Fundam. appl. Nematol., 1994, **17** (5), 423-431

Saprophytic and predacious abilities in *Arthrobotrys oligospora* in relation to dead and living root-knot nematodes

Eefje den Belder and Ellis Jansen

Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, 6700 GW Wageningen, The Netherlands.

Accepted for publication 3 November 1993.

Summary – An adhesive hyphae forming isolate of *Arthrobotrys oligospora* clearly responded to the condition of its food source, i.e. living, inactivated or dead second-stage juveniles (J2) of the root-knot nematode *Meloidogyne hapla*. Second-stage juveniles (J2) immobilized by heating and only able to move the anterior region or the stylet, were surrounded by ring structures similar to fully mobile juveniles. However, ring structures were principally developed around the moving head. The fungus penetrated dead, but intact J2 (obtained after treatment with gamma-irradiation or sodium azide), through its buccal cavity with a corkscrew-like structure. Dead J2 with a broken cuticle were totally overgrown by the fungus with thin vegetative hyphae. Evidently, the isolate of *A. oligospora* switched between nutritional modes while exploiting different food sources. The saprophytic and predacious ability appeared not to be mutually exclusive. Addition of dead juveniles to a fungal colony prior to live juveniles did not affect attachment or the development of trophic hyphae through the latter. But one day after addition of the living juveniles, the proportion of live juveniles with ring structures raised in comparison with all juveniles added at the same time. The development of trophic hyphae in killed J2 was delayed in the presence of live J2. The results refute the commonly held assumption that poor conditions for saprophytic growth are a prerequisite for a predacious mode of feeding.

Résumé – *Capacités saprophytique et prédatrice chez* Arthrobotrys oligospora *vis-à-vis de juvéniles de* Meloidogyne *morts ou vivants –* Un isolat d'*Arthrobotrys oligospora* formant des hyphes adhésifs réagit nettement à l'état de sa source de nourriture, en ce cas des juvéniles de deuxième stade (J2) de *Meloidogyne hapla,* vivants, inactivés ou morts. Les J2 rendus immobiles par chauffage et uniquement capables de mouvoir leur stylet sont entourés par des structures annulaires identiques à celles produites dans le cas de J2 parfaitement mobiles; cependant ces structures sont surtout présentes autour de la région céphalique, demeurée seule mobile. Le champignon pénètre dans les J2 morts mais restés intacts – obtenus après traitement aux rayons gamma ou à l'azide de sodium – à travers la cavité buccale à l'aide de structures en tire-bouchons. Les J2 morts ayant une cuticule altérée sont totalement recouverts par de fins hyphes végétatifs. A l'évidence, l'isolat de *A. oligospora* passe de l'un à l'autre type de prise de nourriture suivant la source de cette dernière. Les modes saprophytique et prédateur n'apparaissent pas s'exclure l'un l'autre. Le fait de placer des J2 morts au contact d'une colonie fongique avant d'y placer des J2 vivants n'affecte pas la formation et l'attache des hyphes trophiques sur ces derniers; mais un jour après l'addition des J2 vivants, la proportion de ces juvéniles pourvus de structures annulaires augmente par rapport au nombre total de juvéniles lorsque placés au même moment. Le développement des hyphes trophiques chez les J2 morts est retardé en présence de J2 vivants. Ces résultats contredisent l'affirmation courante suivant laquelle de mauvaises conditions de croissance saprophytique constituent un préliminaire obligé pour un mode de nutrition prédatrice.

Key-words : *Arthrobotrys, Meloidogyne,* adhesive hyphae, morphogenesis, saprophytic predacious, nutritional modes.

Many fungi show a degree of flexibility in nutritional modes throughout their life-cycle (Lewis, 1972; Luttrell, 1974; Cooke & Whipps, 1980; Cooke & Whipps, 1987). Nematode-capturing fungi are also able to switch from one nutritional mode to another. Knowledge about the nutrition of facultative nematophagous fungi, however, is fragmentary (Blackburn & Hayes, 1966; Hayes & Blackburn, 1966; James & Nowakowski, 1968; Nordbring-Hertz, 1968). Nevertheless, nematophagous fungi may capture bacteria, Tardigrada, amoeba and other living soil organisms as well as digest dead nematodes and bacteria (Nordbring-Hertz & Stålhammar-Carlemalm, 1978; Fermor & Wood, 1981).

