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Competitive interactions between two nematophagous fungi during infection and digestion of the nematode *Panagrellus redivivus*

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Individuals of the free-living nematode *Panagrellus redivivus* were infected with conidia of the endoparasitic nematophagous fungus *Drechmeria coniospora*. At different stages of infection the nematodes were added to an agar-plate culture of the predatory fungus *Arthrobotrys oligospora* which contained traps. Initially, nematodes infected with *D. coniospora* were invariably captured, penetrated and colonized by *A. oligospora*. Then both fungi used the nematode contents for development of hyphal biomass outside the nematode. But inside nematodes in which infection by *D. coniospora* was advanced development of *A. oligospora* was markedly hampered. In dual-infected nematodes (colonized from opposite ends), the hyphae of *A. oligospora* were often observed to be dead or degenerate when in close proximity to living mycelium of *D. coniospora*, indicating that the latter acts as an antagonist against *A. oligospora* under these conditions. The interaction between trophic hyphae of *D. coniospora* and *A. oligospora* involved thickening of the cell wall of *A. oligospora* at the sites of contact and degradation of the hyphal compartments and shrivelling of the cell wall of *A. oligospora*. The observed interactions are discussed in relation to mycoparasitism and possible ecological function.

Arthrobotrys oligospora Fresen. is able to grow in a dual feeding-mode namely saprotrophic or predacious. The predacious stage is induced by environmental stimuli (Nordbring-Hertz, 1973, 1977) and is characterized by the development of numerous capturing organs, the so-called traps. These specialized hyphal structures capture living nematodes with the aid of an adhesive layer on the outside of the trap cell wall and subsequently penetrate and digest the prey within 72 h (Veenhuis *et al.*, 1989*b*).

Drechmeria coniospora W. Gams & H. B. Jansson (1985) is an almost obligatory endoparasite of nematodes, infecting the host by means of adhesive conidia (Jansson, von Hofsten & von Mecklenburg, 1984). During colonization of the nematode by trophic hyphae, outgrowth of conidiophores occurs and numerous conidia are formed outside the cuticle at the expense of the host (Dijksterhuis *et al.*, 1991). However, the fungus can also grow on complex media, albeit very slowly, producing large numbers of conidia (Lohmann & Sikora, 1989).

Compared with *A. oligospora*, *D. coniospora* attracts nematodes more strongly (Jansson, 1982*a*) and decreases nematode numbers faster in sterile soil (Jansson, 1982*b*) which reflects the obligate nature of parasitism by this fungus. However, development of trophic hyphae during the early stages of infection of nematodes by *A. oligospora* and subsequent digestion of nematode contents is faster compared with *D. coniospora* which shows abundant conidia production after 96 h (Veenhuis *et al.*, 1989*b*; Dijksterhuis *et al.*, 1991). This prompted us to study the possible competitive interactions between these fungal species as representatives of two major groups of nematophagous fungi, facultative and obligate parasites, during infection of individual nematodes. Therefore we decided to infect individual nematodes with both fungi and investigate the infection process with light- and electron microscopy. In this contribution the interaction between both fungi inside the nematode is presented and discussed in relation to mycoparasitism and possible ecological function.

MATERIALS AND METHODS

Organisms and growth conditions

Arthrobotrys oligospora ATCC 24927 (obtained from Professor Nordbring-Hertz, University of Lund, Sweden) was maintained on 2% cornmeal agar surfaces at 20 °C. Traps were induced on mycelium grown on dilute (one-tenth strength) cornmeal agar by the addition of a drop of nematode suspension (Veenhuis *et al.*, 1985*a*). *Drechmeria coniospora* (obtained from Professor Wyss, University of Kiel, Germany) was kept on 2% cornmeal agar surfaces (Dijksterhuis *et al.*, 1990). For studies of the fungal–nematode interactions, the nematode *Panagrellus redivivus* Goodey, grown axenically on soya-peptone liver extract (Nordbring-Hertz, 1977) or xenically on rolled oat meal, was used.

