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# Fungal Parasites of the Cereal Cyst Nematode *Heterodera avenae* in Southern Sweden[1]

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*Abstract:* Fungal parasites of the cereal cyst nematode *Heterodera avenae* were isolated from four sites in southern Sweden. In all, 15 different fungi were isolated from different stages of the nematode life cycle. Among the egg parasites, *Verticillium chlamydosporium* was common in young cysts on roots, whereas an unidentified species of *Verticillium* (*Verticillium* sp. 1) was the dominating species in cysts from soil, especially if the soil had been stored for 8–12 months. *V. chlamydosporium* was frequently isolated from eggs in cysts from soil, when analyzed shortly after sampling. *Verticillium* sp. 1 is distinct from *V. chlamydosporium* because it does not produce dichtyo-chlamydospores in the aerial mycelium and because it grows at 6 C where *V. chlamydosporium* fails to grow. *Paecilomyces lilacinus, Microdochium bolleyi, Cylindrocarpon* sp., and several nonsporulating fungi were also isolated from eggs in cysts from soil. Between 10 and 20% of the eggs in cysts collected in the field were infected with fungi. In a pot test between < 1 and 29%, with a mean of 13%, of females on roots became infected, always by *Nematophthora gynophila.* Resting spores of *N. gynophila* extracted directly from field soil, collected at the four sites, varied from 3 to 49 spores/gram of air dried soil.

*Key words:* egg parasites, female parasites, resting spores, ecology, taxonomy, storage temperature.

Cyst nematodes are serious pests of many cultivated crops around the world. Current means of control are crop rotation and the use of resistant varieties and chemicals. During recent years, biological control has been considered as a method of reducing the numbers of these organisms in soil. Natural regulation of the soil densities of the cereal cyst nematode *Heterodera avenae* occurs in monoculture areas in southern England (8). The most important parasites of cyst nematodes in these areas are the fungi *Nematophthora gynophila* Kerry & Crump and *Verticillium chlamydosporium* Goddard. In Peru, control of the root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood in the field was obtained by applying the fungus *Paecilomyces lilacinus* (Thom) Samson to nematode-infested soil (4).

Cyst nematodes at different stages of their life cycles can be infected by fungi. Females exposed on the root surface are attacked by zoospore-producing fungi, such as *N. gynophila,* a lagenidiaceous fungus, and *Catenaria auxiliaris* (Kuhn) Tribe (5,14). Also, *V. chlamydosporium,* a hyphomycete, has been isolated from females (5). Eliminating the nematode at the young female stage when few if any eggs have been produced is an effective way of limiting cyst nematode populations (5). *V. chlamydosporium* also has been frequently isolated from eggs of *H. avenae* and *H. schachtii* Schmidt (8,15) as well as from females of the root-knot nematode *Meloidogyne arenaria* (Neal) Chitwood (10). In California, *Fusarium oxysporum* Schlecht. and *Acremonium strictum* W. Gams were the main parasites in eggs of *H. schachtii* (11).

In the present study, the occurrence of fungal parasites of *H. avenae* in four different fields in southern Sweden is reported. Parasites of females, eggs from new cysts on roots, and eggs from mature cysts extracted from soil were studied. The number of *N. gynophila* resting spores in the soils was also assessed.

## Materials and Methods

*Soil status and collection of field samples:* Soil was collected from four fields in southern Sweden, at Alnarp (Alnarp 1 and Alnarp 2), Vårgårda, and Våxtorp, where the influence of resistant varieties on the population density of *H. avenae* is being investigated by the Swedish University of Agricultural Sciences. At the time of sampling, the fields at Alnarp and Vårgårda had been grown for several years in a crop rotation of 3 years of susceptible cereals and 1 year with a non-host. At Våxtorp, resistant barley had been grown continuously for 6 years. Soil nematode numbers

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varied widely over the years. The infestation level was high at Alnarp 1, 23 eggs/gram soil, while it was low at the other three sites, approximately 3 eggs/gram soil (S. Andersson, pers. comm.). Soil samples were collected from four replicate plots in each field. Cores were taken to 20 cm deep on a zig-zag pattern over the plots and mixed to give one composite sample from each field. The soil was sampled at Alnarp and Vårgårda in November 1981 and at Växtorp in March 1982 and stored at 2 C until used 8–12 months later. Egg parasites were also studied using soil from Alnarp 1 and 2 and from Alnarp 3, a field adjacent to Alnarp 2, at storage times of 0–5 months.

