FUNGI ASSOCIATED WITH CYSTS OF *HETERODERA GLYCINES* IN AN ALABAMA SOIL

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ABSTRACT

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A preliminary survey of fungi associated with the cyst-nematode *Heterodera glycines* Ichinohe in an Alabama soybean soil indicates the presence of several specific fungal pathogens as well as a population of miscellaneous incidental species that are at most only weakly parasitic. *Fusarium oxysporum* Schlecht., *F. solani* (Mart.) Sacc., and *Exophiala pisciphila* McGinnis and Ajello are implicated as major pathogens while *Neocosmospora vasinfecta* Smith, *Phoma multirostrata* (Mathur, Menon and Thirum.) Dorenb. and Boerema and *Verticillium leptobactrum* W. Gams are considered to be possibly involved in degradation of cyst cuticle.

Additional key words: Cyst-nematode pathology, soil fungi, population dynamics, biological control, pest management

RESUMEN

Morgan-Jones, G., y R. Rodríguez-Kábana. 1981. Hongos encontrados en quistes de *Heterodera glycines* en un suelo de Alabama. Nematropica 11: 69-74.

Un reconocimiento preliminar de los hongos encontrados en asociación con el nematodo enquistador *Heterodera glycines* Ichinohe en un suelo de un campo con soya demostró la presencia de varios hongos patógenos específicos así como otras especies fungosas de parasitismo debil y que se hallaron sólo ocasionalmente. *Fusarium oxysporum* Schlecht., *F. solani* (Mart.) Sacc., y *Exophiala pisciphila* McGinnis y Ajello fueron implicados como patógenos importantes mientras que se considera que *Neocosmospora vasinfecta* Smith, *Phoma multirostrata* (Mathur, Menon y Thirum.) Dorenb. y Boerema y *Verticillium leptobactrum* W. Gams, son especies que posiblemente esten involucradas en la descomposición de la cutícula de los quistes.

Palabras claves adicionales: patología de nematodos enquistadores, hongos del suelo, dinámica de poblaciones, control biológico, manejo de plagas.

INTRODUCTION

There has, during the last decade, been increasing interest in the association of various fungi with cyst nematodes, belonging to the genus *Heterodera* Schmidt, in soil. A number of these fungi have been recognized as capable of parasitizing two long-lasting stages in the nematode lifecycle; namely the sedentary female and the cysts with the eggs within. Up to comparatively recently very little was known about parasites of nematode eggs, as witnessed by the fact that Barron (1) listed but one fungus, *Rhopalomyces elegans* Corda, as a confirmed pathogen.

To date investigators have predominantly concerned themselves with fungal diseases in population of *Heterodera avenae* Wollenweber and *H. schachtii* Schmidt. The latter is a troublesome pathogen of sugar beets while the former is a pest of cereal crops.

Tribe (10, 11) reviewed current knowledge of cyst nematode pathology as well as report his own survey of European populations of *H. schachtii.* Tribe's investigations, together with those of Kerry (6), Graham and Stone (5) and Kerry and Crumb (7) of *H. avenae* from England, documented a number of fungi to be parasites of females and cysts. *Catenaria auxiliaris (Kühn)* Tribe and *Entomophthora*-like fungi were listed as pathogens of females while *Verticillium chlamydosporium* Goddard and an unnamed so-called 'contortion fungus' were considered to be major egg pathogens. *Cylindrocarpon destructans* (Zinssmeister) Scholten, a group of isolates referred to collectively as 'black yeasts' and a so-called 'crystal-forming fungus' were recorded as minor egg pathogens. More recently Nigh (8) described *Acremonium strictum* W. Gams and *Fusarium oxysporum* Schlect as virulent pathogens of *H. schachtti* eggs in California.

Apart from reports by Tribe (10), based on very limited sampling, of the occurrence of the 'contortion fungus' in eggs of *H. glycines* from Illinois, U.S.A., and of a *Pythium* species in cysts from North Carolina, no information exists on fungi associated with this nematode.

In the present publication results of a preliminary survey of disease of *H. glycines* cysts and eggs in Alabama are reported.

MATERIALS AND METHODS

Cysts were extracted by suspending air dry soil in water followed by passage of the suspension through a 100 mesh stainless steel sieve. The yellow to brown mature cysts with eggs retained in the sieve were then transferred to a Petri dish with water. The soil was collected in September 1980 in a soybean field near Summerdale, Baldwin County, Alabama. The field was heavily infested with race 3 of *H. glycines* and had been in soybean for the past three years. The soil was air dried and kept at room temperature until extraction of

the cysts. Three hundred cysts, selected at random, were hand-picked under a 15x stereoscan microscope and divided into three equal sets in sterile distilled water.

Cyst disease. One set of 100 cysts was plated directly on to potato-dextrose agar (PDA) with added streptomycin (100 ug/ml) in Petri dishes. A second set of 100 cysts was plated out on the same medium following surface sterilization by immersion in a 10% Chlorox® [5.25% (w/w) NaClO] solution for three minutes. Plates were incubated at 25 C for three days and examined for growth of fungal hyphae. Isolations, for purposes of identification, were made from resultant colonies.

Egg disease. Each cyst of the third set of 100 was gently pulled apart between mounted needles in a drop of water on a glass slide and its condition classified as: A, parasitized (substatially diseased - $>$ 50% eggs affected); B, partially parasitized ($<$ 50% eggs diseased); C, empty or containing lysed or shriveled eggs; D, healthy (containing mostly unaffected eggs).

Following examination and classification individual eggs that were clearly parasitized, as evidenced by the presence of fungal hyphae within them, were removed by means of a capillary pipette and plated out on the same medium as used for the cysts. Emergent fungi were isolated by hyphal transfers onto fresh plates.

RESULTS

Nine fungi were found to be intimately associated with cysts (Tables 1 and 2). Several fungi occur with considerable degree of consistency, particularly *Fusarium oxysporum* Schlecht., *F. solani* (Mart.) Sacc., and *Neocosmospora vasinfecta* Smith. These were present in significant numbers both when the cysts were plated directly and when surface sterilized. *Verticillium leptobactrum* W. Gams and *Phoma multirostrata* (Mathur, Menon and Thirum.) Dorenb. and Boerema were present only in cysts plated directly while *Exophiala pisciphila* McGinnis and Ajello was evident only in surface sterilized cysts.

Twenty percent of the cysts examined in detail were found to contain parasitized eggs (8% in condition A, 12% in condition B); 28% contained empty or lysed or shriveled eggs while 52% were found to be healthy.

DISCUSSION

The presence of only small numbers of *Alternaria alternata* (Fr.) Keissler, *Penicillium steckii* Zaleski and *Trichoderma harzianum* Rifai, fungi that are exceedingly common in Alabama soils, leads us to conclude that they are not invasive of the cysts.

We consider it possible that *N. vasinfecta, P. multirostrata* and *V. leptobactrum* might be implicated in cyst cuticle degradation. The latter two

Table 1. Occurrence of fungi on cysts (100) of *Heterodera glycines* (race 3) plated directly on PDA[x].

Fusarium oxysporum	69
Fusarium solani	12
Neocosmospora vasinfecta	9
Penicillium steckii	5
Phoma multirostrata	8
Trichoderma harzianum	4
Verticillium leptobactrum	10

[x]27 bore no fungi

Table 2. Occurrence of fungi on cysts (100) of *Heterodera glycines* (race 3) after sterilization[x].

Alternaria alternata	2
Exophiala pisciphila	10
Fusarium oxysporum	23
Fusarium solani	17
Neocosmospora vasinfecta	9

[x]69 bore no fungi.

species are not particularly common in soil. Their isolation during the present study represents, incidentally, the first record of their occurrence in North America. *P. multirostrata* is known previously from Indian soils, from stems of cucumber, melon and tomato plants under greenhouse cultivation in the Netherlands and Dorenbosch and Boerema (2) report its isolation from an egg mass of a root-knot nematode in a glasshouse experiment, also in the Netherlands. *V. leptobactrum* has been previously isolated from dead wood in Poland, from forest-floor humus in England and from the carpophores of basidiomycetes in France and the Netherlands (4).

All three of the remaining fungi encountered, *E. pisciphila, F. oxysporum* and *F. solani,* appear to be implicated in cyst-nematode pathology. *E. pisciphila* and *F. oxysporum* are the principal parasites. Of ten individual eggs bearing discernible hyaline hyphae plated out nine gave colonies of *F. oxysporum,* the other *F. solani.* The presence of *E. pisciphila* is easily observed as clusters of pale to mid-brown, subglobose to elongate cells within the con-

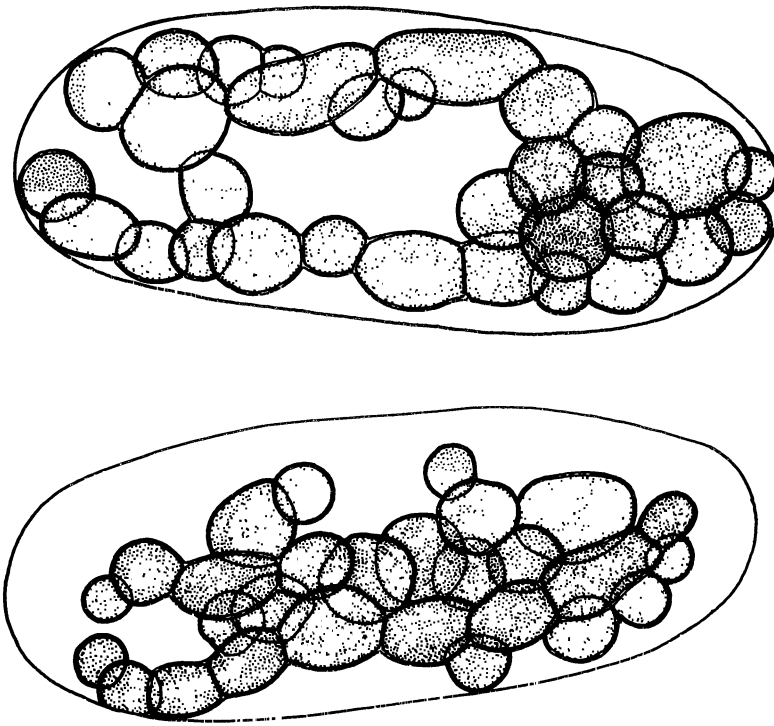

Fig. 1. Swollen hyphal elements of *Exophiala pisciphila* within eggs of *Heterodera glycines* (x 1250).

sumed egg (Fig. 1). It has been repeatedly isolated from such eggs during the course of this investigation. Of the 100 cysts examined in detail nine contained eggs clearly invaded by this fungus.

F. oxysporum appears to be as aggressive a parasite of *H. glycines* eggs as it is those of *H. schachtti* as documented by Nigh (8) and Nigh, Thomason and Van Gundy (9).

Tribe (10,11) reported the infrequent occurrence of *E. pisciphila* in *H. schachtti* eggs in England and Germany and considered it a minor pathogen. In Alabama it appears to be more frequently encountered and it is certainly very effective and complete in its parasitism once it enters the egg. This fungus was originally discovered in Alabama causing systemic mycosis in catfish (3). It has also been isolated in this state from a diseased swim bladder of red snapper (Morgan-Jones, unpublished).

The overall percentage of diseased cysts found in the population examined is somewhat above average compared with most previously published results.

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