Interaction experiments

P. redivivus was initially infected with conidia of *D. coniospora* by adding the nematodes directly to a fungal culture (Dijksterhuis *et al.*, 1990). The efficiency of adherence of conidia of *D.*

coniospora to nematodes was 100% within 10 min. At different stages of infection, 2–3, 8 and 15–16 h after adherence of conidia, nematodes were selected and transferred to trap-containing cultures of *A. oligospora* by means of a thin wooden stick. After capture, nematodes and the adhered traps with a minimum of vegetative mycelium connected to it, were removed from the agar surface and transferred to distilled water (Veenhuis *et al.*, 1989*a*) or to Noble agar surfaces. Samples were taken at 10–12, 22·5–24 and 46–48 h after capture and prepared for electron microscopy. The infection process was followed by means of optical microscopy. In addition, nematodes were captured by traps following infection by *D. coniospora* after 11–13 h. Infected animals were washed twice in a drop of sterile distilled water. Subsequently the individual nematodes were placed in 50 µl distilled water in separate wells of a microtitre plate and the infection process was followed with an inverted light microscope.

Optical and electron microscopy

Video-enhanced optical microscopy was performed at the laboratory of Professor Wyss, Christian Albrechts University, Kiel, Germany, using a Reichert-Jung Polyvar optical microscope equipped with a Hamamatsu DVS 300 image-processing system (Wyss & Zuncke, 1986). For electron microscopy, infected nematodes were embedded in 2% water agar, fixed in 1·5% potassium permanganate in water for 20 min at room temperature and processed as described before (Dijksterhuis *et al.*, 1990). Ultrathin sections were examined in a Philips CM 10 electron microscope.

Interactions on an agar surface

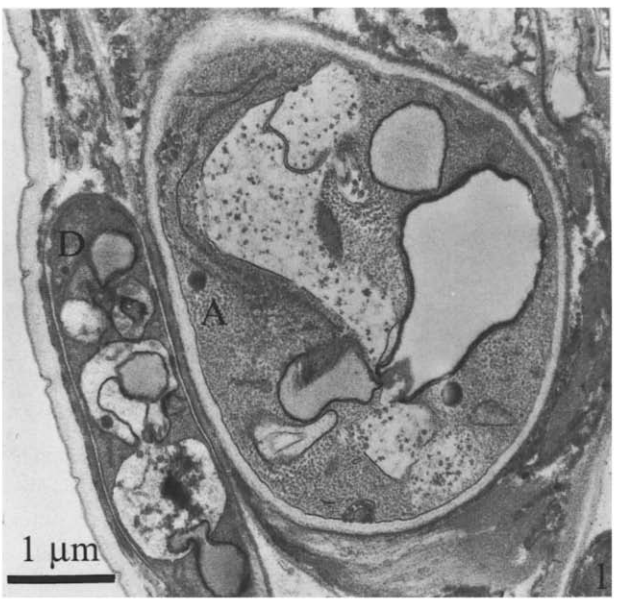
The possible interaction between saprotrophically-grown vegetative hyphae of both fungi was studied in agar plate cultures. *D. coniospora* was grown on agar plates for 15 d prior to the introduction of small pieces of agar which contained hyphae of *A. oligospora*. The cultures were investigated by optical microscopy at the interaction zone of both species.