Both the ability to grow saprophytically and their predacious habit are prerequisites for those nematophagous fungi that capture nematodes with structures developed along the vegetative hyphae. Hence the effect of fungi on nematodes does not depend solely on predation, but also on the ability of the fungus to grow and compete saprophytically (Pramer, 1964). However, the significance of nutritional versatility of nematophagous fungi in relation to their use in biological control is difficult to assess.

ISSN 1164-5571/94/05 $ 4.00 / © Gauthier-Villars - ORSTOM

Arthrobotrys oligospora has been found in a broad range of niches (Gray, 1983; Dackman *et al.,* 1987; Fritsch & Lysek, 1989). Comparison of isolates of *A. oligospora* with respect to radial growth *in vitro,* formation of ring structures, attraction of nematodes and the capture of *Meloidogyne hapla* on agar medium or in sterile soil, showed intraspecific variability (Jansson & Nordbring-Hertz, 1979; den Belder, unpubl.). Hence, individual isolates may have different saprophytic and predacious properties.

The ability of a fungus to form traps may reflect its evolutionary adaptation to nutritional stress imposed by competition with other soil micro-organisms for energy sources (Cooke, 1963 *a, b*). He also suggested that the evolution of predacious efficiency or the ability to reduce soil populations of nematodes has been generally accompanied by a loss of characteristics associated with an efficient saprophytic existence in the soil such as rapid vegetative growth and good competitive saprophytic ability.

The present study was initiated to obtain a better understanding of the relative predacious and saprophytic abilities of *A. oligospora.* This isolate may be considered as an effective predator because it captures nematodes by its morphologically undifferentiated vegetative hyphae, at low temperatures and under poor nutritional conditions (den Belder & Jansen, 1994).

On the basis of comments and conclusions by various authors (Cooke, 1963 *b*; Jansson & Nordbring-Hertz, 1979; Jansson, 1982; Jaffee & Zehr, 1985) it was anticipated that the ability of this isolate to use non-living material to be limited due to an inverse relation between saprophytic and predacious abilities.

Factors affecting or regulating switches from the saprophytic to the predacious feeding mode are largely unknown (Jaffee & Zehr, 1985; Quinn, 1987). Also in other well-studied nematophagous fungi such as the egg parasites *Paecilomyces lilacinus* and *Verticillium chlamydosporium* or in entomophagous fungi little is known of the switches which are responsible for changes in the trophic state (Jatala, 1986; De Ley, 1992).

Adhesive network forming fungi such as *A. oligospora* are considered as facultative predators not capturing prey under nutrient rich conditions (Cooke, 1963 *a, b*). Consequently it was postulated that attachment of live *Meloidogyne* spp. to *Arthrobotrys oligospora* and subsequent formation of ring structures around, and trophic hyphae through the nematode body, should be suppressed in the presence of added dead juveniles.

Materials and methods

Organisms

Arthrobotrys oligospora (CBS 289.82) was cultured on corn meal agar (Oxoid, CMA 1:1, 1.5 %) in Petri-dishes (diam. 88 mm) at 25 ± 1 °C with monthly transfers to fresh medium. Individual 4-mm plugs cut from the periphery of the actively growing stock colony were placed upside down in small Petri-dishes (Lux, diameter 44 mm) on CMA 1:10. The Petri-dishes used in the experiments had an oval hole in the bottom lid (length 35 mm, width 18 mm) covered by a coverglass glued to the lid, thus facilitating microscopic observations with an inverted microscope. Fungus cultures (28 day-old) on corn meal agar 1:10, were inoculated with freshly extracted J2 of *M. hapla.*

Meloidogyne hapla was reared on tomato plants (*Lycopersicon esculentum* Mill. cv. Moneymaker). Second-stage juveniles were harvested and surface sterilized as described by den Belder and Jansen (1994).

Response of the mycelium of *A. oligospora* to vitality of *M. hapla*

Experiment 1

The response of the fungus was studied after addition of different substrates : J2 of *M. hapla* living or either partly immobilized, either dead with either an intact or damaged cuticle. The different qualities of J2 were obtained by applying them a variety of physical stresses. The following treatments were included :

1) Axenic, healthy 2 day-old J2, actively moving (control).

2) Axenic J2 suspended in sterile water and incubated for 24 h at 35 °C, slightly moving the anterior region of the body or the stylet but lacking clear rhythmic muscular movements along the body.

