FUNGAL COLONISATION OF *HETERODERA GLYCINES* CYSTS IN ARKANSAS, FLORIDA, MISSISSIPPI AND MISSOURI SOILS

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ABSTRACT

Morgan-Jones, G., B. Ownley Gintis and R. Rodríguez-Kábana. 1981. Fungal colonisation of *Heterodera glycines* cysts in Arkansas, Florida, Mississippi and Missouri soils. Nematropica 11: 155-164.

A survey of fungi associated with populations of cysts of *Heterodera glycines* from soybean field soils in four states in the Mississippi Valley and the southeastern United States indicates a generically diverse mycoflora, but one that is restricted in numbers of taxa and overlapping. Genera common in cysts from all localities include *Fusarium, Gliocladium, Neocosmospora* and *Phoma.* A species of *Stagonospora,* which we believe to be implicated as a significant natural parasite of *H. glycines* eggs, was also present in the four localities. Other fungi recovered with frequency from cysts in some, but not all soils surveyed, include species of *Chaetomium, Codinaea heteroderae, Exophiala pisciphila,* species of *Thielavia, Verticillium lamellicola* and a fungus referred to as a 'black yeast'. Several other incidental fungi were found rarely.

Additional key words words: nematode egg parasites, cyst-nematode pathology, soil fungi, fungal antagonisms, biological control, population dynamics.

RESUMEN

Morgan-Jones, G., B. Ownley Gintis y R, Rodríguez-Kábana, 1981. La colonización de quistes de *Heterodera glycines* por hongos en suelos de Arkansas, Florida, Mississippi y Missouri. Nematropica 11: 155-164.

Resultados de un reconocimiento de los hongos asociados con de quistes de *Heterodera glycines* Ichinohe provenientes de campos de soya de cuatro estados del valle del Mississippi y del sureste de los Estados Unidos indicaron que existe en los quistes una micoflora genéricamente diversa aunque limitada en número de taxa. Los géneros más comunes en quistes de todas las localidades fueron *Fusarium, Gliocladium, Neocosmospora* y *Phoma.* Tam-

bién, en las cuatro localidades se detectó una especie de *Stagonospora* que creemos ser un parásito natural e importante de huevos de *H. glycines*. Otros hongos recolectados con frecuencia en quistes de algunos de los suelos examinados, pero no en todos, fueron especies de *Chaetomium, Codinea heteroderae, Exophiala pisciphila,* especies de *Thielavia, Verticillium lamellicola* y un hongo que denominamos "levadura oscura." Aparte de los hongos mencionados también se detectaron otras especies fungosas que aparecieron infrecuentemente y de manera incidental.

Palabras claves adicionales: parásitos de huevos de nematodos, patología de nematodos enquistadores, hongos del suelo, antagonismo entre hongos, control biológico, dinámica de poblaciones.

INTRODUCTION

The association of fungi with mature cysts of nematodes belonging to the genera *Globodera* and *Heterodera* Schmidt in agricultural soils has, to some degree, been documented by van der Laan (10, 11) Willcox and Tribe (12), Bursnall and Tribe (1), Goswami and Rumpenhorst (5), Morgan-Jones and Rodríguez-Kábana (6) and others. Tribe (9) reviewed the state of knowledge of cyst-nematode pathology and the possible or potential role of fungi as pathogens of these organisms. Much, however, remains to be learned about the fate and ecology of cysts remaining in soil over an extended period of time and of the factors controlling levels of egg viability. The status and precise role of various fungi in contact with cysts is not entirely clear although there is evidence that some species are implicated in degradation and others in direct parasitism of the cysts and their contents. Interactions and antagonisms among soil fungi found in association with cysts during the various phases of development and maturation are largely unknown, as are the specific conditions necessary for fungal invasion. The role of fungi in progressive degradation of cysts in the soil is likewise an area where research is needed in order to fully understand fungal-nematode relationships and the possible significance of fungi in dictating cyst-nematode population dynamics.