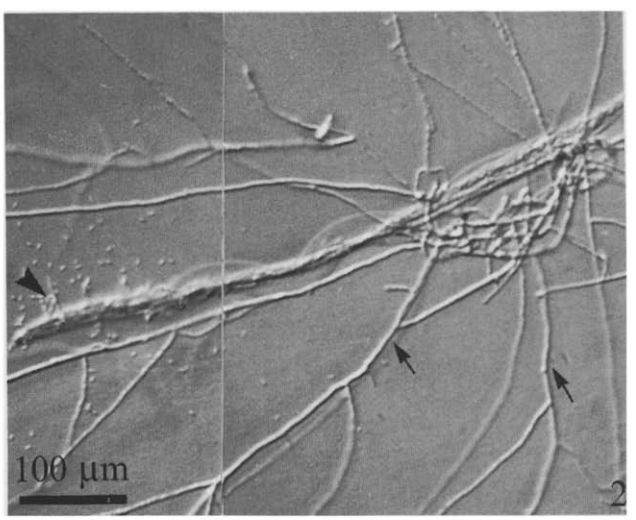
RESULTS

Addition of the nematode *Panagrellus redivivus* directly to agar surfaces containing *Drechmeria coniospora* resulted in the adhesion of numerous conidia to the nematodes (Dijksterhuis *et al.*, 1990), preferentially to the anterior or posterior end (Jansson & Nordbring-Hertz, 1983). The nematodes were not hampered in their movements by conidia and/or developing trophic hyphae inside the body during initial stages of infection. Following their transfer to trap-containing cultures of *Arthrobotrys oligospora* these nematodes generally were captured successfully after contact. However, 15–16 h after the initial adhesion of conidia, movements of the nematode were substantially reduced due to prolonged infection and capture of some nematodes occurred. Even guiding of the animals to the traps with the aid of a small wooden stick no longer resulted in adherence.

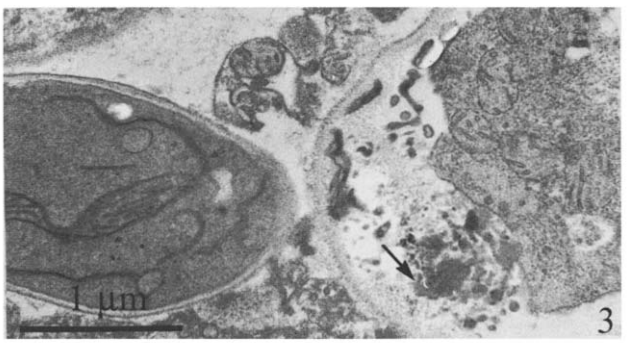
Capture occurred at all possible sites on the nematode. This is of crucial importance for our investigations since the place of interaction of trophic hyphae inside the nematode depends on the location of the trap in relation to the adhesion site of the spores (usually the head). Specifically, we tried to obtain animals trapped at the middle part of the body or at the tail (in the case of females). This allowed both fungi to develop sufficient amount of hyphal mass inside the nematode body.

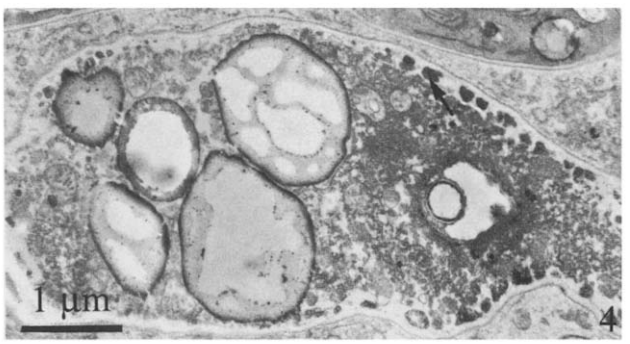
Nematodes captured during the initial stages of infection by the endoparasite (2–3 h or 8 h after adhesion of conidia) were effectively penetrated and colonized by *A. oligospora*. At that time the development of hyphae of *D. coniospora* was confined to a very small part of the animal, but not prevented as a result of the faster development of *A. oligospora*. Ultrathin





Figs 1, 2. Occurrence of living hyphae of two nematophagous fungi in one nematode. **Fig. 1.** Ultrathin section of trophic hyphae of *A. oligospora* (A) and *D. coniospora* (D) inside a nematode. Note the different appearance of both hyphae with respect to hyphal diameter and thickness of the cell wall. **Fig. 2.** Production of fungal biomass outside the host; vegetative hyphae on trap cells in case of *A. oligospora* (arrow) and conidiophores and conidia by *D. coniospora* (arrowhead).