*Assessment of fungal parasites of females:* Pots, 12-cm-d, were filled with the field soils from each site and planted in late May with three germinated seedlings of susceptible oats. Pots were kept outdoors in a plunge and irrigated frequently to keep the soil moist in order to favor zoospore-producing fungi in the soil (7). One pot containing soil from each site was sampled 7, 8, and 9 weeks after planting. Roots were washed free from soil with a fine jet of water. Samples of the root mass were cut out and examined using a dissecting microscope. All females were picked off the roots and recorded whether infected or healthy. Because this procedure was time consuming, only part of the root mass was examined when females were abundant. Infected females were examined with a light microscope to identify fungal parasites.

*Assessment of fungal parasites of new cysts:* Sampling of females and new cysts on oats in the pots described previously continued through autumn. Samples were taken 13, 15, and 19 weeks after planting. New cysts were picked off the roots. Soft and flaccid cysts were cut open and their eggs examined for hyphae. If eggs in a cyst contained hyphae, all eggs from that cyst were washed three times with sterile water containing 50 ppm chlortetracycline (CTC). Single eggs were then transferred with a capillary pipette to water agar containing 50 ppm CTC. After 24 hours at 20 C, eggs were examined for fungal growth. Infected eggs were transferred to 2% malt extract agar to allow the fungus to grow out for identification.

*Assessment of fungal parasites in eggs from mature cysts from soil:* Fungal parasites in eggs were investigated by the method of Kerry and Crump (6) with slight modifications. Three 250-gram subsamples of moist field soil were extracted for cysts with a modified Seinhorst cyst elutriator. All intact cysts in a sample were collected in a test tube in water, and the eggs were released by carefully crushing the cysts so as not to break the egg shells (12). Cyst debris was separated from eggs by passing the homogenate through a 250-μm-pore nylon sieve and collecting the eggs on a 20-μm-pore nylon sieve with the aid of a vacuum pump. Eggs were washed three times with sterile water and collected in a test tube of sterile water containing 50 ppm CTC. The volume was adjusted to about 200 eggs/ml. One milliliter of suspended eggs was then transferred to each of three plates containing water agar supplemented with 50 ppm CTC. The agar in the plates had been dried to allow the water from the egg suspension to be absorbed. Plates were incubated at 20 C for 24 hours. Longer incubation allowed mycelia from infected eggs to overgrow surrounding eggs. One hundred eggs on each plate were examined for fungal growth. Seven fungus-infected eggs from each plate were transferred to 2% malt extract agar to allow the fungus to grow out for identification.

*Count of N. gynophila resting spores in soil:* A slight modification of a centrifugation method (3) was used to estimate the numbers of *N. gynophila* resting spores in soil samples. A 25-gram sample of field soil in 30 ml water was shaken vigorously for 1 hour. The soil suspension was washed through sieves of decreasing pore size to remove most of the soil particles and finally collected on a 10-μm-pore nylon sieve. The > 10-μm fraction was transferred to a solution of saturated magnesium sulfate and centrifuged at low speed (4 *g*) to sediment any soil particles. The supernatant was sieved through a 10-μm-pore sieve, and the remaining residue on the sieve was again washed onto a saturated magnesium sulfate solution and centrifuged a second time at a higher speed (87 *g*). The supernatant was discarded leaving 0.5–2.0 ml suspension, depending on the texture of the soil. Resting spores in 10-μl samples were counted with a microscope at 125 × magnification.

