Infection of *Neoaplectana* and *Heterorhabditis* (Rhabditida : Nematoda) with the predatory fungi, *Monacrosporium ellipsosporum* and *Arthrobotrys oligospora* (Moniliales : Deuteromycetes)

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SUMMARY

Infective juveniles of *Neoaplectana bibionis, N. carpocapsae, N. glaseri, N. intermedia* and *Heterorhabditis heliothidis* were susceptible to infection by the nematophagous fungi *Arthrobotrys oligospora* and *Monacrosporium ellipsosporum.* These fungi could attach, infect and digest the nematodes within 72 hours. The results indicate that attention should be given to the presence of nematophagous fungi during the application of these nematodes.

RÉSUMÉ

L'infestation de Neoaplectana *et* Heterorhabditis *(Rhabditida : Nematoda) par les champignons prédateurs* Monacrosporium ellipsosporum *et* Arthrobotrys oligospora *(Moniliales : Deuteromycètes)*

Les juvéniles infestants de *Neoaplectana bibionis, N. carpocapsae, N. glaseri, N. intermedia* et *Heterorhabditis heliothidis* peuvent être infestés par les champignons nématophages *Arthrobotrys oligospora* et *Monacrosporium ellipsosporum.* Ces champignons sont capables de se fixer, d'infester et de digérer les nématodes en 72 heures. Ces résultats devraient conduire à porter attention à la présence de champignons nématophages lors des traitements à l'aide de ces nématodes.

Biotic factors influencing populations of entomogenous nematodes have been little studied. Nematodes of the genera *Neoaplectana* and *Heterorhabditis* are being commercially produced today and used as biological control agents of insects (Poinar, 1985). Infective stage juveniles of these two genera are being applied to soil, mushroom houses and manure beds for control of specific insects. In all of these habitats, but especially the latter two, the nematodes are likely to encounter predatory fungi which could reduce their number and hinder their control efforts (Barron, 1977). In a previous study, Poinar and Jansson (1986) showed that the adhesive conidia of the nematophagous fungus, *Drechmeria coniospora* would rarely attach and never penetrate the infective stages of *Neoaplectana* and *Heterorhabditis.* The purpose of the present study was to determine if the immunity shown by the infective juveniles of *Neoaplectana* and *Heterorhabditis* to *D. coniospora* was also present against some of the commonly encountered predatory nematophagous fungi.