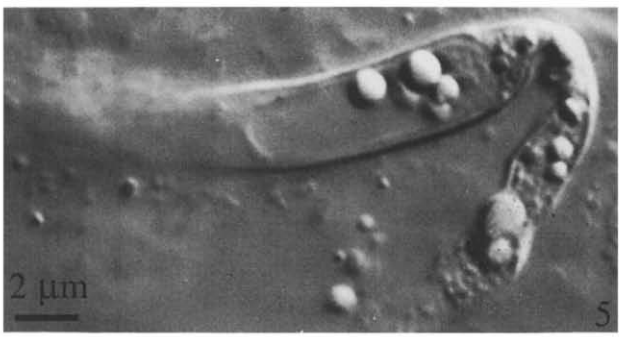


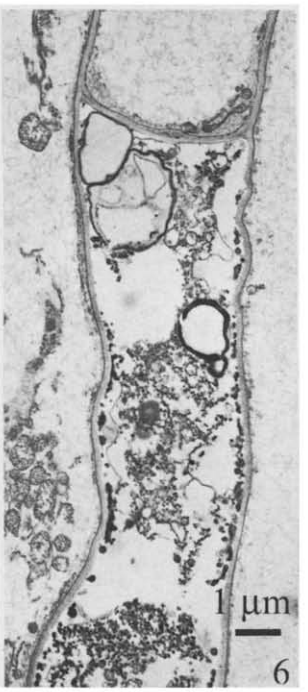


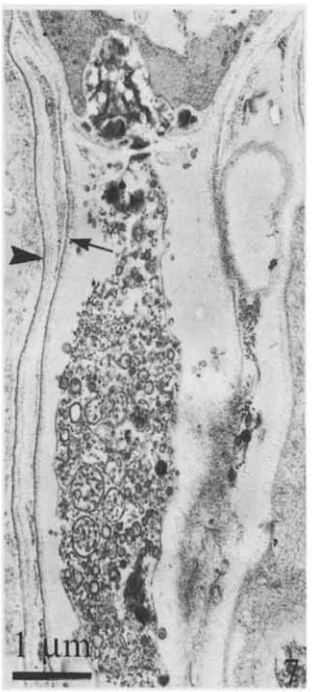
Figs 3, 4. Killing of hyphae of *A. oligospora* by *D. coniospora*. **Fig. 3.** Thickening of the cell wall of *A. oligospora* where it is in close contact with *D. coniospora*. Note the electron dense inclusions (arrow). **Fig. 4.** Disintegration of cell contents in parallel with appearance of dark material at the inner side of the cell wall (arrow).