3) SON treatment : axenic J2 suspended in sterile water and sonicated in ± 60-second bursts with a Branson Sonic Power sonicator until all juveniles appeared to be broken when examined through a light microscope (40 bursts, 60 Watt).

4) SDS- treatment : axenic J2 heated in 5 ml sodium dodecyl sulphate-containing 0.1 M tris-HCl buffer at 100 °C in a water bath for 2 min (STP buffer; Redigarri *et al.,* 1986); they appeared to be broken when examined through a light microscope.

5) SDS+ treatment : following the SDS-treatment, J2 were washed in sterile water three times in a centrifuge (5000 rpm, 10 min); appearing broken when subsequently observed through a light microscope.

6) SON/SDS- treatment : axenic J2 were suspended in buffer (0.05 M tris-HCL containing 1 mM phenylmethylsufonyl fluoride, Ph = 7) and sonicated in 60-second bursts with a Branson Sonic Power sonicator until J2 appeared to be broken when examined with a light microscope. The nematode suspension was centrifuged for 10 min at 2000 rpm and washed in the tris buffer before being treated with the SDS containing buffer at 100 °C in a water bath for 2 min.

7) SON/SDS+ treatment : After the SON/SDS-treatment the J2 were washed in sterile water in a cen-

trifuge (5000 rpm, 10 min) to remove residual detergent.

8) 100 °C heating : axenic J2 were heated at 100 °C in a water bath for 2 min; the J2 appeared broken when observed with a light microscope.

9) NaN_3 treatment : axenic J2 were killed by suspension in sodium azide (0.1 M NaN_3) for 24 h and were washed subsequently in sterile water in a centrifuge (5000 rpm, 10 min). Sodiumazide inhibits the respiration process (Schlegel, 1986). The juveniles appeared intact when observed through the light microscope.

Experiment 2

Also the response of the fungus was studied, after addition of axenic irradiated J2 of *M. hapla*. They have been exposed to gamma irradiation from a cobalt-60 source (at the Pilot plant for Food Irradiation, Wageningen) in a small amount of sterile water in a Greiner. The doses, ranging from 0.50 to 100 kGy, were realized by varying distance from the source and exposure time.

This range was chosen using results on irradiation of other species : about 1 kGy was needed to immobilize *Ditylenchus dipsaci* (Green & Webster, 1965), 7 and 13 kGy was needed to kill juveniles of *Trichinella spiralis* and *Panagrellus* sp. respectively (Myers & Dropkin, 1959; Myers, 1960) and about 10 kGy was needed to break the juveniles of *Ditylenchus dipsaci* (Green & Webster, 1965).

In preliminary studies no differences were found in the reaction of 28 day-old fungal colonies to J2 irradiated with doses from 0.50, 1.00, 1.50 and 1.75 kGy, in comparison to untreated juveniles. In a second series, juveniles exposed to 2, 4, 20 and 100 kGy were added to 28 day-old fungal colonies.

In Experiment 1 and 2 about 100 J2 were added to 28-day old fungal colonies (volume 80-100 µl). Each treatment was repeated three times and as a control axenic J2 were added to the fungal colonies.

Fungal activity was observed at regular time intervals with a light microscope (Zeiss Axiovert 10) and a distinction was made between : J2 captured by adhesive hyphae and surrounded by ring structures, J2 infected via the body orifices and J2 surrounded by a loose mesh.

Response of the mycelium of *A. oligospora* to simultaneous addition of live/dead nematodes

In order to analyze the effects of the presence of dead nematodes on the capture and infection of live nematodes, gamma-ray killed J2 were added together with or 14 days before the live J2.

Aliquots counting 50 or 500 gamma-ray killed J2 (10 kGy) were pipetted over a 1 cm^2 area left and right from the centre of the Petri-dish. In each test, a drop adjusted to contain about 50 living nematodes was added to the fungal cultures. Assays usually consisted of three replicate plates and the experiment was repeated twice.

In all experiments, the response of the fungus was observed at regular time intervals with a light microscope. A distinction was made between : juveniles surrounded by ring structures, J2 infected via the body orifices and J2 surrounded by a loose mesh (and in the live nematodes attachment to hyphae was included).

Development of trophic hyphae was quantified by counting the number of J2 containing trophic hyphae.