TABLE 1. Percentage of *Heterodera avenae* infected by *Nematophthora gynophila* in a pot test and numbers of resting spores of *N. gynophila* in field soil. n.d. = not determined.

| Field | Infected females (%) Weeks after sowing 7 | 8 | 9 | Spores/ gram air dried soil |
|---|---|---|---|---|
| Alnarp 1 | 22 | 13 | <1 | 17 |
| Alnarp 2 | 29 | 21 | <1 | 49 |
| Vårgårda | 11 | 18 | 11 | 10 |
| Våxtorp | 11 | 4 | n.d. | 3 |

## RESULTS

*Pathogens of females:* Resting spores of *N. gynophila* were found in soil from all four fields studied (Table 1). Alnarp 2 had the highest count with 49 spores/gram air dried soil, while Våxtorp only had 3 spores/gram soil. In the pot experiment, the percentage of infected *H. avenae* females, assessed 7, 8, and 9 weeks after sowing, ranged from < 1 to 29%, with a mean of 13% (Table 1). Females parasitized by *N. gynophila* were found in soil samples from all four fields. A single female infected with *Catenaria* sp. was detected.

*Parasites of eggs in new cysts:* Eggs in new cysts on roots in the pot experiment were often infected by fungi. All eggs were infected in 10 of 75 flaccid cysts; in 23 flaccid cysts some of the eggs (range 1–55%) were infected while 42 cysts lacked fungi. Only three fungal species were found in cysts with all eggs infected, and all eggs in a cyst were always infected by the same fungus. *V. chlamydosporium* was the species most frequently isolated, while an unidentified *Verticillium* species and *Paecilomyces lilacinus* were isolated from one cyst each. In cysts with only a portion of the eggs infected, several different fungi were often isolated from a single cyst, such as *Cylindrocarpon* sp., *Periconia macrospinosa* Lefebvre & Johnson, and different nonsporulating fungi. Cysts with all eggs infected often contained few eggs. Three of the ten cysts contained only 10 eggs each. No empty egg shells were present in these cysts.

*Infection of eggs in mature cysts from soil:* Fungal parasitism of eggs in cysts from the fields was 10–20% (Table 2). Percentage of infection and species of active fungi varied between subsamples of the composite sample from each site. The numbers of cysts extracted from soil were similar for each subsample. *Verticillium* sp. 1 was iso-

TABLE 2. Fungal infection of eggs from cysts of *Heterodera avenae* from soil stored for 8 months (Våxtorp) or 12 months (other fields).

| Field | Fungus | Number of isolates per subsample | | | % of total number of isolates | % infected eggs (± SE) |
|---|---|---|---|---|---|---|
| Alnarp 1 | *Verticillium* sp. 1 | 15 | 6 | 8 | 46 | |
| | Sterile spp. | 4 | 7 | 13 | 38 | |
| | *V. bulbillosum* | 0 | 5 | 0 | 8 | 20 ± 1.2 |
| | *Acremonium* sp. | 0 | 3 | 1 | 6 | |
| | *Exophiala pisciphila* | 1 | 0 | 0 | 1 | |
| Alnarp 2 | Sterile spp. | 1 | 12 | 8 | 46 | |
| | Slow-growing sp. 1 | 11 | 1 | 7 | 41 | |
| | *Verticillium* sp. 1 | 0 | 0 | 3 | 7 | 13 ± 3.1 |
| | *V. chlamydosporium* | 2 | 0 | 0 | 4 | |
| | *Cylindrocarpon* sp. | 1 | 0 | 0 | 2 | |
| Vårgårda | Sterile spp. | 14 | 22 | 12 | 89 | |
| | *Verticillium* sp. 1 | 3 | 0 | 0 | 5 | 10 ± 1.8 |
| | *Mortierella* sp. | 2 | 0 | 0 | 4 | |
| | *Fusarium* sp. | 1 | 0 | 0 | 2 | |
| Våxtorp | Sterile spp. | 6 | 5 | 11 | 39 | |
| | *Verticillium* sp. 1 | 0 | 12 | 8 | 36 | |
| | *Microdochium bolleyi* | 8 | 1 | 0 | 16 | 12 ± 3.1 |
| | *Mortierella* sp. | 1 | 1 | 0 | 4 | |
| | *Paecilomyces lilacinus* | 1 | 0 | 0 | 2 | |
| | *Cylindrocarpon* sp. | 0 | 1 | 0 | 2 | |

