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Loss of Virulence of the Endoparasitic Fungus *Drechmeria coniospora* in Culture[1]

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Parasitic fungi in culture frequently lose pathogenicity. For example, the virulence of certain entomogenous fungi decreases during in vitro culture over long time periods (12). Virulence of fungal isolates has been restored by serial passage of the organism through the insect host (13), through mutagenesis by ionizing radiations (1), and by genetic manipulation. In an overview of random genetic drift in populations of obligatory entomogenous insects, Mackauer (10) concludes that parasitic drift in organisms transferred from their natural ecosphere and subjected to the artificial environment of the laboratory is a problem of paramount importance. Loss of virulence has not been documented for nematophagous fungi.

Hazards imposed on the environment and humans by chemical pesticides emphasize the need for alternate means for controlling agricultural pests. Management of plant-parasitic nematodes through natural enemies is an active research area. Since many of the efficient nematode antagonists are obligate parasites, knowledge of their behavior under laboratory conditions is required.

Previously, *Drechmeria coniospora* was reported to infect *Meloidogyne incognita* (6) and two other plant-parasitic nematode species (5). *D. coniospora* reduced damage by *M. incognita* in greenhouse and microplot field trials (7). This is a report of the loss of pathogenicity of isolates of *D. coniospora* and on studies investigating this phenomenon.

Organisms: Drechmeria coniospora cultures were from several sources, but all were derived from a Canadian culture isolated by G. C. Barron and held in the American Type Culture Collection as ATCC #18767 or from a Swedish culture isolated by H-B. Jansson and held in the American Type Culture Collection as ATCC #48243. ATCC #48243 is further designated as SO-1 obtained from Sweden in 1983, SO-2 obtained from Sweden in 1986, and SO-3 passed through *Panagrellus redivivus* three times. All of the preceding strains were maintained on diluted cornmeal agar (CMA 1:10; 1.5% agar). In addition, a culture derived from ATCC #48243, designated SO-4, was maintained continuously on *P. redivivus* on water agar (1.5%) for 3 months by weekly passage to healthy nematodes. In each passage the fungus infected the nematodes and conidia produced were collected and used to infect new nematodes.

The microbivorous nematodes in this study were *P. redivivus* and *Caenorhabditis elegans* axenically cultured in heme media (11) or liver extract medium (15). They were harvested and washed as described by Jansson et al. (8). The *Meloidogyne* species used were *M. hapla* and *M. incognita* (from M. Harrison, Cornell University) and *M. incognita* race 3 (from M. McClure, University of Arizona). Second-stage juveniles of *Meloidogyne* were collected from egg masses held in aerated Baermann funnels (4).

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TABLE 1. Adhesion to nematodes and their infection by *Drechmeria coniospora.*

Fungus isolate†	*Meloidogyne hapla*		*M. incognita* (Cornell)		*M. incognita* race 3		*Caenorhabditis elegans*		*Panagrellus redivivus*	
	A	I	A	I	A	I	A	I	A	I
ATCC #18767	+	−	+	−	+	−	+	+	+	+
ATCC #48243	+	−	+	−	+	−	+	+	+	+
SO-1	+	−	+	−	+	−	+	±	+	±
SO-2	+	−	+	−	+	−	+	±	+	±
SO-3	+	−	+	−	+	−	+	+	+	+
SO-4	+	−	+	−	+	−	+	+	+	+

A = attachment. I = infection. + = adhesion or infection occurs; − = no infection; ± = 5-day delay in infection.
† SO-1 = Sweden, 1983. SO-2 = Sweden, 1987. SO-3 = passed three times through *Panagrellus.* SO-4 = continuous culture on *Panagrellus.*

Adhesion and infection: Adhesion and infection of nematodes by conidia of *D. coniospora* were studied as previously described (9). Water agar containing nematodes and *D. coniospora* was examined daily for 8 days to determine the infection. The criterion for infection was the observation of spore-bearing conidiophores emerging from the nematode cadaver.

