# FUNGI ASSOCIATED WITH CYSTS OF POTATO CYST NEMATODES IN PERÚ

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ABSTRACT

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A survey of fungi associated with the potato cyst nematodes *Globodera pallida* (Stone) Behrens and *Globodera rostochiensis* (Wollenw.) Stone in potato (*Solanum tuberosum* L.) growing areas in the vicinity of Arequipa, Perú, revealed the presence of an extensive mycoflora colonizing cysts. Just over 62% of 800 cysts examined bore fungi. *Cylindrocarpon destructans* (Zinssm.) Scholten, *C. gracile* Bugn., *Fusarium* spp., *Gliocladium roseum* Bainier, and *Ulocladium atrum* Preuss were the most frequent species encountered.

*Additional key words: fungal ecology, biological control, pest management, Heteroderidae, microbial interactions.*

RESUMEN

Morgan-Jones, G., R. Rodriguez-Kabana y P. Jatala. 1985. Hongos asociados con los quistes del nematodo del quiste de la papa en el Perú. Nematrópica 16:21-31.

Se efectuó un reconocimiento de los hongos asociados con los quistes de los nematodos *Globodera pallida* (Stone) Behrens y *Globodera rostochiensis* (Wollenw.) Stone en campos de papa (*Solanum tuberosum* L.) en las cercanias de Arequipa, Perú. El estudio reveló la presencia de una abundante micoflora colonizando los quistes de los nematodos. El 62% de los quistes examinados no tenían hongos. *Cylindrocarpon destructans* (Zinssm.) Scholten, *C. gracile* Bugn., *Fusarium* spp., *Gliocladium roseum* Bainier y *Ulocladium atrum* Preuss fueron las especies fungosas aisladas de los quistes con más frecuencia.

*Palabras claves adicionales: ecología de hongos, combate biológico, manejo de plagas, Heteroderidae, interaciones microbianas.*

## INTRODUCTION

Although a considerable number of investigations have been conducted on the association between opportunistic soil fungi and cysts of species of *Heterodera* Schmidt, especially *H. avenae* Wollenw., *H. glycines* Ichinohe, and *H. schachtii* Schmidt (2,3,5,6,7,11,13,15,16,17,23), comparatively little is known about the activity of fungi vis-à-vis cysts of species of *Globodera* Mulvey and Stone (24). The intensive studies of fungal colonization of cysts of *Heterodera* accomplished in recent years (5,6,16) have not been matched, to date, by similar studies on *Globodera.*

As a result of surveys on *Heterodera,* it is now well established that a number of soil-inhabiting fungi, belonging mainly to the class Hyphomycetes, consistently colonize cysts of that genus and, in many instances, are thought to exercise a controlling influence on nematode populations through destruction of eggs and developing larvae by various means (14). The number of fungi implicated in this activity is rather small compared to the total known soil mycoflora. Regular occurrence of particular taxa in this ecological niche, irrespective of geographical location, is considered to be more than coincidental and had led to the belief that possession of specific physiological capabilities, such as ability to degrade chitin or to biosynthesize mycotoxins, may be prerequisites that enable some fungi to exploit sedentary nematode reproductive structures as a food source (6,14). Species found to be commonly associated with cysts of *Heterodera* belong to such genera as *Exophiala* Carmichael, *Fusarium* Link, *Gliocladium* Corda, *Paecilomyces* Bainer, *Phoma* Saccardo, and *Verticillium* Nees (3,5,6,11,13,15,17,19).

In the published reports of fungi occurring in cysts of *Globodera,* low levels of colonization were encountered for which no convincing explanation has been advanced. This is surprising given the fact that *Globodera* and *Heterodera* are closely related, constituting as they do the family *Heteroderidae* Skarbilovich.