TABLE 3. Fungal infection of eggs from cysts of *Heterodera avenae* from soil stored 2 months (Alnarp 1), 5 months (Alnarp 2), fresh soil (Alnarp 3).

| Field | Fungus | % of total number of isolates | % infected eggs (± SE) |
|---|---|---|---|
| Alnarp 1 | *V. chlamydosporium* | 73 | |
| | *Verticillium* sp. 1 | 20 | 20* |
| | Sterile pink | 7 | |
| Alnarp 2 | *Paecilomyces lilacinus* | 43 | |
| | *Verticillium* sp. 1 | 36 | |
| | *V. chlamydosporium* | 8 | 30 ± 13 |
| | *Cylindrocarpon* sp. | 6 | |
| | Sterile spp. | 6 | |
| Alnarp 3 | *Verticillium* sp. 1 | 79 | |
| | *V. chlamydosporium* | 10 | |
| | Sterile pink | 5 | 33 ± 7.4 |
| | Sterile spp. | 5 | |
| | *Cylindrocarpon* sp. | 1 | |

* Only one subsample.

lated most frequently (Table 2); it was isolated from infected eggs from all four fields, and it was the most common species in eggs from two fields.

Different nonsporulating fungi dominated among the isolates as a whole in soil stored 8–12 months. A beige, slow-growing mycelium (slow-growing sp. 1) was isolated from 41% of infected eggs at Alnarp 2. This fungus resembles the "contortion fungus" that Tribe (13) reported as a major parasite of eggs of *H. schachtii.* Nonsporulating fungi did not dominate when the soil was processed after 0–5 months storage. *V. chlamydosporium* was isolated from 73% of the infected eggs in a soil sample taken at Alnarp 1 in November 1981 and analyzed 2 months later (Table 3). A sample taken the same month at Alnarp 2 and analyzed after 5 months yielded *P. lilacinus* from 43% of the infected eggs; *Verticillium* sp. 1 and *V. chlamydosporium* were also frequently isolated (Table 3). A sample taken at Alnarp 3 (in April 1982) and processed immediately yielded *Verticillium* sp. 1 in 79% of the isolates and *V. chlamydosporium* from 10% of the infected eggs (Table 3). Few nonsporulating fungi were isolated from these eggs.

## DISCUSSION

A species of *Verticillium,* here called *Verticillium* sp. 1, was the most common fungal egg parasite found infecting eggs in cysts of *Heterodera avenae* in southern Sweden. This *Verticillium* sp. cannot be classified to any of the described species (W. Gams, pers. comm.). It has not been reported as a parasite of cyst nematodes, but might previously have been identified as *V. chlamydosporium.* Isolates that do and do not produce chlamydospores have been included in *V. chlamydosporium* (8,13), and *V. chlamydosporium* has been considered as a species complex (2). *Verticillium* sp. 1 differs from *V. chlamydosporium* in several respects. A few submerged dichtyo-chlamydospores are produced in agar, but none on the aerial mycelium. Furthermore, it grows more slowly than *V. chlamydosporium* at room temperature, and it grows at 6 C where *V. chlamydosporium* fails to grow. Two subgroups of *Verticillium* sp. 1 could be distinguished on the basis of conidial morphology. One is characterized by more globose and usually catenate conidia (2–3 μm), while the other has more elongate and capitate conidia (2 × 3–4.5 μm).