Also the relative length of every nematode body containing trophic hyphae was determined at intervals (days 1, 2, 3, 6, 10, 14, 17, 22, 29). Five classes were distinguished : 0, 25, 50, 75 and 100 % body length filled with hyphae. T_{50} and T_{95} (days after addition when 50 and 95 % of the nematode body length was filled with trophic hyphae) were estimated and subsequently analyzed by analysis of variance followed by Student's t-test for pairwice comparison of treatments ($\alpha = 0.05$; den Belder & Jansen, 1994).

Light and fluoresence microscopy

Observations were made using a Zeiss Axiovert 10 light microscope (objective, plan-neofluar 20 × long distance) and differential interference contrast (DIC). Images were documented using Kodak Ektachrome 160. In several cases a fluorescent mycology stain was used (Fungi-Fluor, Polysciences, Inc., Warrington) to visualize trophic hyphae and observations were made using the same microscope with fluorescence illumination (filter block 2 Fl, 365-440 nm).

Low-temperature scanning electron microscopy

For detailed observations of fungal structures, cryo-SEM was employed. Specimens were mounted on custom designed copper stubs and immediately frozen by immersion in a nitrogen slush (60 K) in the EMSCOPE SP2000 Cryogenic-Preparation System, etched by conductive heating and subsequently sputtered with gold for 2 min. (den Belder *et al.*, 1993). The frozen specimens were transferred to the scanning electron microscope (Jeol JSM 35C) which had been modified with a cryo-stage to maintain specimens at a temperature of 110 K.

Results

Response of the mycelium of *A. oligospora* to vitality of *M. hapla*

Experiment 1

Significant differences were observed in the reaction of the fungus to axenic juveniles and juveniles killed by sonification, heating or a respiration inhibitor (Table 1). Juveniles immobilized after 24 h at 35 °C except for slight head and stylet movements still induced ring

Table 1. *Effects of physio-chemical treatments of second-stage juveniles of* Meloidogyne hapla *on the response of the mycelium of* Arthrobotrys oligospora *(CBS 289.82).*

Treatment of nematodes		Days-after addition	No reaction of the fungus*	Vegetative hyphae*	Corkscrew-like structures*	Ring structures*
None	Active juveniles	1				90
		7				100
35 °C heating	Partly paralysed	1				90
	juveniles with intact cuticle	7				100
SON**	Broken juveniles	1		25		
		7		100		
SDS-***	Broken juveniles	1	100			
	with SDS-residues	7	100			
		21	100			
SDS +	Broken juveniles	1		40		
		7		100		
SON/SDS-	Broken juveniles	1	100			
	with SDS residues	7	100			
		21	100			
SON/SDS +	Broken juveniles	1		40		
		7		100		
100°C heating	Broken juveniles	1		25		
		7		100		
NaN_3	Dead juveniles with	1			80	
	intact cuticle	7			100	

* % of juveniles
** SON = sonification
*** SDS = sodium dodecyl sulphate

structures. None of the dead J2 stimulated the development of ring structures. In treatments where SDS was not removed from the J2 (SDS- and SON/SDS-) the fungus did not respond to the presence of juveniles (Fig. 1A). In those treatments where J2 were broken due to sonification or heating (SON, SDS +, SON/SDS + and 100 °C heating), the J2 were surrounded by many vegetative hyphae resulting in a mesh totally overgrowing the J2 (Fig. 1B, 2A).

In treatments where the J2 were dead but not broken (NaN_3) the fungus responded by forming a corkscrew-like structure penetrating the buccal cavity (or in some cases the anus) of the nematode (Fig. 1C). The hypha forming this spiral structure tended to be of the same diameter as the vegetative hyphae (Fig. 2B). Subsequently trophic hyphae developed throughout the nematode body (Fig. 2C). In some cases vegetative hyphae grew closely alongside the nematode body but never as many as in the SON, SDS + or the SON/SDS + treatments.

Experiment 2

Irradiated juveniles (0.5 to 1.75 kGy) or untreated juveniles did not differ as inducers of capture and ring structure development in *A. oligospora* (CBS 289.82) when added immediately after the irradiation of the juveniles. However, increased radiation doses applied to juveniles (2 kGy and higher) resulted in significant differences in the reaction of the fungus. The fungus reacted positively by forming a corkscrew-like structure, approaching the buccal cavity of the nematode similarly as in the case juveniles killed with NaN_3 (Fig. 2B). At 2 kGy, also ring structures were induced around all moving J2 (60 %) (Table 2). Irrespective of the formation of these ring structures, also 70 % of all J2 were approached by corkscrew-like structures. Both types of responses occurred finally on 30 % of the J2 (Fig. 2D). At 4, 20 and 100 kGy ring structures were not formed at all, neither at the start of the experiment nor after digestion of the nematode body. The J2 were invaded only after the formation of the corkscrew-like structures near the buccal cavity and in some cases the anus (Table 2).