Cysts of *Globodera rostochiensis* (Wollenw.) Stone, the potato cyst nematode, originating from potato fields in England (including the Channel Island of Jersey), the Netherlands, and Perú, were examined for the presence of fungi by van der Laan (25,26). In the first paper, *Phoma exigua* Desm. [as *Phoma tuberosa* Melhus, Rosenbaum, and Schultz] was reported to occur with regularity in cysts of Dutch origin. All told, 8 fungal species were found in the various samples. These were, in addition to *P. exigua: Penicillium dangeardii* Pitt [as *Penicillium vermiculatum* Dang.] and *Pseudoeurotium ovale* Stolk from the English material; *Colletotrichum coccodes* (Wallroth) Hughes [as *Colletotrichum atramentarium* (Berk. and Br.) Tabenhaus] and *Humicola grisea* Traaen [as *Monotospora daleae* Mason] from Dutch material; and *Exophiala jeanselmei* (Langer.) McGinnis and Padhye var. *heteromorpha* (Nannf.) de Hoog [as *Margarinomyces heteromorpha* Mangenot], an undescribed species of *Scopulariopsis* Bainier, and *Xanthothecium peruvianum* (Hansen) Arx and Samson [as *Anixiopsis stercoraria* Hansen] from the Peruvian material.

*Humicola grisea* was also encountered in a sample of *G. rostochiensis* collected in Denmark (26).

The presence of *Colletotrichum coccodes* in cysts was typified by small black spots, but van der Laan indicated that he did not consider this to be a likely parasite (26). The fungi isolated from cysts were tested for their pathogenicity to cysts, but with uneven results. In the survey work, intact whole cysts were surface sterilized with 70% alcohol and plated onto cherry and malt agar. No assessment could be made as to whether or not individual taxa acted as parasites and colonized eggs or were present as saprophytes. The presence of fungal biomass within a cyst does, however, as we have pointed out elsewhere (14), render a possibility for damage to eggs by toxic metabolites. In connection with the studies cited above, it is interesting to note that *Pseudoeurotium ovale* and *Humicola grisea* have more recently been reported to occur in cysts of *Heterodera glycines,* as have other species of *Exophiala* and *Phoma* (6).

Populations of cysts of *Globodera rostochiensis,* as well as some of *Heterodera avenae* and *H. schachtii,* were examined for disease and for the presence of naturally occurring antagonists by Willcox and Tribe (27). A study of over 5,000 cysts of *G. rostochiensis* from 20 different sources (19 from potato growing areas of England and one from Bolivia) revealed a very low level of disease, and no cysts containing an appreciable number of dead eggs were encountered. This corroborated unpublished observations made previously in England by F. G. W. Jones which indicated little disease in *G. rostochiensis* cysts. This was considered to be somewhat at variance with the results obtained by van der Laan (26). Low disease incidence in *G. rostochiensis,* an introduced nematode in England, thus contrasted with a much higher level in species of *Heterodera,* such as *H. avenae* and *H. schachtii,* native to that country. That an introduced nematode is necessarily less prone to fungal invasion and disease that an indigenous one is not, however, always the case as our own study (16) in Columbia has indicated. In that country newly introduced *Heterodera glycines* was found to be readily colonized by fungi. No individual fungus was named as being associated with *G. rostochiensis* in the study by Willcox and Tribe (27).

Two zoosporic fungi, which have been described as obligate parasites of *Heterodera* females, namely *Catenaria auxilaris* (Kuhn) Tribe and *Nematophthora gynophila* Kerry and Crump, have not been found on *G. rostochiensis* but the latter has been encountered in laboratory populations of *G. pallida* (10). Kerry and Crump (12) showed *N. gynophila* to be pathogenic not only to *H. avenae,* which is frequently found to be parasitized by it in wheat fields in England, but to six other species of *Heterodera.* This pathogenicity did not, however, extend to *G. rostochiensis.*

The capacity of three fungi known to be pathogenic to insects, *Beauvaria bassiana* (Bals.) Vuill., *Metarrhizium anisopliae* (Metschn.) Sorok., and *Paecilomyces farinosus* (Holm) A. H. S. Brown and G. Smith, to colonize and destroy cysts of *G. rostochiensis* has been investigated (18) but with negative results. All three have been isolated from soil and *M. anisopliae* has been found in cysts of both *G. rostochiensis* and *H. schachtii* (4). Rademacher and Schmidt (19) considered *M. anisopliae* [as *Isaria destructor* Metschn.] to be an important parasite of cysts and eggs of *H. schachtii.*