The present paper reports a survey of the mycoflora associated with cysts of *Heterodera glycines* Ichinohe in a number of soybean field soils located in Arkansas, Florida, Mississippi and Missouri, U.S.A. It is essential in the first instance to establish what fungi are present before an assessment can be made of their roles, activity and interactions. In this survey a study has been made primarily of whole cysts but some attention has been paid to any obvious egg parasites encountered. Fungi recorded are those present within unopened cysts, whether growing saprophytically or parasitically in this particular niche. Our aim has been to determine whether or not there is some constancy in the mycoflora associated with the cysts of *H. glycines* in soils of different composition, cropping history and geographical location.

MATERIALS AND METHODS

Cysts were extracted from four air-dried soil samples, received from different localities, by suspending each soil separately in water followed by passage of the suspensions through a 100 mesh stainless steel sieve. Cysts from each soil were kept separate throughout the investigation. Mature, mid-brown cysts retained within the sieve were transferred to Petri dishes containing sterile distilled water. 250 cysts from each soil sample, selected at random, were hand picked under a 15X stereoscan microscope with sharp-pointed forceps (care being taken not to cause any rupture of the cyst wall) and removed in turn to a Nalgene® (Fisher) membrane filter unit [pore size 0.20 μm]. Enough 10% Chlorox® [5.25 % (w/w) NaClO] solution (*ca.* 10 ml) to cover the cysts was added. Following gentle agitation for two minutes a vacuum aspirator was attached to the filter unit and the Chlorox solution drawn off (this ensures approximately equal surface sterilization treatment time for each cyst). Sterile distilled water containing streptomycin (0.05%) was then added, agitated, and removed in the same manner. The latter procedure was repeated twice. Individual cysts were then removed and plated directly onto potato-dextrose agar (PDA) with added streptomycin (100 μg/ml) in Petri dishes, four per dish, and placed as wide apart as possible near the periphery of each dish. Plates were incubated at 25C for five days and examined for fungal colonies.

A set of 10 cysts was removed from the surface of the agar from colonies of fungal species occurring with a frequency of more than 5% of the total sample. Each cyst was placed in a drop of lactophenol on a glass slide and pulled apart between mounted needles. Exposed eggs were then examined microscopically for the presence of endogenous fungal hyphae.

The sources of soil samples were as follows:

Arkansas; from soybean field in Marianna, Lee County. The field had been in Forrest soybeans for three years and in cotton before that.

Florida; from soybean field in Jay, Escambia County.

Mississippi; from soybean field in Pontotoc County.

Missouri; from soybean field in Lee Farm, Pemiscot County. The field had been in soybeans for five years, the last three in the Bedford variety.

All samples were collected in the summer 1981.

RESULTS

A total of twenty seven different fungi were encountered associated with cysts. In almost all instances cysts gave pure cultures of the respective fungi, indicating a mutual exclusivity on the part of individual fungi occupying each cyst. It seems possible that, once a given fungus enters and its biomass occupies the cyst cavity, even though there might be no direct parasitism of the eggs, a second fungus is unable to enter.

The mycoflora was found to be, by and large, remarkably consistent in all four populations (Table 1). In three of the samples about half the cysts

Table 1. Occurence of fungi in cysts (250 in each sample) of *Heterodera glycines* plated on PDA after surface sterilization.

Arkansas (132 cysts bore no fungi)	
Exophiala pisciphila	11
Fusarium oxysporum	9
Fusarium solani	34
Gliocladium catenulatum	7
Gliocladium roseum	10
Myrothecium verrucaria	4
Neocosmospora vasinfecta	7
Stagonospora heteroderae in ed.	25
Phoma macrostoma	3
Thielavia basicola	5
Trichoderma harzianum	2
Black yeast (I)	10
Florida (89 cysts bore no fungi)	
Chaetomium indicum	5
Codinaea heteroderae	9
Fusarium oxysporum	25
Fusarium solani	74
Gliocladium roseum	7
Macrophomina phaseoli	1
Neocosmospora vasinfecta	10
Phoma multirostrata	3
Phoma macrostoma	6
Rhizoctonia solani	1
Stagonospora heteroderae in ed.	38
Thielavia basicola	3
Thielavia terricola	5

contained fungi while in the fourth, from Florida, almost two thirds of the cysts gave fungal colonies. Three fungi, in particular, were found to occur with considerable frequency, namely *Fusarium oxysporum* Schlecht., *Fusarium solani* (Mart.) Sacc., and *Stagonospora heteroderae* Morgan-Jones in ed. Other fungi encountered in significant numbers in some, but not all cyst samples, include *Exophiala pisciphila* McGinnis and Ajello, *Gliocladium roseum* Bain., *Neocosmospora vasinfecta* Smith and a fungus referred to

