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# Fungal Parasites of the Potato Cyst Nematode *Globodera rostochiensis*: Isolation and Reinfection

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*Abstract:* Fungal parasitism of eggs of the potato cyst nematode *Globodera rostochiensis* was < 1, 3, and 17% at three sites in Sweden. The fungi isolated most frequently from infected eggs were a *Septocylindrium*-like fungus (19%), *Exophiala* spp. (17%), and *Cylindrocarpon* spp. (13%). *Verticillium suchlasporium* was isolated from infected eggs at a low frequency (4%). In laboratory experiments *V. suchlasporium* infected 93% of the eggs within cysts after 10 days on dilute corn meal agar. This species showed chitinase and protease activity. Infection of eggs by the *Septocylindrium*-like fungus was moderate, whereas *Cylindrocarpon destructans* and *Cladosporium cladosporoides* did not infect eggs. No chitinase activity was found in these fungi, but protease activity was recorded in all. Growth of the fungi in cysts did not influence the number of physiologically disordered eggs.

*Key words:* egg parasite, infection, chitinase, physiological disorder, *Globodera rostochiensis, Verticillium suchlasporium, Cylindrocarpon destructans.*

Eggs of cyst and root-knot nematodes are parasitized frequently by fungi in the soil. The mycoflora of *Heterodera* spp. and *Meloidogyne* spp. has been examined many times, but few studies have dealt with the mycoflora of *Globodera* spp. (14). Willcox and Tribe (16) examined a large number of cysts from several localities in Great Britain and found a very low level of disease. One explanation was that the potato cyst nematode (PCN), *Globodera rostochiensis* Wollenw., was introduced to Great Britain and thus had escaped its indigenous antagonists. Several fungi were associated with PCN cysts in Peru, where the potato and these cysts are native (13), and in other countries (3,9,11). In these studies, the associated fungi may have grown parasitically on viable eggs or saprophytically on dead eggs as well as on nutrients present from decomposition of the internal organs of the female following egg differentiation.

The nematode egg shell is rigid and contains a chitin–protein complex (17). Penetration of the egg shell by parasitic fungi probably involves chitinases and proteases (2,5,8).

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The purpose of this study was to examine the extent of fungal parasitism of PCN at three localities in Sweden and to test the parasitic ability of isolated fungi in vitro.

## MATERIALS AND METHODS

The three sites chosen for this study, one in northern (Skellefteå) and two in southern Sweden (Mårtorp and Sövde), are part of an investigation of the population dynamics of *G. rostochiensis* under nonhost conditions (1). A composite soil sample was taken at each site after harvest and stored at 2 C until used.

To estimate fungal parasitism of nematode eggs, cysts were extracted from one subsample (150 g soil) per composite sample with a modified Seinhorst cyst elutriator. About 150–200 cysts per sample remained after empty and damaged cysts had been discarded. Eggs were released from cysts (6), suspended in soft agar (0.8%), and spread onto 10 malt agar plates supplemented with 50 mg chlortetracycline (CTC) per liter (4). About 30 eggs were spread on each plate. The plates were incubated at room temperature (20 C) for 14 days. The numbers of healthy and infected eggs on all plates were counted with a dissecting microscope. Fungal colonies were identified to genera or species directly on the plates and were subcultured only when their identity was uncertain.

The infection ability of selected fungi was studied in vitro. Potato cyst nematode

cysts were collected from a greenhouse soil, rinsed in sterile water, and put in a streptomycin sulphate solution (150 mg/liter) for 1 hour. The cysts then were rinsed in sterile water and transferred to potato dextrose agar plates supplemented with 50 mg CTC/liter. Cysts free of fungal growth after 1 week were used. The fungi to be tested were grown on diluted (1:10) corn meal agar (1.5% agar); one cyst was placed close to the advancing mycelium. Ten plates were used for each fungus and for the fungus-free control. Plates were incubated at room temperature for 10 days. The cyst content was examined at 400× magnification with interference phase contrast microscopy. Healthy, fungus-infected, and physiologically disordered eggs were counted. Physiologically disordered eggs were those with a disordered content without visible hyphae. They are often described as shrivelled, coagulated, or partly lysed (15).

Chitinase activity of fungal isolates was studied on a solid medium with 0.2% colloidal chitin as the only carbon source, whereas protease activity was studied on a solid skim milk medium (5). Enzyme activity was shown by clearing of the medium.

## Results and Discussion

The level of fungal parasitism of eggs in the field was 17% at the northern site and 0.4 and 3% at the southern sites. A low level of infection, usually not exceeding 5% of the total number of eggs, seems to be a common characteristic of the European PCN populations (13,16). A rapid decline in the nematode population under a non-host crop was found at the northern site (1). The density of viable eggs decreases by 80–90% during the first year after cultivation of susceptible potatoes. At the other sites the decline was normal, about 40%. The higher incidence of egg parasitism at the northern site cannot fully explain the rapid decline, but it is possibly one of the factors involved.