Up to the mid-1970's there was general agreement that *G. rostochiensis* had no effective fungal antagonists in agricultural soils in temperate regions (22). The observations of Kerry and Crump (12) supported this contention. In 1978, however, Goswami and Rumpenhorst (8) reported an unidentified fungus, together with *Fusarium oxysporum* Schlecht. and *F. solani* (Mart.) Sacc., associated with cysts of *G. rostochiensis* in West Germany. Judging from the available description and its morphology within eggs as seen in the published illustrations, the unidentified fungus, which was not isolated in pure culture, probably belonged to the *Exophiala*-complex and was possibly *E. jeanselmei, E. mansonii* (Castell.) de Hoog, or *E. pisciphila* McGinnis and Ajello. A similar level of cyst colonization to that reported by van der Laan (26) was detected. The *Fusarium* isolates could not, in inoculation experiments, be shown to be capable of parasitizing eggs and it was concluded that the unnamed fungus was largely responsible for the destruction of larvae within eggs. The fungus was reported to infect various pathotypes of both *G. rostochiensis* and *G. pallida.*

Tribe (24) discussed the rarity of parasitism of *G. rostochiensis* and broached the possible reasons for this. Two possible explanations were offered. These were the absence of a vulval cone and the fact that the vulval aperture in the cyst wall is small, together with the presence of a layer of cuticle in the female of *G. rostochiensis,* additional to those possessed by *Heterodera* as demonstrated by Shepherd, Clarke, and Dart (20). A reduced aperture and the extra layer of cuticle were thought to offer effective barriers to penetration and ultimate colonization of the cyst interior, including eggs.

We have had opportunity to collect and study populations of cysts of *Globodera pallida* and *G. rostochiensis* from a number of potato-growing sites in the vicinity of Arequipa in southern Peru and to examine them for the presence of fungi. This is of especial interest since the potato plant and the nematodes are native to Peru and potato cultivation in the area goes back to pre-Columbian times. Damage to some potato cultivars from nematode infestation is sometimes severe in the area but, overall, there appears to be some measure of natural suppression of nematode populations in place at least in some soils. It is of interest to determine if an associated mycoflora is implicated in regulating nematode population dynamics in an area where the nematodes, host plant, and indigenous soil fungi have co-existed for a long period of time.

## MATERIALS AND METHODS

Soil samples were collected from 8 separate potato fields at locations within approximately a 15 km radius of Arequipa during April 1984, and air dried. Cysts were extracted seven days after samples were collected, and treated in the manner described previously (15). Following surface sterilization, cysts were plated onto potato dextrose agar (PDA) with added streptomycin (100 μg/ml) in Petri dishes. Cysts were examined for the presence of fungal colonies after incubation for five days at 25C. Cysts giving rise to fungal colonies were aseptically removed from the Petri dishes and examined microscopically, following crushing with a dissecting needle in a drop of lactophenol. Cysts not bearing fungi were examined similarly. Fungi emerging from cysts were subcultured onto separate plates and identified. One hundred cysts from each locality were combined to make a total sample of 800 [this included both *G. pallida* and *G. rostochiensis,* with the latter predominating].

## RESULTS

In terms of number of taxa, a rather sizable mycoflora was found associated with the cysts. All told, some 28 species (Table 1) were recognized, all belonging to the class Hyphomycetes. The majority, however, were present infrequently. Of 800 cysts examined, 302 [37.75%] yielded fungal colonies. In all cases, each colonized cyst appeared to be occupied by but a single fungus.