Table 1. (continued)

Missouri (123 cysts bore no fungi)	
Chaetomium gracile	7
Cladosporium cladosporioides	3
Curvularia lunata var. *aeria*	2
Exophiala pisciphila	9
Fusarium equiseti	5
Fusarium lateritium	6
Fusarium oxysporum	18
Fusarium solani	46
Gliocladium roseum	7
Neocosmospora vasinfecta	12
Phoma multirostrata	2
Rhizoctonia solani	2
Stagonospora heteroderae in ed.	24
Thielavia basicola	5
Black yeast (I)	16
Mississippi (121 cysts bore no fungi)	
Curvularia lunata	3
Gliocladium catenulatum	5
Gliocladium roseum	9
Fusarium lateritium	2
Fusarium oxysporum	10
Fusarium solani	57
Phoma macrostoma	7
Stagonospora heteroderae in ed.	26
Trichoderma harzianum	1
Verticillium lamellicola	8
Black yeast (I)	11
Black yeast (II)	3

herein as 'black yeast (I)'. *Phoma macrostoma* Mont., *Phoma multirostrata* (Mathur, Menon and Thirum.) Dorenb. and Boerema, and *Thielavia basicola* Zopf, were each recorded in more than one sample, but in small numbers, while *Codinaea heteroderae* Morgan-Jones and *Verticillium lamellicola* (R.E.V. Smith) W. Gams were each present in only one sample but in slightly larger numbers.

Observation of individual cysts by dissection to expose eggs indicated

penetration of the eggs with regularity by four of the fungi recorded. In all forty examined, from which colonies of *Stagonospora heteroderae* had been obtained, a number of eggs were found to be penetrated by pale-brown, torulose hyphae and the larvae were obliterated. The hollow interior of most of these cysts was plugged with a stromata-like tissue composed of a closely appressed mass of mid-brown, isodiametric cells. Similar invasion of eggs was also apparent in cysts occupied by *Exophiala pisciphila, Codinaea heterode-rae* and the fungus referred to as 'black yeast (I)'*. Of nineteen *E. pisciphila*-parasitized cysts examined (ten from the Arkansas samples and all nine from the Missouri sample) thirteen were found to contain eggs in which ellipsoid to globose, swollen, pale-brown hyphal cells were evident.

Cysts invaded by *Fusarium* spp., contained mostly healthy eggs with intact normal-looking larvae. Occasional eggs which appeared abnormal were observed; some with larvae containing large, irregularly-shaped oil drops, others where lysing and shrivelling was apparent. Five eggs from two cysts (out of a total of eight examined), from which colonies of *Verticillium lamellicola* were derived, were found to contain hyaline fungal hyphae.

DISCUSSION

It is evident from this, and previous studies, that a distinct but restricted mycoflora is associated with cysts of the genus *Heterodera* Schmidt in various soils. Bearing in mind the hundreds of fungi known to be ubiquitous and cosmopolitan in a wide variety of soils, as well documented by Domsch, Gams and Anderson (3), this particular mycoflora is indeed extremely limited in numbers. Whether or not this indicates an invariably high degree of specialization on the part of individual component members, or well developed, but unspecialized opportunistic capacity, has yet to be elucidated. There is some indication that at least two, if not three, elements are involved in this mycoflora.

Firstly, fungi that are able to enter the cysts early and grow saprophytically on cyst contents, including, possibly, the multilayered endocuticle, but leaving unaffected the thick, resistant, exocuticle and the tough egg shell membrane with the larva folded in loops within. *Fusarium, Gliocladium* and *Neocosmospora* might fall in this category. Entry by such fungi into eggs would require a weakening predisposition by physiological disorder.