Development of trophic hyphae through the nematode body occurred both following either development of ring structures or development of corkscrew-like structures.

Sixteen days following addition trophic hyphae were developed in all J2 radiated with 2 to 20 kGy gamma irradiation. However in 20 % of the nematodes irradiated with 100 kGy no development of trophic hyphae occurred.

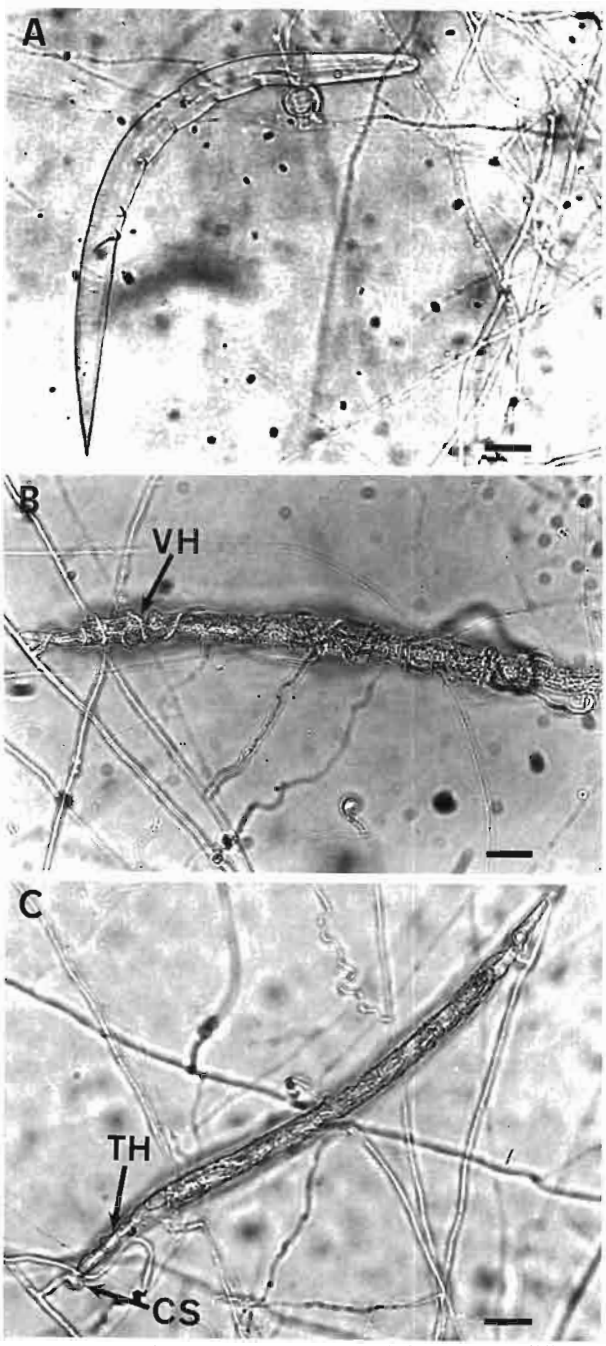

Fig. 1. Arthrobotrys oligospora *(CBS 289.82) on differently treated second-stage juveniles of* Meloidogyne hapla. *A : After SDS- or SON/SDS- treatment of juveniles the fungus did not respond to the presence of juveniles; B : After sonication of juveniles, the fungus formed a meshwork of vegetative hyphae (VH); C : After NaN_3 treatment of juveniles (in which the nematodes were dead but not broken) the fungus responded by forming a corkscrew-like structure (CS) approaching the buccal cavity (or in some cases the anus). Subsequently trophic hyphae (TH) were formed.* (Bar = 30 µm.).