The most frequently occurring species was *Cylindrocarpon destructans* (Zinssm.) Scholten [*Cylindrocarpon radicicola* Wollenw.], the anamorphic state of the ascomycete *Nectria radicicola* Gerlach and Nilsson. Cysts giving rise to colonies of this fungus were seen to bear eggs containing abundant pale-brown hyphae. Some swollen, chlamydospore-like cells were also occasionally evident within the eggs. Many eggs were almost totally engorged by such hyphae, which obliterated the larvae. In many instances, there was also evidence that the egg shell had been degraded, giving the eggs a buckled appearance. The fungus was apparently able to penetrate egg shells with little difficulty. Two other species of *Cylindrocarpon* Wollenw., namely *C. didymum* (Hartig) Wollenw., and *C. gracile* Bugn., were also found in the samples, but in lower numbers. In several cysts colonized by *C. gracile,* pale brown chlamydospores were observed

Table 1. Fungal colonization of cysts of potato cyst nematodes, Arequipa, Perú [800 cysts examined; 498 bore no fungi = 62.25%].

| Species | No. colonized cysts | % frequency[z] |
|---|---|---|
| *Aspergillus sydowii* | 1 | 0.33 |
| *Cladosporium cladosporioides* | 8 | 2.6 |
| *Cylindrocarpon destructans* | 67 | 22.2 |
| *Cylindrocarpon didymum* | 3 | 0.99 |
| *Cylindrocarpon gracile* | 11 | 3.6 |
| *Drechslera australiensis* | 3 | 0.99 |
| *Exophiala pisciphila* | 7 | 2.2 |
| *Fusarium moniliforme* | 10 | 3.3 |
| *Fusarium oxysporum* | 27 | 8.9 |
| *Fusarium semitectum* | 46 | 15.2 |
| *Fusarium solani* | 32 | 10.6 |
| *Gliocladium catenulatum* | 4 | 1.3 |
| *Gliocladium roseum* | 16 | 5.3 |
| *Humicola grisea* | 5 | 1.7 |
| *Paecilomyces lilacinus* | 6 | 1.98 |
| *Paecilomyces variotii* | 2 | 0.7 |
| *Penicillium chrysogenum* | 2 | 0.7 |
| *Penicillium fellutatum* | 6 | 1.98 |
| *Penicillium restrictum* | 3 | 0.99 |
| *Penicillium rubrum* | 3 | 0.99 |
| *Phoma americana* | 4 | 1.3 |
| *Ramichloridium schulzeri* | 3 | 0.99 |
| *Scolecobasidium tschawytschae* | 4 | 1.3 |
| *Stachybotrys chartarum* | 2 | 0.7 |
| *Trichocladium asperum* | 8 | 2.6 |
| *Trichoderma harzianum* | 4 | 1.3 |
| *Trichoderma longibrachiatum* | 2 | 0.7 |
| *Ulocladium atrum* | 13 | 4.3 |

[z]% frequency of occurrence in terms of the total number of cysts colonized (302).

within eggs but in the case of *C. didymum* no apparent hyphal penetration of eggs occurred.

Species of *Fusarium,* a genus closely related to *Cylindrocarpon,* accounted for another sizable portion [38%] of the cysts colonized, *F. semitectum* Berk. and Rav., and *F. solani* (Mart.) Sacc., being the commonest. *Gliocladium roseum* Bainier and *Ulocladium atrum* Preuss were two other fungi found with some frequency. Examination of the contents of cysts invaded by these fungi did not reveal any consistent pattern of egg parasitism as evidenced by the presence of endogenous fungal hyphae. Shrivelled, partly lysed and coagulated eggs, some containing fungal hyphae, were occasionally seen among apparently healthy ones.

Several cysts bearing *Exophiala pisciphila* had eggs containing pale brown hyphae, but among the other less abundant taxa, most eggs in the cysts colonized by them appeared unaffected. Cysts which did not bear fungi had mostly normal-looking eggs but some showed a condition similar to that described by Tribe (22) as "oily degeneration."

## DISCUSSION

This survey indicates that cysts of *Globodera* are invaded by opportunistic soil fungi to much the same degree as those of *Heterodera,* at least in the area investigated by us. It is interesting to note that the level of colonization was of the same order of magnitude as that encountered in previous surveys of cysts of *H. glycines* conducted in our laboratory (14,16). It is possible that in geographical areas such as northern Europe, where *Globodera* is not native, fungi fail to colonize cysts consistently and in significant numbers. As we have alluded to in the introduction, however, it does not always follow that where a nematode is newly introduced colonizaton levels will be low, since our experience with *H. glycines* in Colombia indicates otherwise (16).

