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Susceptibility of *Neoaplectana* spp. and *Heterorhabditis heliothidis* to the Endoparasitic Fungus *Drechmeria coniospora*

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Abstract: Adhesive conidia of the nematophagous fungus, *Drechmeria coniospora* (Drechsler) W. Gams and Jansson (Moniliales: Deuteromycetes), would occasionally attach but never penetrate the infective stages of insect parasitic *Neoaplectana carpocapsae, N. glaseri, N. bibionis, N. intermedia,* and *Heterorhabditis heliothidis* (Rhabditida). However, adult and pre-infective stages of *Neoaplectana* spp. became infected by the fungus.

Key words: Drechmeria coniospora, entomogenous nematodes, nematophagous fungus, *Heterorhabditis heliothidis, Neoaplectana bibionis, N. carpocapsae, N. glaseri, N. intermedia, Rhabditis* sp.

Nematodes of *Neoaplectana* spp. (reasons for maintaining *Neoaplectana* are presented elsewhere (7)) and *Heterorhabditis* spp. are being commercially produced for use as biological control agents of insects (8). The infective third-stage juvenile that survives without feeding is the stage released in nature. This stage actively searches for a host, enters and kills the host, and develops on the host cadaver. Abiotic and biotic factors influence the survivability of these infective-stage juveniles. Abiotic factors including temperature, humidity, and soil texture have been reported previously (5). Of the biotic factors, disease-producing organisms including nematophagous fungi may be exceedingly important.

Since soil is the natural habitat for neoaplectanid and heterorhabditid nematodes, their use against soil insects constitutes a natural application. However, soil application brings them into contact with a multitude of soil microorganisms, including nematophagous fungi.

Nematophagous fungi include predators and endoparasites (1). The former group is characterized by saprophytic growth in the soil and production of hyphal trapping devices. The latter group infects nematodes via conidia. Most are obligate parasites and are not able to exist saprophytically in soil. Only the conidiophores, which are nurtured by nematode cadavers, are found outside the host.

The fungus *Drechmeria coniospora* infects microbotrophic (6) plant and animal parasitic nematodes (2). Adhesion of *D. coniospora* conidia to the cuticle of *Panagrellus redivivus,* growth of trophic hyphae inside the nematode, and production of conidiophores and conidia have been studied (4). Our objective was to determine the susceptibility of insect parasitic nematodes of *Neoaplectana* spp. and *Heterorhabditis heliothidis* to the fungus *D. coniospora.*

MATERIALS AND METHODS

Cultures of *Drechmeria coniospora* (Drechsler) W. Gams and Jansson (basionym *Meria coniospora* Drechsler) CBS 615.82 were maintained on diluted corn meal agar (Difco, CMA 1:10, 1.5% w/v agar) at 25 C. After 8–10 weeks conidia were washed off the petri dishes with about 10 ml sterile water. Approximately 10^9 conidia in 5 ml sterile water were applied to the surface of the agar in each dish. Dishes were left open until the excess moisture evaporated.

The nematodes used in this study included 1) a control consisting of a hermaphroditic *Rhabditis* sp. maintained on nutrient agar plates seeded with a mixture of bacteria, 2) infective-stage juveniles of *Neoaplectana carpocapsae* (strain 42) stored in water at 8 C, 3) developing stages of *N. carpocapsae* (strain 42) grown on dog food agar in association with the symbiotic bacterium, *Xenorhabdus nematophilus,* 4) infective-stage juveniles of *N. glaseri* (strain F1) stored in water at 20 C, 5) infective-stage juveniles of *N. bibionis* (strain SN) stored in water at 8 C, 6) infective stages of *N. intermedia* (strain SC) stored in water at 8 C, 7) pre-infective stages of *N. intermedia*

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TABLE 1. Adhesion to, and infection of, certain insect parasitic nematodes by *Drechmeria conispora*.

Nematode species	Nematode stage	Adhesion	Infection
Neoaplectana carpocapsae	Developing stages	Common	Common
Neoaplectana carpocapsae	Infective juveniles	Rare	None
Neoaplectana bibionis	Infective juveniles	Rare	None
Neoaplectana glaseri	Infective juveniles	Rare	None
Neoaplectana intermedia	Infective juveniles	Rare	None
Neoaplectana intermedia	Pre-infective juveniles	Common	Rare
Heterorhabditis heliothidis	Infective juveniles	Rare	None
Rhabditis sp. (control)	Developing stages	Common	Common

(strain SC) stored in Ringer's solution at 20 C, and 8) infective stages of *Heterorhabditis heliothidis* stored in water at 20 C. All nematodes were grown in wax moth larvae (*Galleria mellonella*) and had been in storage for 1–3 months.