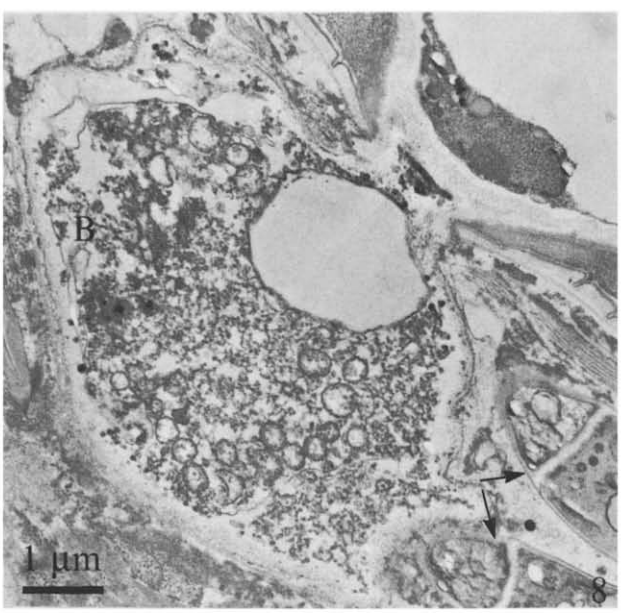
sections revealed that the ultrastructure of both fungi initially was not different from that observed during the infection process of each individual species in separate nematodes (Fig. 1). The hyphae of the two fungi were easily discerned; *A. oligospora* showed wider hyphae ($\geqslant$ 3 µm *v.* 1·5–2 µm) with a thicker cell wall (100 nm *v.* 50 nm). Furthermore, *D. coniospora* exhibited a more electron dense appearance of the cytosol as a result of the procedure for the preparation of samples. The other characteristics of trophic hyphae were similar to those described earlier, including accumulation of lipid droplets and branch formation in the case of *D. coniospora* (Dijksterhuis *et al.*, 1991) and frequent anastomoses in the case of *A. oligospora* (Veenhuis *et al.*, 1989*b*). Both fungi were able to produce biomass outside the nematode at the expense of the host, namely vegetative hyphae on trap cells (in the case of *A. oligospora*, Veenhuis *et al.*, 1989*a*) and conidiophores and conidia (by *D. coniospora*, Fig. 2). This latter phenomenon occurred after 40–48 h of infection on a limited part of the nematode compared with those infected with the endoparasite alone.

However, invariably dead hyphae of *A. oligospora* were observed in the interaction zone between both fungi, i.e. near or amidst the invading mass of hyphae of *D. coniospora* resulting from many conidia adhering to one specific site on the animal. This restricted the colonization of trophic hyphae of *A. oligospora* to only part of the nematode. The extent of this limited development decreased with the stage of infection of the nematode by *D. coniospora*. At later stages development of trophic hyphae of vegetative mycelium of *A. oligospora* was









Figs 5–8. Killed hyphae of *A. oligospora*. **Fig. 5.** Light micrograph of terminal stage of interaction showing the shrivelled cell wall. **Fig. 6.** Ultrathin section of such a hypha. **Fig. 7.** Death of intra-hyphal cell of *A. oligospora*, the cell wall of this hypha (arrow) clearly discernible from the other one (arrowhead) **Fig. 8.** Killed infection bulb (B). Note the close appearance of *D. coniospora* (arrows).

grossly restricted in most cases. In a microtitre plate experiment, 40 nematodes, captured 11–13 h after infection by *D. coniospora* were studied. Only in three cases development of vegetative hyphae on adhered trap cells occurred; all other nematodes showed no outgrowth. However, inside a number of these nematodes hyphae of *A. oligospora* were observed, indicating that the cuticle was successfully penetrated by the fungus. For this experiment, nematodes were selected with trap(s) adhered randomly to the body surface. Animals with traps attached near the conidia showed no development of vegetative hyphae on the trap-cell except in one case, where only a few hyphae were formed.

The interaction between both fungi included inhibition of further development, active killing and degradation of trophic hyphae of *A. oligospora* by *D. coniospora*. During contact between the hyphae, only close proximity of the attacking hyphae near the killed one was observed. Ultrathin sectioning revealed that in a number of cases distinct thickening of the cell wall of *A. oligospora* was observed at the side facing *D. coniospora* (Fig. 3) which was characterized by the presence of electron dense inclusions. During later stages of the interaction disintegration of cell contents occurred in parallel with the presence of dark material near the inner side of the cell wall (Fig. 4). This material was still visible when the cytoplasm and organelles were totally distorted and only dark granular material and remnants of lipids and membranes were observed (Fig. 4). The cell wall showed structural irregularities and occasionally hyphae had a shrivelled appearance (Fig. 5, LM, and Fig. 6). Hyphae of *D. coniospora* were never observed inside cells of *A. oligospora*, indicating that the interaction did not involve penetration and intra-hyphal growth. Development of new hyphae of *A. oligospora* was often observed in dead ones but also these cells died (Fig. 7). This type of intrahyphal cell development was also observed in vegetative cultures (liquid medium and agar surfaces) and during cell–cell interactions in capture organs (Veenhuis, Nordbring-Hertz & Harder, 1985*b*). In nematodes captured at later stages of infection (15–16 h) occasionally dead penetration bulbs were observed (Fig. 8) which indicates that colonization of the nematode by hyphae of *A. oligospora* failed after penetration.