Secondly, fungi that are clearly capable of invading healthy eggs in significant numbers and can therefore be considered as bona fide pathogens. *Exophiala pisciphila* and *Stagonospora heteroderae,* among the fungi encountered in this study, belong here. The former fungus was implicated in a similar manner in a survey made in Alabama by Morgan-Jones and

*This fungus is believed to belong to the *Exophiala*-complex (it possesses budding and germinating cells similar to *E. jeanselmei* (Langer.) McGinnis and Padhye) as is that referred to in Table 1 as 'black-yeast (II)'. Lack of conidiation precludes positive indentification at present.

Rodriguez-Kabana (6). The fungi referred to here as 'black-yeasts' might also be involved. In this connection it is interesting to note that similar fungi have been repeatedly encountered in cysts but there is some confusion as to their proper identity. As far back as 1929 Korab isolated a fungus from cysts of *Heterodera schachtii* Schmidt in the Ukraine which Jaczewski [in Goffart (4)] identified as *Torula heteroderae* Jaczewski [now accepted as being possibly *Phialophora malorum* (Kidd and Beaumont) McColloch]. Rozypal (7) found what might be the same fungus (named *Trichosporium populneum* Lambotte and Fautrey by him) as a principal parasite of *H. schachtii* cysts in Moravia. More recently van der Laan (11) found what was determined as *Margarinomyces heteromorpha* (Nannf.) Mangenot in cysts of *Globodera rostochiensis* from Peru. Schol-Schwarz (8) considered *M. heteromorpha* to be a synonym of *Rhinocladiella mansonii* (Castellani) Schol-Schwarz but de Hoog (2) has recently determined, after examining Nannfeldt's and Mangenot's original strains, *M. heteromorpha* to be typical *Exophiala jeanselmei. Rhinocladiella mansonii* is now classified in *Exophiala* Carmichael as *E. mansonii* (Castellani) de Hoog. Goswami and Rumpenhorst (5) and Tribe (9) also report 'black-yeast'-like fungi from cysts of *G. rostochiensis* and *H. schachtii* respectively. The former authors considered their fungus to be similar to *Phialophora malorum* while two of Tribe's isolates were identified as *Exophiala pisciphila* and others he considered to be possibly *Exophiala mansonii.* Although it is now impossible to be certain of the exact identity of the fungi on which these prior reports are based, with the exception of Tribe's records of *E. pisciphila,* it is clear that *Exophiala-Phialophora* type hyphomycetes are involved in cyst-nematode egg pathology.

Thirdly, a succession of fungi could be involved in long-term degradation of cyst exocuticle in soil. Such fungi would be expected to have demonstrable chitinolytic activity. Among the fungi found by us *Chaetomium* spp., as well as the egg parasites, might well be operative in this regard.

In addition to the matter of cyst nematode pathology, and infection of eggs in particular, the wider question of the possible relationship between nematode activity and disease of host substrate, i.e. root tissue, must be addressed. There is clearly a consistent association between such potential pathogens of the soybean plant as *Fusarium oxysporum, R. solani* and *Neocosmospora vasinfecta* and the cysts of *Heterodera glycines.* Evidence from our work and that of others suggests that these fungi are not normally invasive of nematode eggs but there is frequent accumulation of fungal biomass within cysts. Whether or not fungi occupying this niche invade swelling sedentary females through the anal aperture or by some other mode of entry remains to be determied. It seems possible that a chain of events might occur. It is not inconceivable, provided there is a ready inoculum in the rhizosphere, that these fungi enter the root tissue following penetration of the cortex by larvae and subsequently enter the female nematode in situ at its feeding site as it becomes transformed into the cream-colored, fertilized state. Alternatively, the fungus might already be present in root tissue before larval penetration or

both situations might be operative. In order to fully understand population dynamics of both fungi and nematodes it is clearly important to establish when, and by what pathway, the various fungi enter the nematode cysts and the condition of the cysts at the time of infection. There is now good reason to believe that some fungi enter only after the cysts have remained in soil for a period of time while others are early invaders. The condition of cysts in soil, particularly the degree to which degradation has occurred, will govern fungal-cyst interactions. Antagonisms among fungi will also undoubtedly, to some extent, dictate invasive potentiality whatever the condition or age of the cyst.

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