The pre-infective and infective stages of *Neoaplectana* spp. and *H. heliothidis* were added directly to the agar plates containing *Drechmeria* conidia at a rate of ca. 1,000 nematodes per plate. Developing stages of *N. carpocapsae* sp. were washed in saline to remove associated dog food medium and were then added to the *Drechmeria* plates at ca. 500 nematodes per plate.

Each nematode treatment was added to a single petri dish of water agar seeded with *Drechmeria* spores. Nematodes were observed twice daily over the following 7 days. Infected nematodes were hand picked, mounted in Ringer's solution on microscope slides, and observed microscopically with interference contrast optics.

RESULTS

Results of exposing nematodes to *D. coniospora* are summarized in Table 1. The most susceptible nematode was *Rhabditis* sp. Spores attached to the heads of females and penetrated through the cuticle (Fig. 1). After the mycelium ramified through the body, hyphae and conidiophores emerged from the cadaver (Fig. 3). In some cases, spherical globules formed within the mycelium, especially in older infections (Fig. 2). This *Rhabditis* sp. was viviparous, and newly hatched juveniles normally destroyed the females. In several instances, conidia of *D. coniospora* ingested by females were released from the intestine by action of the juveniles; these conidia sometimes attached to and infected the juveniles (Fig. 4).

Developing stages of *N. carpocapsae* were the next most susceptible nematodes after *Rhabditis* sp. Spores attached to the heads, necks, and tails of developing juveniles (Figs. 5–7). Larger females also ingested spores (Fig. 8), but no infections were observed to occur via the alimentary tract. All stages of nematodes became infected following direct penetration of the fungus through the cuticle (Fig. 9).

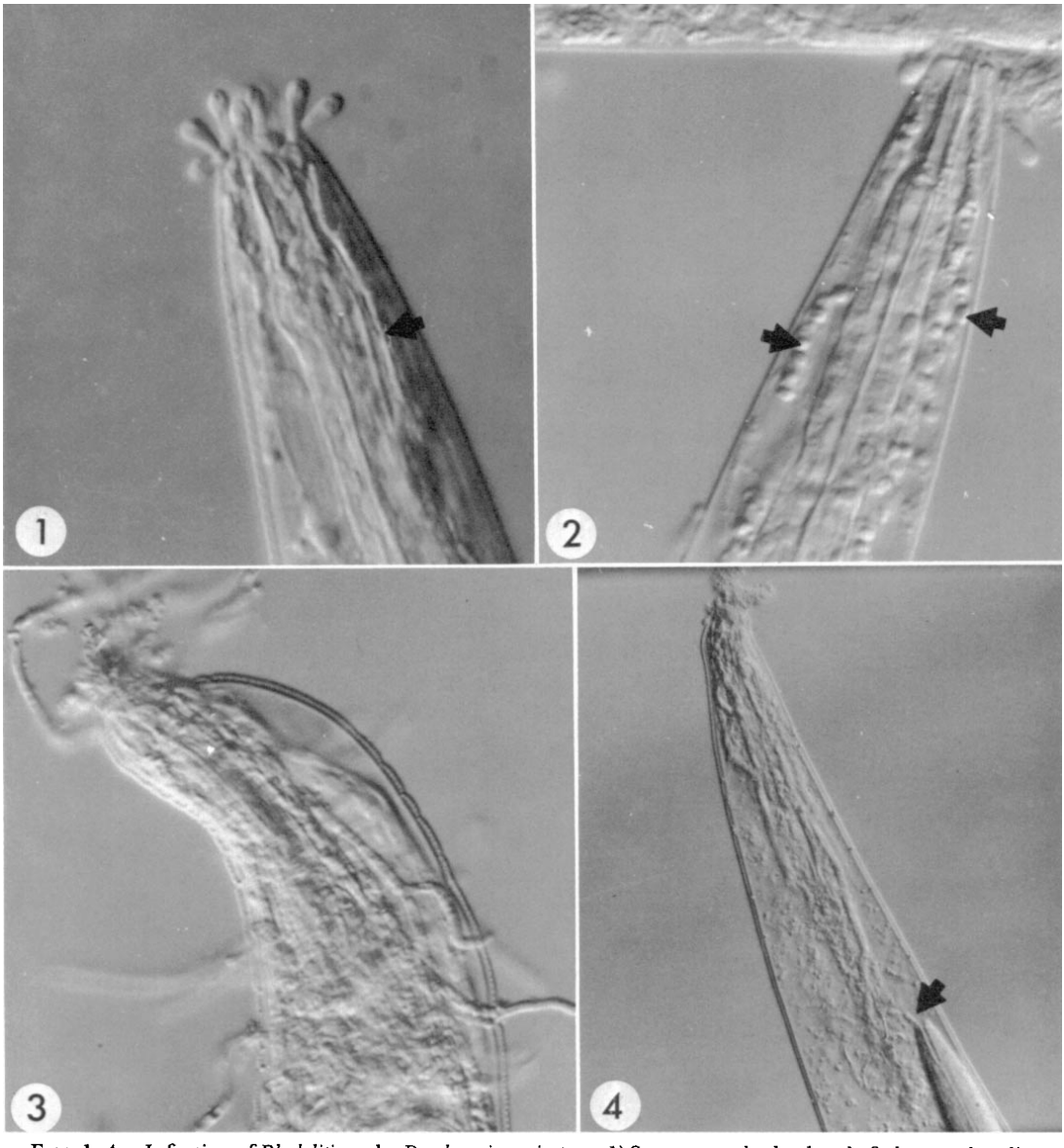
Infective stages of nematodes were the most resistant to fungus infection. Spores rarely adhered to infective stages, but when they did, attachment was to heads, necks, and tail tips (Figs. 10–12). Penetration of infective stages was never observed; however, pre-infective stages of the SC strain of *Neoaplectana intermedia* were penetrated. These nematodes developed to the infective stage but later died (Fig. 13). Spores occasionally attached to cuticles of pre-infective nematodes and remained attached to the loose ensheathing cuticle after the infective stages formed.