The identity of fungi isolated from eggs was determined only at the northern site because the level of parasitism there was much higher than at the other sites. A *Septocylindrium*-like fungus was isolated from 19% of the infected eggs. This fungus is similar to that described by Gams and Domsch (7) as *Septocylindrium*, but it is no longer regarded as *Septocylindrium* (W. Gams, pers. com.). However, it has not yet been given a new name. *Exophiala* spp., *Cylindrocarpon* spp., *Varicosporium* sp., and *Trichocladium opacum* (Corda) Hughes were isolated from 17, 13, 9, and 8% of the eggs, respectively. *Humicola grisae* and *Cladosporium cladosporoides* were found in 2% of the eggs. *Verticillium suchlasporium* W. Gams & Dackman was isolated from 4% of the eggs. This fungus is a common egg parasite of the cereal cyst nematode, *Heterodera avenae*, in Sweden (6), and *V. chlamydosporium* Goddard, a related species, was the most common egg parasite of *H. avenae* in suppressive soils in England (10). It is therefore noteworthy that *V. suchlasporium* was isolated also from PCN eggs, although at a low frequency.

Four fungi were chosen for the in vitro infection study: *V. suchlasporium* because it is a very common parasite on *Heterodera* spp. and has shown a high capacity for infection on these nematodes (5); *Cylindrocarpon destructans* (Zins.) Scholten, which is another common egg parasite; the *Septocylindrium*-like fungus because it was the fungus most frequently isolated from eggs in this study; and *Cladosporium cladosporoides* (Fres.) de Vries, which was isolated from few eggs and thought to grow mainly saprophytically. The fungi varied significantly in their ability to infect eggs within cysts in vitro. *Verticillium suchlasporium* was highly virulent, infecting over 90% of the eggs after 10 days (Fig. 1A). The *Septocylindrium*-like fungus infected about half of the eggs, whereas the levels of infection caused by *C. destructans* and *C. cladosporoides* were not significantly different from the control.

The incidence of physiologically disordered eggs also was recorded. It has been proposed that this disorder is caused by fungal metabolites or depletion of oxygen by fungi growing in the cyst (12,15). For unknown reasons as many as 40% of the

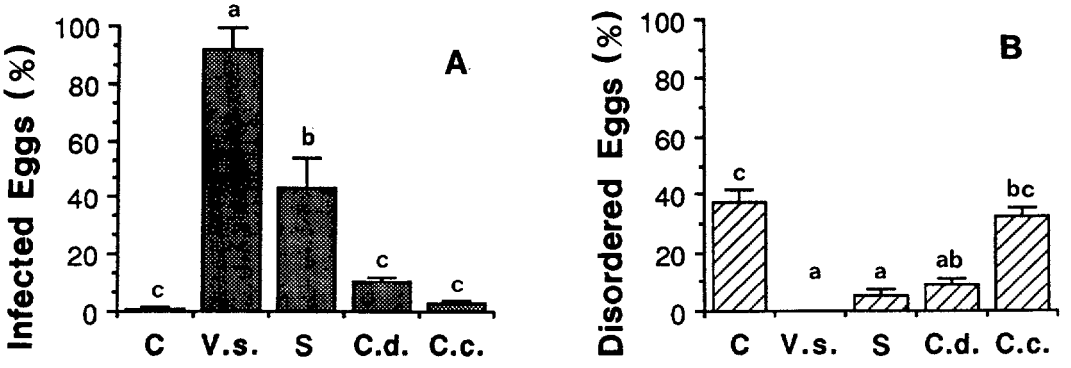


FIG. 1. In vitro infection of eggs within cysts of *Globodera rostochiensis* by different fungi. A) Infected eggs. B) Physiologically disordered eggs, as a percentage of total number of eggs. Error bars indicate S.E.M. (n = 10), C = control, V.s. = *V. suchlasporium*, S = *Septocylindrium*-like fungus, C.d. = *C. destructans*, and C.c. = *C. cladosporoides*. Treatment means followed by different letters are different at the $P = 0.05$ level. Arcsin transformed percentage data were subjected to analysis of variance and the significance of the individual treatment differences tested for using Duncan's multiple-range test.

eggs in control cysts were physiologically disordered (Fig. 1B). Fungi were not found in these cysts, and the degeneration may have been autogenous. The incidence of disordered eggs in cysts exposed to fungi was no higher than that of the control cysts; i.e., none of the isolates tested caused physiological disorder in the eggs. No such eggs were found in cysts exposed to *V. suchlasporium*.

Because of the high incidence of disordered eggs in control cysts, some of the infected eggs may have been dead before infection took place and the process should therefore by regarded as saprophytic rather than parasitic. However, there should be no doubt about the high parasitic ability of *V. suchlasporium*, whereas that of the *Septocylindrium*-like fungus could be questioned.

The infection ability of a fungus probably depends upon the formation of enzyme complexes, e.g., chitinase and protease, which aid in the penetration of the egg shell. Only *V. suchlasporium* showed chitinase activity, but protease activity was observed for all four fungi. These results together with other investigations (2,5) indicate that chitinase may be an important enzyme in the infection process.

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