In order to study whether this interaction also occurred under vegetative growth conditions, we introduced *A. oligospora* to an agar surface containing colonies of *D. coniospora* which had been grown for 15 d (diam. less than 1 cm). Hyphae of *A. oligospora* showed no changes in growth patterns in the interaction zone between both fungi, but actually grew through these colonies. No evidence for inhibition of growth or avoidance reactions was found.

DISCUSSION

The investigations presented in this paper clearly show that infection of the nematode *Panagrellus redivivus* by the endoparasitic fungus *Drechmeria coniospora* restricts or even prevents development of trophic hyphae of the predatory fungus *Arthrobotrys oligospora* inside the prey. Hyphae of the latter are killed in a necrotrophic interaction in which the following aspects are important: (i) a close proximity of hyphae of both species, (ii) increase in thickness of the cell wall of *A. oligospora* hyphae at the site of contact and (iii) degradation of cell contents and shrivelling of the cell wall of *A. oligospora*.

This was somewhat unexpected because several species of *Arthrobotrys* have been reported to be pathogenic to different fungal species, although hyphae of the mycoparasitic *Geotrichum* were also able to attack *A. oligospora* (Tzean & Estey, 1978). The interaction between *D. coniospora* and *A. oligospora* hyphae does not show the involvement of specialized hyphal cells (e.g. coiling hyphae) which were observed during mycoparasitism of *Arthrobotrys* sp. on (among others) the plant pathogen *Rhizoctonia solani* (Persson, Veenhuis & Nordbring-Hertz, 1985; Persson, 1991). Secondly, there was no penetration of the *A. oligospora* cell wall and subsequent intra-hyphal growth as has been observed during the necrotrophic interaction of *Verticillium biguttatum* and *R. solani* (van der Boogert *et al.*, 1989).

With respect to hyphal contact and subsequent coagulation/vacuolation and lysis of host cytoplasm, the interaction we observed most resembles mycoparasitism of *Pythium* sp. on different fungal species (Laing & Deacon, 1991). However, in that study intra-hyphal growth was reported in 69 of the 148 interactions and also hyphal coil cells were observed in a small number of cases.

The observation that hyphae of *A. oligospora* were not killed on an agar surface probably illustrates the difference between trophic and vegetative hyphae. The former facilitate digestion, uptake of nematode contents and subsequent transport of nutrients to hyphal cells outside the nematode (Veenhuis *et al.*, 1989*a*). However, the presence of many hyphae in a small compartment (the nematode) for a prolonged period of time might also explain the occurrence of interactions not observed on agar surfaces.

Infection of individual nematodes by more than one fungus is reported by Barron (1977); he describes that zoosporangia of the endoparasitic fungus *Catenaria* aborted in the presence of hyphae of different fungal species, especially *Harposporium* sp. This interaction was characterized by the distortion of cellular contents without penetration of the sporangia by hyphae of *Harposporium* sp. The author suggested that this effect is caused by antibiotic secretions. In our study, infection of nematodes with two fungi was studied in the laboratory; however, its possible occurrence in soil is likely because (i) nematodes infected with *D. coniospora* can move freely for at least 15 h before their movements gradually slow down (Dijksterhuis *et al.*, 1991) and (ii) *A. oligospora* is known to be one of the most abundant nematophagous fungi in soil (Gray, 1988). Restriction or prevention of concurrent infection by means of hyphal killing by *D. coniospora* might be a strategy for the endoparasite to reduce loss of prey in favour of the widespread facultative predatory fungi.

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