Table 2. *Effects of gamma irradiation of second-stage juveniles of* Meloidogyne hapla *on the response of the mycelium of* Arthrobotrys oligospora *(CBS 289.82).*

Dose of gamma-irradiation (kGy)	Days after addition	Nematodes surrounded by ring structures (%)	Nematodes approached by a corkscrew-like structure (%)
0	1	60	0
	10	100	0
1.75	1	60	0
	10	100	0
2	1	60	30
	10	60	70
4	1	0	60
	10	0	100
20	1	0	60
	10	0	100
100	1	0	70
	10	0	95

Response of the mycelium of *A. oligospora* to simultaneous addition of live/dead nematodes

At the first observation, one day after addition of living nematodes, all juveniles of *M. hapla* were captured by the hyphae of the 28 day-old fungal cultures irrespective of addition of 50 or 500 irradiated J2 or the timing of the addition of the live J2 (Table 3). The presence of live nematodes did not affect the proportion of dead nematodes invaded through a corkscrew-like structure. The proportion of the irradiated juveniles with a corkscrew-like structure in front of the buccal cavity increased from 60 % after one day to 90 % two days after addition of nematodes.

Subsequent development of ring structures around the J2 differed significantly between treatments : addition of dead J2 prior to live J2, stimulated the ring structure development around the latter. One day after addition of the live nematodes (no dead nematodes were added) about 15 % more J2 were surrounded by ring structures than when dead nematodes were added two weeks earlier ($P < 0.0001$).

Development of trophic hyphae in those J2 that were added alive was not significantly influenced by the presence of dead J2 neither when 50 nor when 500 juveniles were added (Table 4). However, development of trophic hyphae through irradiated nematodes was significantly slower if live J2 were added at the same time. This resulted in significantly more days needed to reach 50 % or 95 % nematode body length filled with trophic hyphae.

Discussion

It is clear from this study that the condition of the nematode, in this case living, inactivated or dead J2 of