Whether or not the attendant mycoflora in the fields surveyed in Perú exercises a significant controlling influence on golden and potato cyst nematode populations is not known, but it seems likely that *Cylindrocarpon destructans* may be contributing towards this because of its abundance and to damage eggs as observed here. Goffart (7) found it repeatedly as a parasite of *H. avenae* in Schleswig-Holstein, Germany, in the early 1930's. There are also a number of other records of its occurrence in association with cysts and eggs. Booth (1) reported an isolate from eggs of *G. rostochiensis* from Scotland. It has been found, at low frequency, in cysts of *H. schachtii* in England (2) where it was noted to be capable of penetrating eggs. Infected eggs were described as being reddish-brown in color due to the content of endogenous hyphae. It had a similar appearance in our Perú material, as indicated above. Kerry (11) and Kerry and Crump (12) found it, again in rather low numbers, in females and eggs of *H. avenae* in England. Although it occurred in soils where the nematode failed to multiply, it was not considered to be of importance because of its scarcity. As a result of studies of a wide diversity of *H. schachtii* cyst samples from England and Germany, Tribe (23) noted *C. destructans* to be reasonably constant in occurrence though at low frequency. Van der Laan (26), although he did not isolate *C. destructans* from cysts of *G. rostochiensis,* tested the ability of an isolate of this species, of unspecified origin, to colonize cysts on agar media and to penetrate eggs. The results were negative. Hams and Wilkin (9) used *C. destructans* in trials against both *G. rostochiensis* and *H. avenae* and their results were also negative. *Cylindrocarpon destructans* is predominantly found in soil colonizing senescing plant roots (21). Some isolates are known to be pathogenic to plants (1). It seems possible that, in Perú, a biotype of the species has been selected for that has the capacity to parasitize *Globodera* eggs.

The occurrence of the other species of *Cylindrocarpon, C. didymum* and *C. gracile,* is also of interest and reinforces a belief that members of this genus occur in association with nematode cysts and eggs consistently. Undetermined species of *Cylindrocarpon* have been reported from eggs of *H. schachtii* in California (17) and *H. avenae* in Sweden (3). *Cylindrocarpon tonkinense* Bugn., was reported by Gintis *et al.* (6) from cysts of *H. glycines* in Alabama and exhibited egg parasitism.

Species of *Fusarium* have frequently been encountered previously as colonizers of nematode cysts (6,7,13,15,17). Microscopic examination of eggs from cysts where *Fusarium* is present gives conflicting results as to the capacity of isolates to penetrate their shell. Nigh *et al.* (17) documented the ability of isolates of *F. oxysporum* from *H. schachtii* to parasitize eggs of that nematode and we have made similar observations on strains of *F. solani* isolated from *H. glycines.* This does not, however, mean that all isolates of these species achieve egg shall penetration. As the results of the present study show, cysts invaded by *Fusarium* spp. sometimes contain eggs that appear unaffected by the fungus. The condition of the eggs vis-à-vis maturation when fungal hyphae come in contact with them will, in part, determine their vulnerability. Those at an early stage of embryonic development, when their shell is not fully differentiated, are more easily penetrated than are mature eggs containing larvae (14). Empirical evidence has accumulated that indicates varying capacities to colonize eggs within fungal species. There undoubtedly exist differing biotypes with divergent physiological capacities. Some of these are able to colonize particular ecological niches such as nematode cysts and eggs while others cannot. It seems reasonable to assume that differing selection pressures favor the dominance of one or other biotype in any given microhabitat. Differences in biotypes may also account for the different degree of colonization in Perú and Europe. Where a nematode is not native a time lapse may well precede the emergence of fungal biotypes adapted to the new host.

A number of other fungi occurring in Perú cysts, albeit in low numbers, have also been consistently recorded previously from nematode cysts and eggs. These include *Exophiala pisciphila* McGinnis and Ajello, *Gliocladium roseum,* and *Phoma americana* Morgan-Jones and White. As more surveys are conducted, in widely different geographical areas and irrespective of cyst nematode host, a consistent pattern of fungal occurrence is emerging although predominating taxa may vary.

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