Materials and methods

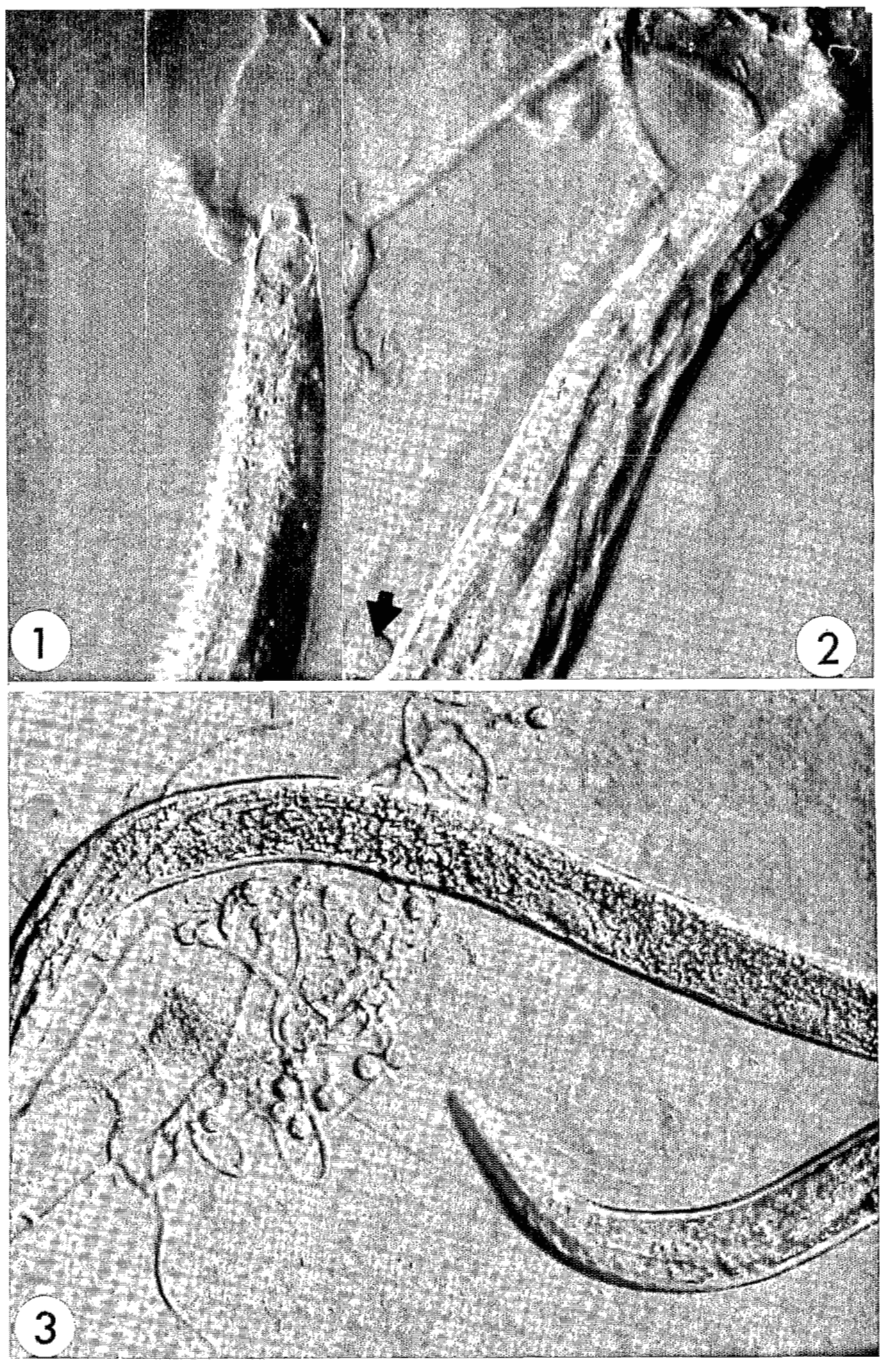
Colonies of *Monacrosporium ellipsosporum* (Grove) R. C. Cooke & Dickinson were received from Dr. R. Mankau at the University of California in Riverside and were grown on diluted corn meal agar plates (CMA 1:10; 1.5 % agar : Difco Laboratories). Cultures of *Arthrobotrys oligospora* frees were recovered from diseased juveniles of *Neoaplectana glaseri* that had been held in storage at room temperature. This fungus was then transferred to diluted corn meal agar plates (CMA 1:10; 1.5 % agar : Difco Laboratories). Fungal cultures were maintained at 20°.

Infective stage juveniles of *Neoaplectana carpocapsae* (strain 42), *N. glaseri* (strain FL), *N. bibionis* (strain SN), *N. intermedia* (strain SC) and *Heterorhabditis heliothidis* that were harvested from wax moth larvae *(Galleria mellonella)* and had been in storage for 1-6 months at 12° were used in this study.