In several instances, infective stages of *H. heliothidis* dying from unknown causes were covered with attached spores (Fig. 14).

DISCUSSION

Two critical steps are necessary for *D. coniospora* to complete its development on nematodes—conidium attachment and germ tube penetration. Once the fungus invades the body cavity of the nematode, successful colonization and sporulation are imminent since there appears to be no known defense reaction or immunity in nematodes to invading fungi.

D. coniospora attached to and penetrated *Rhabditis* sp. and developing stages of *N. carpocapsae*; however, spore attachment and penetration were slower and involved fewer spores with developing stages of *N. car-*

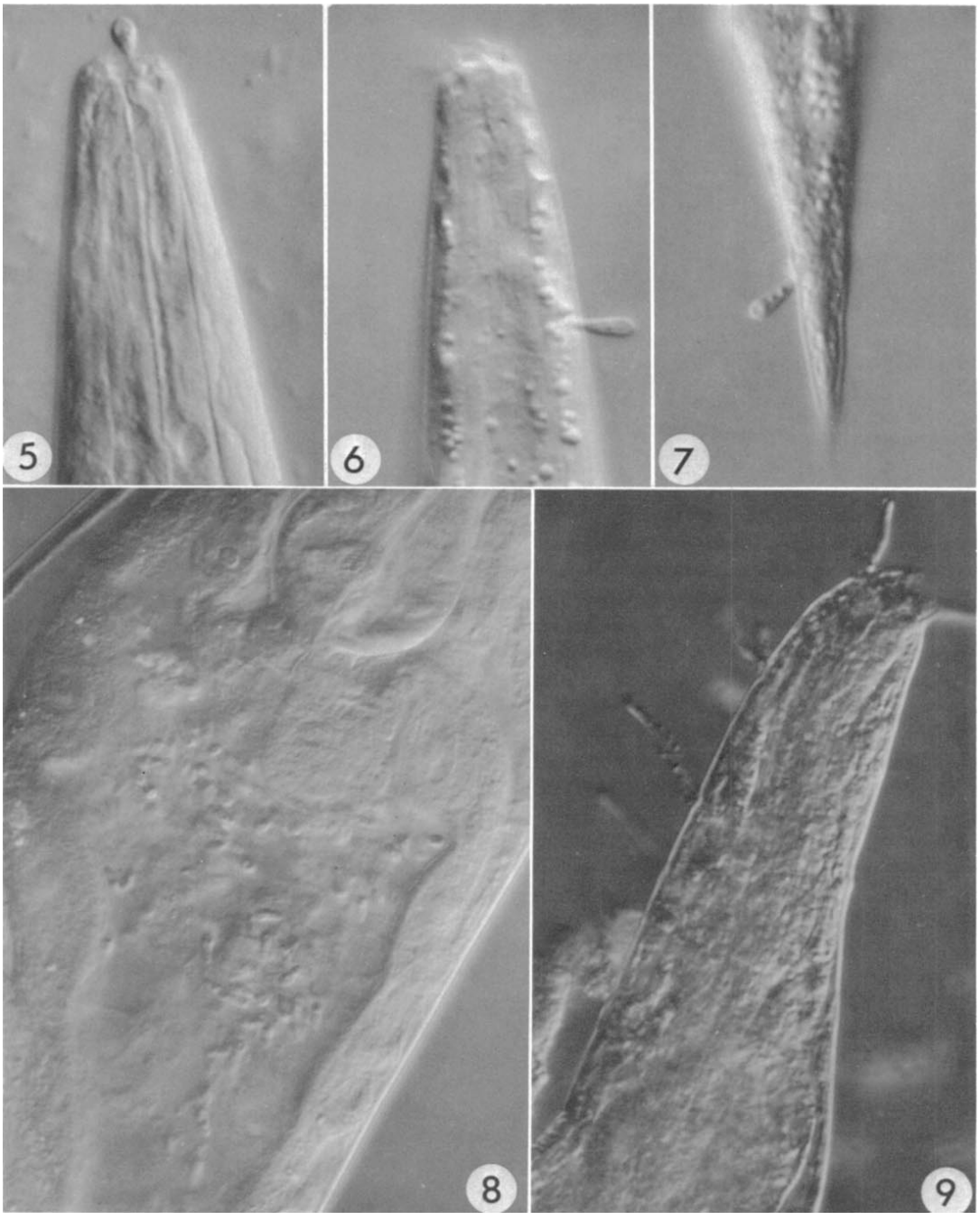


FIGS. 1–4. Infection of *Rhabditis* sp. by *Drechmeria coniospora*. 1) Spores attached to head of a hermaphrodite. Note mycelium (arrow) has already penetrated the host's body. 2) Infected hermaphrodite. Note mycelium has broken down, leaving globules produced by the fungus (arrows). 3) Mycelium and conidiophores emerging from a hermaphrodite. 4) Spore attached to the head of a juvenile (arrow) still inside the dead infected hermaphrodite.

pocapsae than with *Rhabditis* sp. Spore attachment and penetration were even less frequent with pre-infective stages of *Neoaplectana intermedia*.

In general, *Drechmeria* spores adhered most frequently in the cephalic region of all the nematodes tested, yet spores were attached to tails, mid-bodies, or neck regions of nematodes. Adhered conidia did not infect infective-stage juveniles. Obviously some structural or chemical cuticular difference allows attachment and penetration of developing juveniles of *N. carpocapsae* and only rare attachment with no penetration of infective juveniles.