Virulence enhancement: Since virulence of entomogenous fungi can be enhanced by passage through insects (13), in a second experiment *P. redivivus* was used as a substrate for growth of *D. coniospora* in an attempt to restore virulence of the isolate. The fungus was passed through the nematode three times on water agar during a time interval of 3 months, and data were taken on attachment of conidia to *C. elegans* and *P. redivivus* and nematode infection (Table 1). The protocol for passage through *P. redivivus* was the same in a second experiment, but the conidia were evaluated against three *Meloidogyne* species (Table 1). Each experiment was replicated at least twice. The Barron (2) method of renewing viability of nematode predatory fungi and endoparasitic fungi after long periods in culture was also attempted. Soil was placed in vials, then sterilized and inoculated with a suspension containing both infected and healthy *P. redivivus.* The soils were allowed to stand at room temperature for 7 days and then stored at 5 C. The fungus was reisolated and evaluated for pathogenicity to juveniles of *M. incognita.*

Drechmeria coniospora spores attached to *P. redivivus* and *C. elegans* within 1 hour in vitro and nematodes were infected within 36 hours, except in trials with cultures SO-1 and SO-2 where infection was delayed.

The results of experiments with *Meloidogyne* in the current trials were in direct contrast to those previously reported (6,7). In experiments in 1983–84, *D. coniospora* isolate SO-1 attached to, and then infected, *M. incognita* from Cornell; in the current test, spores of the same isolate attached to, but no longer penetrated, *M. incognita* from Cornell (Table 1). This was also true for *M. incognita* race 3, against which *D. coniospora* had given significant control (7). In fact, several combinations of fungus isolates from the American Type Culture Collection and the 1986 culture from Sweden were not parasites on the three *Meloidogyne* isolates, demonstrating that *D. coniospora* kept continuously in culture lost its virulence to *Meloidogyne.* All attempts to restore virulence of the several *D. coniospora* isolates to *Meloidogyne* species were negative (Table 1).

The current study demonstrates loss of virulence in nematophagous fungi. One approach to preventing the loss of virulence may be cryopreservation of cultures. Cryopreservation studies were performed recently on *D. coniospora,* and although conidia survived several different methods of freezing, the normal infection period was altered and it took longer for spores to infect *P. redivivus* (14). *Meloidogyne* was not tested in these trials, so it is uncertain whether the spores were still pathogenic to them.

Generally, micro-organisms remain stable and viable for years when cryopreserved (3). This method is used in many

laboratories to maintain stability of bacteria, cell lines, and myelomas producing monoclonal antibodies. For the long-term study of nematophagous fungi, subsamples of cultures should be frozen when the isolate is obtained from nature.

Why the fungus was able to infect bacteriophagous nematodes but not the root-knot nematode is not understood. *D. coniospora* in culture over a period of time may have lost the ability to penetrate the cuticle of *Meloidogyne* species. These results would infer a specific decrement in cuticle penetrating enzymes (i.e., collagenase and [or] elastinase); however, the observed 5-day delay in infection by fungus isolates SO-1 and SO-2 held in our laboratory in vitro suggests a different interpretation. The results indicate a loss of virulence of *D. coniospora* maintained continuously on diluted cornmeal agar. The same isolate that had been cryopreserved (ATCC #48243) was more virulent though it did not infect *Meloidogyne*. Its virulence may be explained by a shorter time interval of this isolate on CMA prior to deposition in the ATCC and cryopreservation. The Swedish isolates SO-3 and SO-4 passed through *P. redivivus* also showed greater virulence to the bacteriophagous nematodes, but still did not regain the capability of infecting *Meloidogyne*.

On the basis of these observations we conclude that maintenance of virulence in nematophagous fungi can best be assured by cryopreservation of subcultures soon after isolation from the natural environment. The loss of infectivity of root-knot nematodes suggests that the two-stage mechanism of the lectin–carbohydrate interaction (6) which controls the sequential events of adhesion and infection has been disrupted.

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