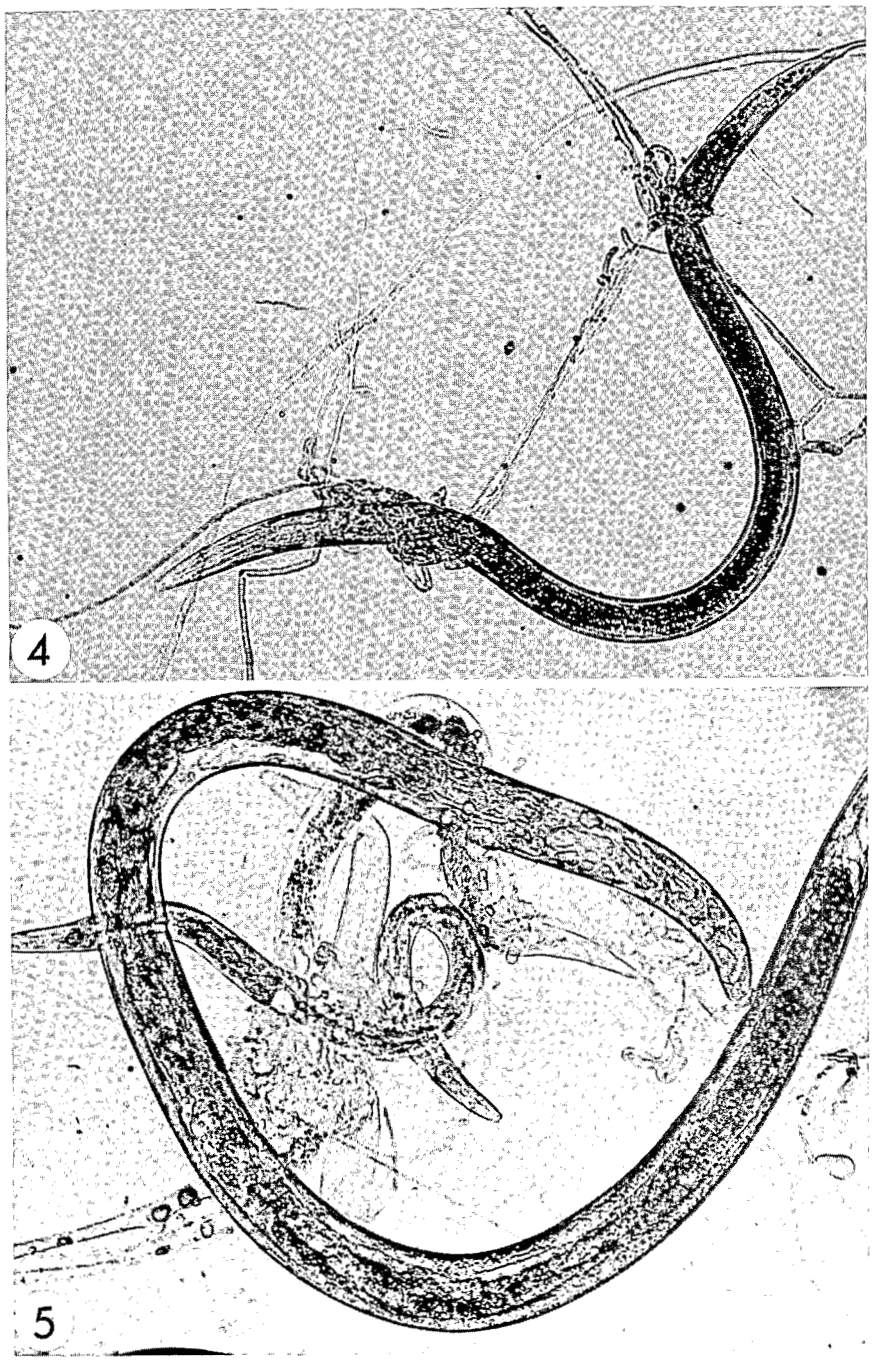
Approximately 1 000 infective stage juveniles of each nematode species were placed on Petri dishes containing 14 day old cultures of *M. ellipsosporum* or *A. oligospora,* respectively. The plates were left on the laboratory bench (at 20°) and observed daily for one week.

Results

Essentially all species of *Neoaplectana* as well as *H. heliothidis* were susceptible to infection by both fungal species. Capture of the nematodes occurred within the first 12 hours after the experiments were initiated.



Figs 1-3. Infection of *Neoaplectana* with *Monacrosporium ellipsosporum*. 1 : Two sticky knobs of *M. ellipsosporum* attached to the head of an infective stage juvenile of *N. carpocapsae;* 2 : Infective stage juvenile of *N. glaseri* with a sticky knob of *M. ellipsosporum* attached to its body (arrow) and hyphae of the fungus ramifying through the nematode's body; 3 : Two infective juveniles of *N. bibionis* surrounding a clump of *M. ellipsosporum*. The juvenile on top has lost its ensheathing cuticle and has fungal knobs attached to its body. The lower juvenile still contains its second stage cuticle which has become fixed to the sticky knobs of the fungus while the nematode attempts to pull away.



