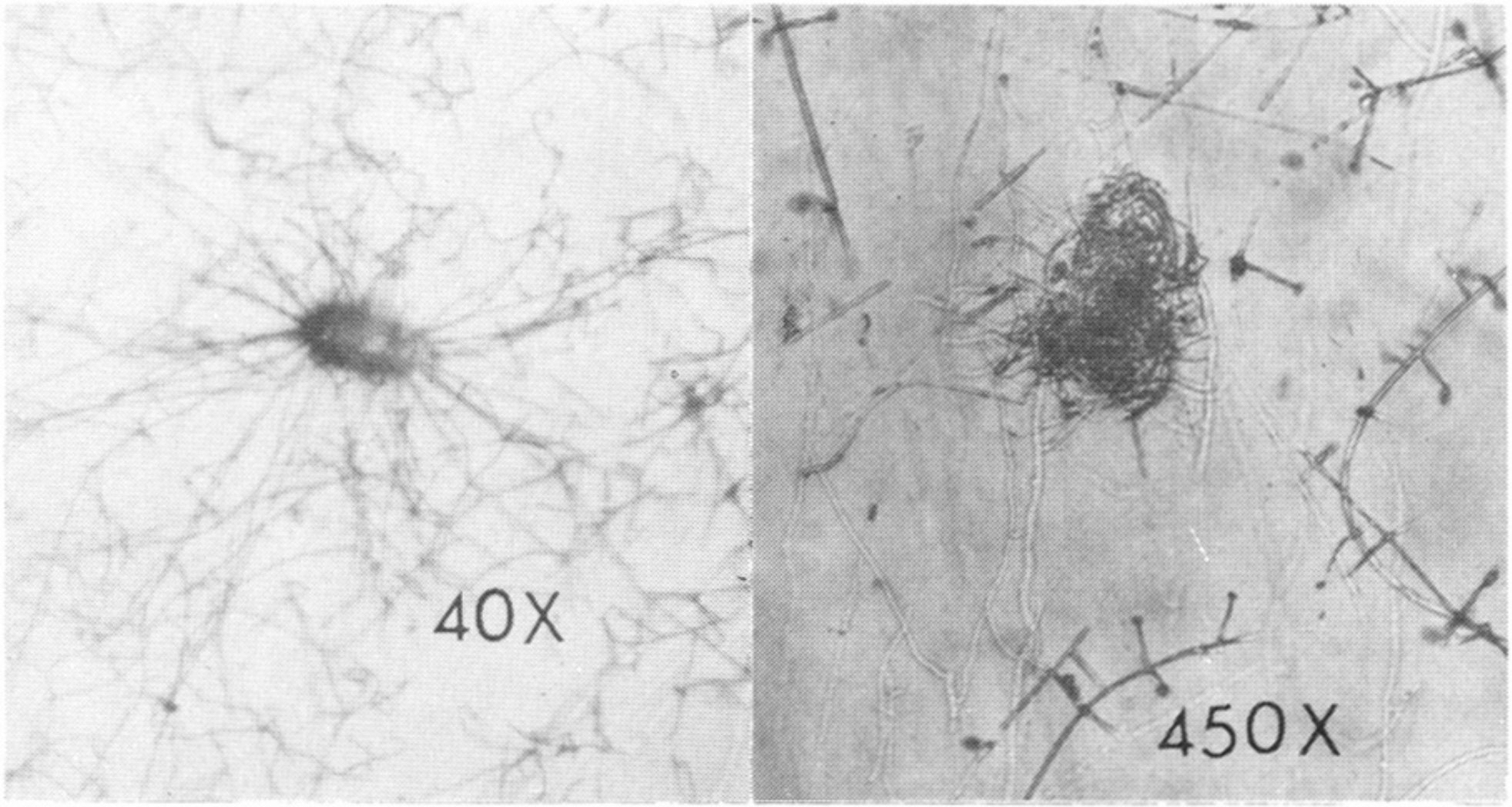

Figure 2. Eggs of *Meloidogyne arenaria* parasitized by *Verticillium leptobactrum*.