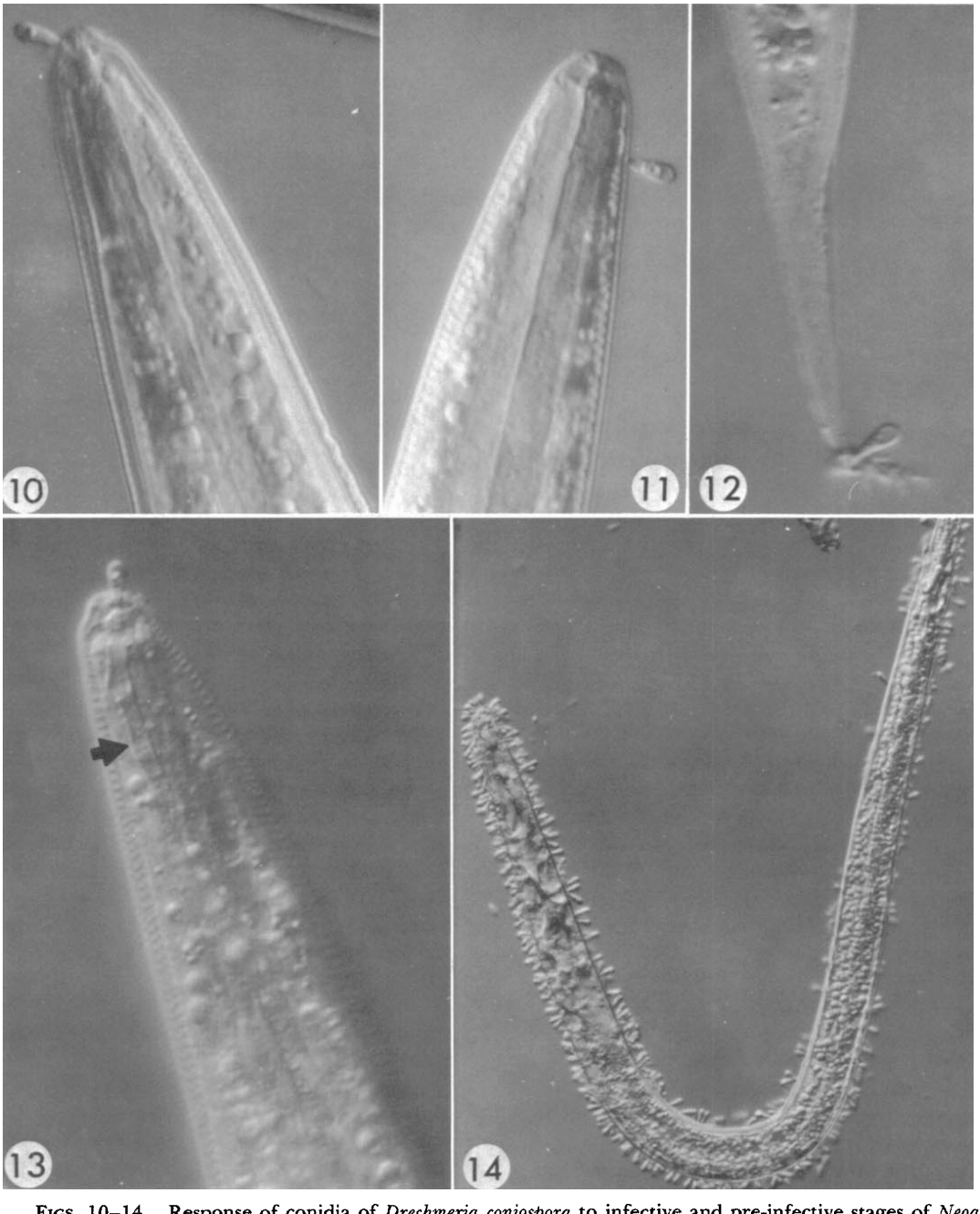
It was previously suggested that a recognition mechanism involving a sialic acid-specific lectin located on the conidia of *D. coniospora* bound the conidia to sialyl resides on the nematode cuticle (3). The ab-



FIGS. 5–9. Infection of noninfective stages of *Neoaplectana carpocapsae* by *Drechmeria coniospora*. 5) Spore attached to the head of a second-stage juvenile. 6) Spore attached to neck region of a second-stage juvenile. 7) Spore attached to tail of a fourth-stage juvenile. 8) Female showing ingested spores in ventricular portion of intestine. 9) Mycelium and conidiophores emerging from a male.

sence or presence of the correct lectin receptor on the cuticle of the nematodes may explain the results obtained in this study.

The apparent immunity of *Neoaplectana* spp. and *H. heliothidis* infective stages to parasitism by *D. coniospora* will be of interest to applicators using these nematodes against soil insects.



FIGS. 10–14. Response of conidia of *Drechmeria coniospora* to infective and pre-infective stages of *Neoaplectana* spp. and *Heterorhabditis.* sp. 10) Attachment of spore to the head of an infective-stage juvenile of *N. intermedia.* 11) Spore attachment to the neck region of an infective-stage juvenile of *N. carpocapsae.* 12) Spore attachment to the tail tip of an infective-stage juvenile of *N. carpocapsae.* 13) Early stages of infection of an infective-stage juvenile of *N. intermedia.* Penetration probably occurred (arrow indicates mycelium in body) while nematode was in pre-infective stage. 14) Spores attached to a previously diseased infective juvenile of *H. heliothidis.* Spores rarely adhered to healthy individuals.

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