Figs 4-5. *Neoaplectana* and *Heterorhabditis* infected with *Arthrobrytris oligospora.* 4 : Infective juvenile of *N. bibionis* captured in the sticky networks of *A. oligospora* in the head and tail region; 5 : Larger infective stage of *N. glaseri* and smaller infective stages of *H. heliothidis* infected with *A. oligospora.* Note mycelium developing inside nematodes.

Monacrosporium ellipsosporum

During routine movement, infective stage juveniles would encounter the sticky knobs of *M. ellipsosporum*. Attachment usually occurred in the cephalic region (Fig. 1) although it could occur anywhere on the body (Fig. 2). Attachment occurred on the ensheathing second stage cuticle as well as on the cuticle of the third stage juvenile if the sheath was lost (Fig. 3). In the latter case, the nematode could often escape by breaking through the attached cuticle and leaving the infection court. Once attachment was made, the nematode was securely held. In no instance was a nematode observed to break free (except in cases when the ensheathing cuticle was caught), and frequently, its struggles would break the knob from its mycelial stalk. The nematode would then crawl away with the attached knob which would subsequently germinate and initiate infection. After 3-4 days, the infectives did not become attached to many knobs, although they were observed rubbing past and against the knobs.

Soon after knob attachment, penetration of the cuticle occurred and the mycelium ramified through the nematode (Fig. 2), eventually breaking through the body wall and producing conidiophores and conidia.

Arthrobotrys oligospora

The trapping mechanism of *A. oligospora* are sticky three dimensional networks which adhere to the nematode cuticle. These sticky networks were effective traps and none of the juveniles were observed breaking free. Even ensheathed individuals could not shed their cuticle and escape from the traps of *A. oligospora*. Attachment of the fungus to the nematode occurred at any site of the nematode surface (Fig. 4). Penetration of the hyphae occurred within six hours after contact and the mycelium quickly ramified throughout the body cavity (Fig. 5). The nematodes were completely consumed in three days, at which time conidiophores were emerging from their cadavers.

Discussion

Infective juveniles of *Neoaplectana* and *Heterorhabditis* were susceptible to infection by both *M. ellipsosporum* and *A. oligospora*. There appeared to be no resistance by the nematode once contact with the fungi had been made. Penetration and nematode death rapidly followed. Only rarely were ensheathing juveniles able to escape the sticky knobs of *M. ellipsosporum* by shedding their cuticle. It is not known whether the ineffectiveness of the knobs of *M. ellipsosporum* in older cultures to attach to the nematodes was due to their loss of adhesive or a change in the nematodes cuticle.

The two predatory nematophagous fungi, *M. ellipsosporum* and *A. oligospora*, differed from the endoparasitic fungus *Drechmeria coniospora* in their ability to infect the nematodes. The adhesive conidia of *D. coniospora* rarely adhered to and never penetrated the infective juveniles of *Neoaplectana* and *Heterorhabditis* (Poinar & Jansson, 1986). In many nematophagous fungi, cuticle recognition via a lectin-carbohydrate relationship has been established (e.g. Rosenzweig & Ackroyd, 1983; Nordbring-Hertz & Jansson, 1984). The traps of *A. oligospora* bear a lectin specific for N-acetyl-galactosamine (Nordbring-Hertz & Mattiasson, 1979) and a sialic acid-specific lectin has been suggested to be localized on the conidia of *D. coniospora* (Jansson & Nordbring-Hertz, 1983). The presence of the corresponding carbohydrates on the nematode cuticle thus dictates the adhesion between the nematode and the fungus. A review of the importance of carbohydrates in nematode-fungal recognition phenomena has recently been presented (Zuckerman & Jansson, 1984).

The results here demonstrate that some predatory fungi pose a direct threat to neoaplectanid and heterorhabditid infective juveniles. Both *M. ellipsosporum* and *A. oligospora* and probaly other predatory nematophagous fungi should be considered as potential threats during the application of these nematodes.

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