*Nematophthora gynophila, V. chlamydosporium,* and *P. lilacinus* are also recognized for nematophagous activity in other countries in Europe (9,15), in the United States (10), and in South America (4).

*Nematophthora gynophila,* a fungus well known for attacking cyst nematode females (8), was isolated from soil samples from all four sites. In our study from < 1 to 29% of females were infected, with a mean of 13%. In one pot experiment, a mean infection level not exceeding 20% was obtained (6), and in another pot test, mean infection rates were 13–14% (7). In a field trial, up to 45% of the females were infected (8), but infection varied widely over the 12-week sampling period, just as in this study (Table 1). Frequent sampling over long periods therefore seems necessary to give an accurate estimate of average female infection. In addition, infected females are fragile, which makes their separation from soil difficult and causes underestimation of the level of infection (6).

Resting spores of *N. gynophila* were extracted from soil samples collected from all four sites, which agrees with a recent survey of *Nematophthora* resting spores in Swedish soils infested with *H. avenae* (S. Andersson, pers. comm.). A positive correlation seemed to exist between the number of resting spores in the soil and the percentage of infected females in the pot

experiment. The highest concentration of spores, 49/gram soil, was found in Alnarp 2, where the highest percentage of diseased females also occurred. Few spores and infected females were found in Växtorp, while the other two fields had intermediate values. Only at Alnarp 2 were spore numbers so high that, according to previous findings (3), *N. gynophila* could be expected to affect the nematode population. High spore numbers were found associated with declining nematode populations and vice versa (3). Nematode cyst populations in our four fields have varied a great deal over the past 5–9 years (S. Andersson, pers. comm.). Because crop rotation is practiced, the decline in the *H. avenae* population is mainly due to the lack of host plants rather than fungal activity (1).

*Verticillium chlamydosporium* parasitizes nematodes in many countries. It has been reported as an important egg parasite on *H. avenae* (8), *H. schachtii* (15), and *Meloidogyne arenaria* (10) and as an important parasite of females on *H. avenae* (8). *V. chlamydosporium* was not a common parasite of eggs from mature cysts isolated from soil in this study. It was the dominant species (73% of infected eggs) in one sample, Alnarp 1 analyzed after 2 months of storage. With longer storage, the frequency of *V. chlamydosporium* appeared to decrease. It was, however, a common invader of eggs in new cysts on roots but was not found in females.

*Verticillium* sp. 1 was the most common fungal egg parasite detected in these four soils. It was isolated from eggs in cysts from all fields and in all samples. Often it was the dominant species, in one sample being recovered from 79% of the infected eggs, but was seldom isolated from new cysts.

Preliminary studies showed that *Verticillium* sp. 1 grew below 6 C, where *V. chlamydosporium* failed to grow. Thus *Verticillium* sp. 1 was probably favored by the low soil storage temperature of 2 C, explaining the low recovery of *V. chlamydosporium*. Extrapolated to the field, *V. chlamydosporium* may be an early invader, active as a parasite of females and eggs in young cysts during the warm period. *Verticillium* sp. 1 might then become more important as the soil temperature decreases and its relative importance may increase during the winter. Alnarp 3 was the only soil sampled in early spring, and in this sample *Verticillium* sp. 1 was the dominant egg pathogen.

The dominance of nonsporulating fungi among the egg parasites in soil stored for 8–12 months could also be an effect of storage at low temperatures, since preliminary studies showed that some of these isolates grew at 2 C.

*Paecilomyces lilacinus* was isolated from 43% of the infected eggs in the sample from Alnarp stored for 5 months. This fungus has not been reported as a parasite of cyst nematodes in Europe before, but a *Paecilomyces* sp. was found in 5% of all infected eggs at one site (8). In Peru, however, *P. lilacinus* has been isolated from eggs of the root-knot nematode, *M. incognita*, and it has provided biological control of *M. incognita* and *Globodera pallida* (4).

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