Table 1. Chitinolytic activity and degree of parasitism of fungi isolated from cysts and eggs of *Heterodera glycines* on eggs of *H. glycines* and *Meloidogyne arenaria.*

	Chitinolytic[x] Activity	Radial[y] Mycelial Growth (cm)	Percent Eggs Parasitized	
			H. glycines	*M. arenaria*
Chaetomium indicum	+	6.0	0	0
Codinea heteroderae	+	1.2	18	29
Gliocladium roseum	+	8.5	-	12
Stagonospora heteroderae	+	3.9	26	12
Thielavia terricola	+	7.5	0	0
Verticillium leptobactrum	+	2.5	80	98
Verticillium lamellicola	+	3.5	65	85
Fusarium oxysporum	-	8.5	0	0
Fusarium solani	-	8.5	0	0
Gliocladium catenulatum	-	5.5	-	5
Neocosmospora vasinfecta	-	7.0	0	0
Phoma macrostoma	-	5.4	35	36
Phoma multirostrata	-	8.5	40	80

[x]Denotes presence (+) or absence (-) of a clearing zone around or beneath colonies growing on chitin-mineral salts agar.
[y]Determined on chitin-mineral salts agar seven days after inoculation.

some of the fungal species was accompanied by formation of fruiting bodies or abundant sporulation. *N. vasinfecta* and *C. indicum* produced perithecia and *T. terricola* cleistothecia which were evident five days after inoculation; *G. roseum, V. leptobactrum* and *V. lamellicola* sporulated profusely but only *G. roseum* exhibited abundant mycelial growth. Cultures of *F. oxysporum* and *F. solani* covered the entire plate surface seven days after inoculation; however mycelial growth though extensive was not abundant and production of macro- and microconidia was poor compared with that of cultures on PDA. The remaining species produced abundant mycelium but their growth was slow.

V. leptobactrum and *V. lamellicola* showed the greatest degree of parasitism against eggs of *H. glycines* and *M. arenaria* (Table 1) while *C. indicum, N. vasinfecta, F. solani,* and *F. oxysporum* did not parasitize eggs of the two nematodes. *P. multirostrata* and *P. macrostoma* parasitized more than 30% of the eggs of both species while eggs parasitized by the remaining species in the study varied from 12-29%. Parasitism of *G. roseum* and *G. catenulatum* on eggs of *H. glycines* could not be assessed accurately because profuse development of spore masses in the cultures obstructed observation.

Large numbers of larvae of the two nematodes were observed seven days after initiation of the experiment only in plates with no fungi and in those with fungal colonies evidencing no parasitism.

DISCUSSION

Fifty percent of the fungal species tested exhibited chitinolytic activity and of these only two were not parasitic on eggs of *H. glycines* or *M. arenaria.* These results suggest a correlation between ability to parasitize nematode eggs and chitinolytic activity. Jackson (5) and Baath and Sonderstrom (1) reported that species of *Verticillium* and *V. chlamydosporium* were able to degrade chitin. Tribe (15) determined that *V. chlamydosporium* was an important parasite of eggs of *H. schachtii* in Europe. Other fungi parasitic of nematode eggs are known to have chitin-degrading properties (3). Stirling and Mankau (14) detected chitinase production in cultures of *Dactylella oviparasitica* Stirling and Mankau and suggested a possible involvement of the enzyme's activities in the process of parasitism. Jatala recently found (6) *Paecilomyces lilacinus* (Thom.) Samson infecting egg masses of *M. incognita* (Kofoid & White) Chitwood and cysts of *Globodera pallida* (Stone) Mulvey & Stone. Baath and Sonderstrom had previously reported that species of *Paecilomyces* Bain exhibited chitinolytic activity (1).

Our results also indicate that the ability of fungi to parasitize eggs is not limited to species possessing chitinolytic activity as observed in our study. Thus, *P. macrostoma* and *P. multirostrata* did not result in clearing of the chitin medium but were nevertheless able to parasitize eggs of both nematode species. This suggests that these fungal species may posses abilities to degrade

other components of the egg shell wall besides chitin. It is also possible that they may exhibit a type of chitinolytic activity not discernible by the methods and criteria used in the present study. Clearly, the mode of action of these fungal parasites needs further investigation.

Our results on *Fusarium* spp. contrast with those of Nigh (13) who found species in the genus growing out of eggs of *H. schachtii*. It is possible that the ability to parasitize nematode eggs by *Fusarium* spp. may vary between isolates of the same species or less likely according to the species of nematodes or even the physiological condition of eggs used for testing.

The only fungal species exhibiting chitinolytic activity that did not attack eggs were *C. indicum* and *T. terricola*. Morgan-Jones and Rodríguez-Kábana have suggested that these fungi may be involved in long term degradation of cyst exocuticle in soil. Species of *Chaetomium* Kunze ex Fr. and *Thielavia* Zopf produce a wide variety of exocellular enzymes which allow them to grow on substrates of widely different composition (3); however, species in these genera have yet not been implicated in parasitism of nematode eggs. There is thus for these species an apparent discrepancy for which we have no explanation at present.

In conclusion our results indicate that several fungal species isolated from cysts of *H. glycines* can parasitize eggs of both *H. glycines* and *M. arenaria*. Also, results suggest that the mode of action of these egg parasites is not limited to the ability to degrade chitin (clearing of medium) and that other mechanisms may be operative in the process of pathogenicity.

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Received for publication:
15.IV.1982
Recibido para publicar: