MYCOFLORA OF YOUNG CYSTS OF *HETERODERA GLYCINES* IN NORTH CAROLINA SOILS

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ABSTRACT

Gintis, B. Ownley, G. Morgan-Jones and R. Rodríguez-Kábana. 1982. Mycoflora of young cysts of *Heterodera glycines* in North Carolina soils. Nematropica 12:295-303.

Sixteen fungi were found colonizing young cream-colored cysts of *Heterodera glycines* Ichinohe on soybean roots in North Carolina soils. Five, namely *Exophiala pisciphila* McGinnis and Ajello, *Fusarium oxysporum* Schlecht., *Fusarium solani* (Mart.) Sacc., *Neocosmospora vasinfecta* Smith, and *Phoma leveillei* Boerema and Bollen, were prevalent in all soil samples surveyed. Several other species of *Phoma* Sacc., were also implicated as early invaders of cysts. Upwards of two thirds of the cysts examined were found to be invaded and many showed signs of disease, bearing discolored, aborted or physiologically disordered eggs. The significance of early colonization is discussed.

Additional key words: cyst-nematode pathology, biocontrol agents, soil fungi.

RESUMEN

Gintis, B. Ownley, G. Morgan-Jones y R. Rodríguez-Kábana. 1982. La micoflora de quistes inmaduros de *Heterodera glycines* en suelos de Carolina del Norte. Nematrópica 12:295-303.

Se encontraron 16 especies fungosas colonizando quistes inmaduros de color crema de *Heterodera glycines* Ichinohe en raíces de soya en suelos provenientes de Carolina del Norte. Cinco de las especies, a saber, *Exophiala pisciphila* McGinnis y Ajello, *Fusarium oxysporum* Schlecht., *F. solani* (Mart.) Sacc., *Neocosmospora vasinfecta* Smith, y *Phoma leveillei* Boerema y Bollen, se hallaron en todas las muestras de suelos examinadas. Varias otras especies de *Phoma* Sacc., también se encontraron involucradas como invasoras iniciales de los quistes. Más de dos tercios de los quistes examinados se hallaron invadidos y muchos mostraban síntomas de enfermedad, conteniendo huevos descoloridos, abortados, o fisiologicamente alterados. El trabajo también contiene una disertación sobre la importancia de la colonización rápida de los quistes por los hongos.

Palabras claves adicionales: patología de nematodos enquistadores, control biológico, hongos del suelo.

INTRODUCTION

In order to fully understand the ecological history of fungi found to be associated with the cysts of *Heterodera glycines* Ichinohe in soybean field soils it is necessary to determine the time and place where colonization is initiated. In previous surveys of fungal associations with cysts conducted in our laboratory (8,9), as well as those conducted elsewhere on other species of *Heterodera* Schmidt and on *Globodera rostochiensis* (Wollenw.) Stone (7,3,12,13,16,17,1,18,11), cysts were extracted, usually by flotation techniques, from soil samples and examined by various means for the presence of fungi. In each case cysts had existed in soils for extended periods of time and no account could be given of the inoculum source of associated fungi nor of the time of invasion relative to preceeding stages of cyst development and maturation. These surveys revealed the existence of a restricted mycoflora of opportunistic fungi capable of invading cysts and, in some cases, effectively functioning as egg parasites causing destruction of larvae.

Very little is known of how soon mature females are invaded by fungi demonstrated to be regularly associated with tanned cysts in soil, including those implicated as successful egg parasites. It is not yet clear how these fungi, some of which are unspecialized soil saprophytes, enter the cysts and at what stage in their development. The manner in which some of these fungi deleteriously affect cysts and eggs is also largely unknown. As each maturing female increases in obesity and ruptures the root cortex it is exposed to the rhizosphere (Fig. 1), as are its vulval cone and anal aperture, while the elongated neck region remains embeded in root tissue. At this stage it is clearly vulnerable to invasion.

Three specialized obligate parasites of females, *Catenaria auxiliaris* (Kühn) Tribe, *Nematophthora gynophila* Kerry and Crump and an unnamed lagenidiaceous fungus (4,5) are known to be capable of invasion within a few days of exposure on the root surface (4,5,14). The first is thought to infect by means of zoospores entering through natural openings or through disrupted cuticle while *N. gynophila* infection, although not observed, probably takes place through the cuticle (6). All three fungi cause similar symptoms leading to the ultimate demise of the female. These fungi are, to date, the only ones known to regularly penetrate females on the root surfaces before they become fully converted into mature cysts.

Extension of a survey of diseased cysts extracted from soil to diseased females and young cysts of *Heterodera schachtii* Schmidt, taken from roots (15), revealed the presence of *Cylindrocarpon destructans* (Zinssmeister) Scholten and *Verticillium chlamydosporium* Goddard, in addition to *Catenaria auxiliaris* and undetermined filamentous fungi. Both *C. destructans* and *V. chlamydosporium* had been implicated as egg parasites within cysts extracted from soil. Also documented was the fact that even within young cysts lysed, shrivelled or decayed eggs occur. This indicated early incidence of disease. At least two phases of cyst-nematode pathology were considered to

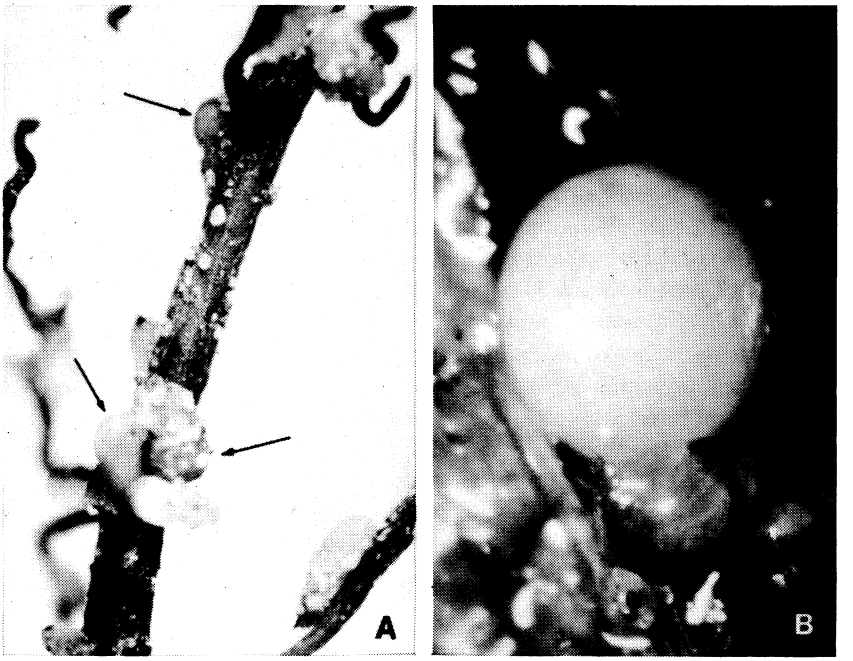

Figure 1. Young cysts of *Heterodera glycines* (indicated by arrows in photograph A) exposed on root surface.

exist. Firstly, females and young cysts on the root surface become diseased, followed by continued degeneration of cysts released into the soil and persisting for some time. Information about the initial phase and relative disease-inducing capacities of various fungi is meager.

At the time of its exposure on the root surface the fourth stage female is white and narrowly flask-shaped, but is soon assumes a swollen, lemon-shaped configuration and becomes cream to pale yellow in color (Fig. 1), at which stage it qualifies for definition as a "young cyst". Although the distinction between a "mature female" and "young cyst" is not sharp, with the externally discernible changes there is usually concurrent degeneration of the internal organs, and eggs containing larvae come to occupy much of the space within the cuticle.

In the present paper an investigation of fungal colonization of young, cream-colored cysts of *Heterodera glycines* while still attached to soybean roots is reported.

MATERIALS AND METHODS

Soil samples from four locations in the coastal plain area of North Carol-

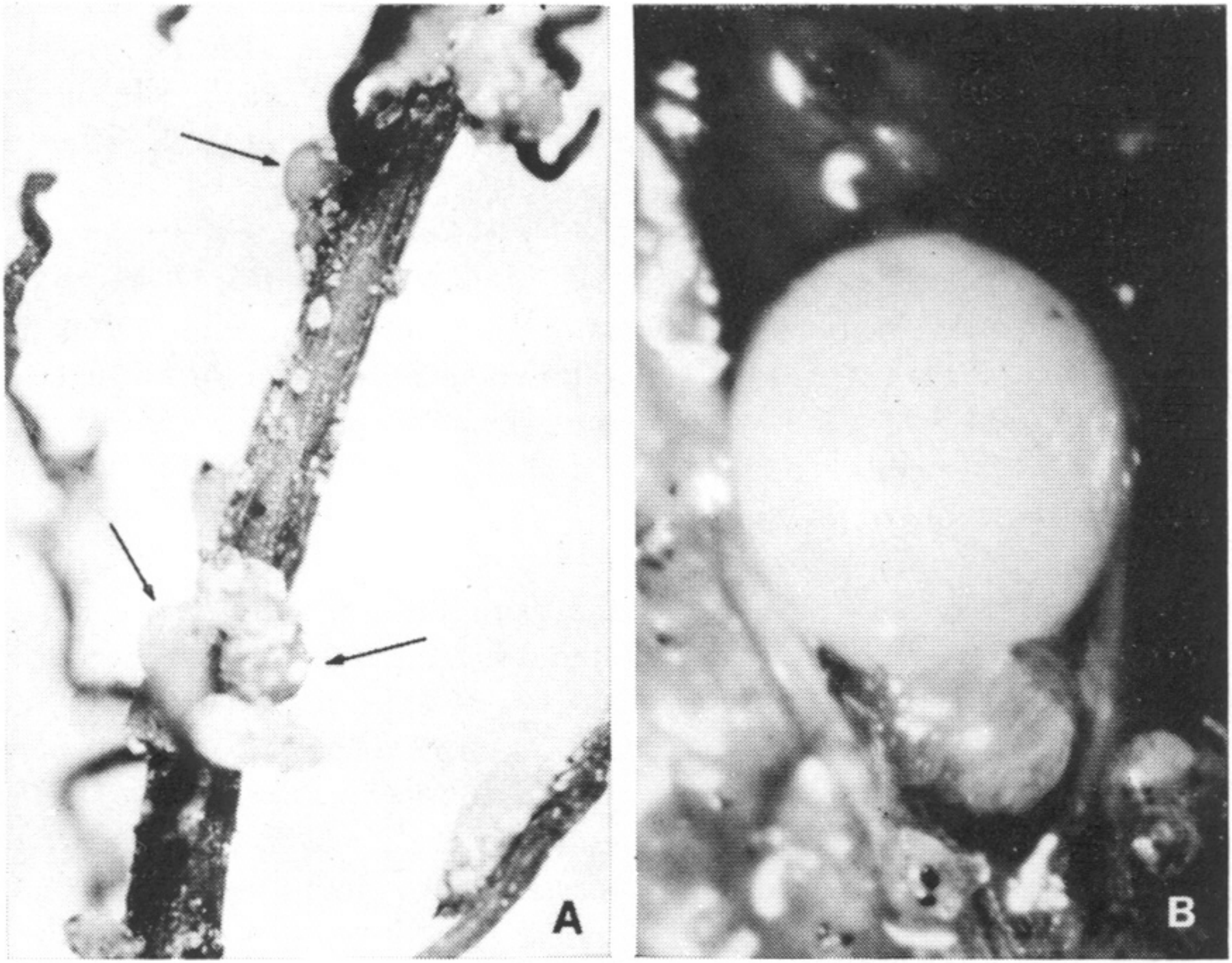

Figure 1. Young cysts of *Heterodera glycines* (indicated by arrows in photograph A) exposed on root surface.

ina, derived from *Heterodera glycines* infected soybean fields, were obtained from Dr. Donald P. Schmitt. The fields had been in soybeans during 1980 and 1981. The geographical sources were as follows: Clayton, Johnston County; Hertford, Perquimans County; Clinton, Sampson County; Goldsboro, Wayne County.

The soil samples were brought into the greenhouse, potted, and planted with Ransom soybeans (*Glycine max* Merr.). Following a five week growth period soybean plants were carefully removed, with the roots intact, from the pots, gently washed in sterile distilled water and examined for the presence of young cysts. Attached cream-colored young cysts were individually detached from the roots by means of a sharp-pointed fine tissue forceps and transferred to Petri dishes containing sterile water. 150 cysts from each soil sample (except the Wayne County sample where only 35 cysts were recovered as a result of a low infestation level), selected at random, were removed in turn to a Nalgene® (Fisher) membrane filter unit and surface sterilized in the manner described previously (9). Individual cysts were removed and plated onto potato-dextrose agar (PDA) with added streptomycin (100μg/ml) in Petri dishes. Plates were incubated for four days at 25 C and examined for the presence of fungal colonies. Cysts producing fungal colonies were removed aseptically and placed in a drop of lactophenol on a glass slide and dissected with mounted needles to expose and release enclosed eggs. Following addition of a coverslip each cyst was microscopically examined for the presence of fungal biomass and the condition of individual eggs, particularly the presence of endogenous fungal hyphae, was determined.

RESULTS

A more or less consistent mycoflora was found to be associated with cysts from all four soil samples (Table 1). A total of sixteen different fungi were encountered. Of 485 cysts examined, fungi were found to be present in 300 and all but seven of these gave pure cultures of the respective fungi. The level of fungal invasion varied with the soil sample; in the Perquimans and Sampson Counties samples a little over two thirds of the cysts examined bore fungi, in the Johnston County sample only a third. The greatest fungal species diversity was encountered in the Sampson County sample this being also the sample where the level of invasion was highest.

Five fungi, namely *Exophiala pisciphila* McGinnis and Ajello, *Fusarium oxysporum* Schlecht., *Fusarium solani* (Mart.) Sacc., *Neocosmospora vasinfecta* Smith, and *Phoma leveillei* Boerema and Bollen occurred in all four samples. Of these, *P. leveillei* occurred consistently in greatest abundance. Occurrence of the *Fusarium* species varied among the samples, *F. oxysporum* predominating in three and being particularly common in the Sampson County sample, while *F. solani* occurred in a significant number of Perquimans County cysts. Other species of *Phoma* Sacc., were found with some

frequency in some, but not all cyst samples. *P. macrostoma* Mont., was recorded in three samples, *P. eupyrena* Sacc., in two, and *P. medicaginis* Malbr. and Roum., var. *pinodella* L.K. Jones in one. Another *Phoma, P. pomorum* Thüm., was found only once, as were several other incidental fungi.

Cysts from which cultures of *Exophiala pisciphila* were derived were in part discolored, brown patches being evident. In each case, upon dissection, cysts were found to be occupied by brown fungal hyphae and many individual eggs had assumed a yellow brown coloration and had been permeated by hyphae. Cysts from which *Phoma leveillei* and *P. macrostoma* were isolated showed black patches and were densely filled with mycelium; some eggs appeared darkish brown and, where *P. macrostoma* was present, frequently reddish brown. Cysts invaded by *Fusarium* species contained discolored, yellowish to pale brown eggs and, in the case of *F. solani*, a number of eggs were found where the wall was clearly pitted and there was evident penetration of the egg membranes by fungal hyphae.

DISCUSSION

This survey has shown that a relatively high number of young cysts can become invaded by fungi soon after their exposure to the soil rhizosphere as a result of the disruption of the root cortex. Detected differences in level of fungal involvement are, presumably, dependent upon availability of inoculum as well as various environmental factors affecting fungal growth and physiological condition of the cysts. The overlap in the components of the participating mycoflora in the survey samples, and its restricted nature in terms of number of species, again indicates a measure of specialization. As has been stated by us elsewhere (9), considering the presence in soil of hundreds of fungal species, the regular association of but a small number of taxa with cysts is certainly significant.

Exophiala pisciphila, as well as species of *Fusarium,* are consistent elements in many surveys in both Europe and North America. Species of *Phoma,* likewise, are encountered with regularity in the southeastern United States. In this regard it is interesting to note that *Phoma exigua* Desm. [as *Phoma tuberosa* Melhus] was frequently encountered in cysts of *Globodera rostochiensis* of Dutch origin (16). *Humicola grisea* Traaen was also isolated from Dutch and Danish cysts.

Some doubt has existed as to the capacity of *Fusarium* species to effectively destroy cyst contents and parasitize eggs. Our observations during the course of this study, however, confirm those of Nigh (10). *F. solani* appears to readily attack and penetrate eggs much as described by Nigh in the case of *F. oxysporum* affecting eggs of *H. schachtii* in California.

The status of some fungi, even those referred to as minor egg pathogens, vis-a-vis pathogenic capacity remains difficult to determine. There is little doubt of the ability of fungi known to be regularly associated with cysts to enter, possibly through one of the natural openings, and thereby come in contact with mucilage containing the breakdown products of internal organ

Table 1. Occurrence of fungi in young cysts of *Heterodera glycines* plated on PDA after surface sterilization.

Johnson County, N.C. (150 cysts - 102 bore no fungi)	
Exophiala pisciphila	6
Fusarium oxysporum	7
Fusarium solani	1
Neocosmospora vasinfecta	2
Phoma eupyrena	10
Phoma leveillei	19
Phoma macrostoma	3
Perquimans County, N.C. (150 cysts - 59 bore no fungi)*	
Exophiala pisciphila	4
Fusarium equiseti	2
Fusarium lateritium	1
Fusarium oxysporum	15
Fusarium solani	26
Neocosmospora vasinfecta	1
Phoma eupyrena	2
Phoma leveillei	40

* 3 cysts produced mixed colonies of *Fusarium solani* and *Neocosmospora vasinfecta*

degeneration, a potential food source. It is even conceivable that the wall of the fourth-stage female or even of a young cyst could be penetrated by fungi having adequate chitinolytic enzymatic capability, more so than would be the case with brown, hardened cyst cuticle. It is also possible that actinomycetes, bacteria or even the known female parasites could exercise chitinolytic activity thus creating an entry pathway for opportunistic fungi present in the rhizosphere. There is indication that no high degree of specialization is necessary as a prerequisite for egg and larval destruction since an appreciable number of fungi can achieve this effect. It seems possible that products of fungal metabolism might well diffuse into eggs and conceivably be toxic to larvae or be inhibitory of hatching. Where there is no discernible degradation of egg shell membranes but where there is clear evidence of physiological egg disorder and larval degeneration [where eggs and/or larvae contain large oil globules, or are shrivelled, coagulated or lysed] such a process is a distinct possiblity. This would explain why, in some of our studies, fungi such as *Phoma* species, isolated from cysts, have lacked chitinolytic capacity (2) [as evidenced by inability to clear chitin agar plates] but yet appear to have a

Table 1. (continued)

Sampson County, N.C. (150 cysts - 28 bore no fungi)	
Acremonium strictum	2
Chaetomium globosum	1
Exophiala pisciphila	7
Fusarium equiseti	1
Fusarium oxysporum	31
Fusarium solani	4
Humicola grisea	1
Neocosmospora vasinfecta	13
Periconia macrospinosa	1
Phoma leveillei	40
Phoma macrostoma	10
Phoma medicaginis var. *pinodella*	8
Scolecobasidium terreum	1
Thielavia terricola	2
Wayne County, N.C. (35 cysts - 2 bore no fungi)*	
Exophiala pisciphila	2
Fusarium solani	1
Fusarium oxysporum	9
Neocosmospora vasinfecta	7
Phoma leveillei	14
Phoma pomorum	1

4 cysts produced mixed colonies of *Exophiala pisciphila* and *Neocosmospors vasinfecta* (one), *Neocosmospora vasinfecta* and *Phoma leveillei* (three).

deleterious effect on eggs. Once an egg is physiologically disordered it becomes predisposed to further decay and fungal entry.

Demonstration that at least a number of fungi are capable of invading freshly exposed young cysts at about the same time as the three known obligate parasites of females or shortly thereafter [these are not known in early, unexposed stages of fourth-stage female development (6)] raises the question of competitive advantage. There are clearly a number of fungi, of various pathogenic capacities, both obligate and facultative, competing for occupation of the same favorable ecological niche and their success or failure in any given circumstance will depend on inoculum potential, adaptability, ability to persist in the absence of the host, synchronization with stages of host development, and antagonisms among participants. Numbers of obligate

parasites, which are totally destructive of the female, may be dictated not only by environmental factors such as availability of water for zoospore dissemination but also by competition from less specialized potential occupiers of the same niche.

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