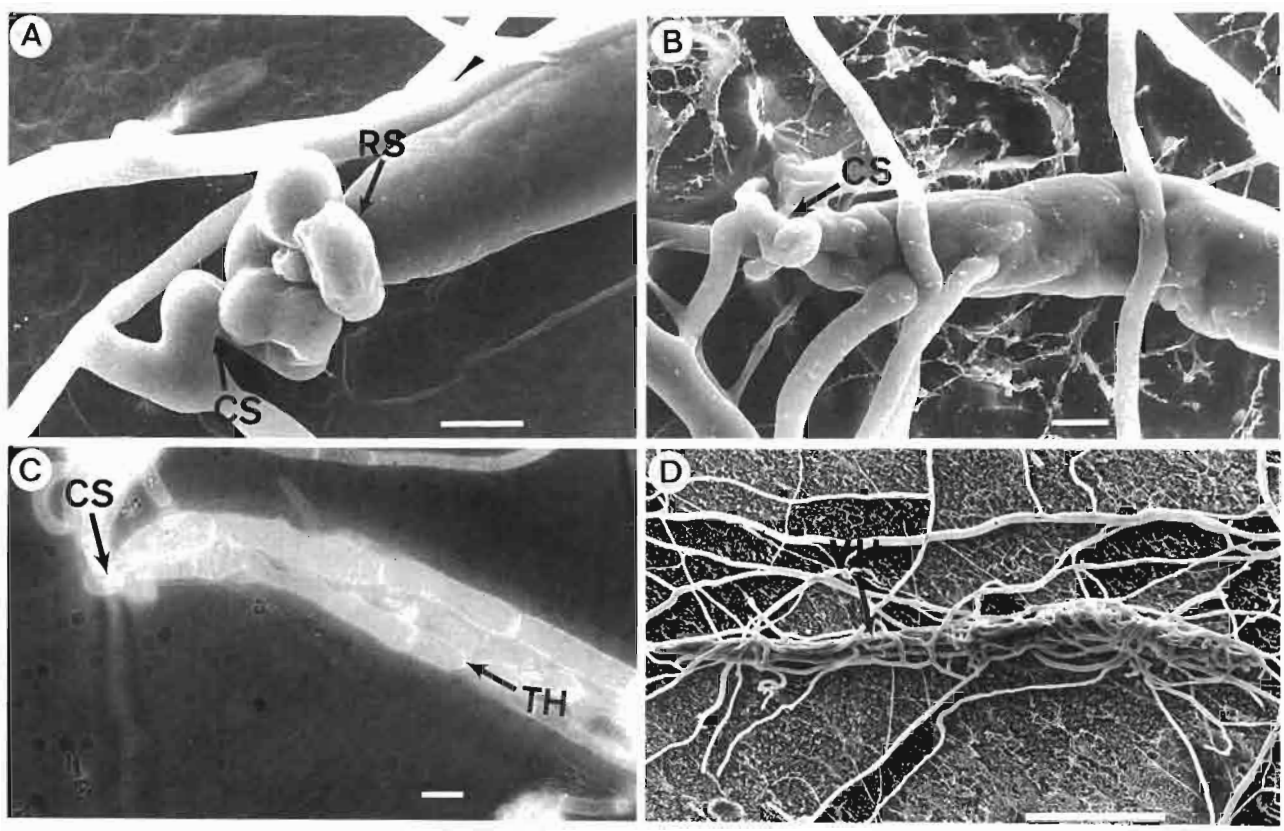

Fig. 2. *A :* Arthrobotrys oligospora *on broken, sonicated second-stage juveniles of* Meloidogyne hapla, *six days after addition of second-stage juveniles to the fungal colony; B : Corkscrew-like structure (CS) of* Arthrobotrys oligospora *approaching the buccal cavity of a second-stage juvenile of* Meloidogyne hapla; *C : Trophic hyphae (TH) of* Arthrobotrys oligospora *in a second-stage larva of* Meloidogyne hapla *killed by gamma-irradiation (CS = corkscrew-like structure); D : simultaneous development of a ring structure (RS) and a corkscrew-like structure (CS) in a gamma-ray irradiated second-stage juvenile of* Meloidogyne hapla *(dose 2 kGy).* (Bar equivalents : A = 100 μm; B, D = 10 μm; C = 5 μm.)

Table 3. *Response of* Arthrobotrys oligospora *(CBS 289.82) on living second-stage juveniles of* Meloidogyne hapla *when gamma-ray killed juveniles were added before or at the same time to four weeks old fungal colonies.*

Addition of nematodes		Results				
		% living nematodes captured by hyphae	% living nematodes surrounded by ringstructures		% dead nematodes with corkscrew-like structure	
At day 1	At day 14	One day after addition	One day after addition	Two days after addition	One day after addition	Two days after addition
0 dead + 50 living	+ 0 living	100	73.1 ± 11.2 *ab*	98.0 ± 1.4 *a*		
50 dead + 50 living	+ 0 living	100	72.2 ± 13.4 *ab*	98.0 ± 1.2 *a*	60.1 ± 12.3 *a*	94.1 ± 1.2 *a*
50 dead + 0 living	+ 50 living	100	88 ± 2.3 *b*	99.1 ± 0.6 *a*	60.7 ± 3.8 *a*	90.6 ± 2.3 *a*
500 dead + 50 living	+ 0 living	100	64.2 ± 2.5 *a*	100 ± 0.0 *a*	58.7 ± 2.7 *a*	88.0 ± 2.0 *a*
500 dead + 0 living	+ 50 living	100	92.3 ± 0.8 *b*	100 ± 0.0 *a*	59.3 ± 3.8 *a*	87.6 ± 5.2 *a*
0 dead + 0 living	+ 50 living	100	75.2 ± 1.9 *a*	100 ± 0.0 *a*		

Different letters indicate significant differences between the means in the column (Student's t-test, $p > 0.05$).

Table 4. *Development of trophic hyphae of* Arthrobotrys oligospora *in second stage juveniles expressed in days after addition when 50 % and 95 % of the nematode body was filled with trophic hyphae (T_{50} and T_{95}).*

Addition of nematodes		Results			
		T_{50}		T_{95}	
At day 1	At day 14	Living nematodes	Dead nematodes	Living nematodes	Dead nematodes
0 dead + 50 living	+ 0 living	10.7 ± 0.9 *a*		20.4 ± 1.6 *a*	
50 dead + 50 living	+ 0 living	11.3 ± 0.3 *a*	15.4 ± 1.3 *a*	23.0 ± 0.6 *a*	30.6 ± 6.9 *a*
50 dead + 0 living	+ 50 living	11.6 ± 0.7 *a*	9.1 ± 0.6 *b*	19.9 ± 1.8 *a*	19.7 ± 1.4 *b*
500 dead + 50 living	+ 0 living	12.7 ± 0.6 *a*	13.7 ± 0.3 *a*	25.2 ± 2.0 *a*	26.5 ± 0.6 *a*
500 dead + 0 living	+ 50 living	12.5 ± 0.1 *a*	9.1 ± 0.2 *b*	23.4 ± 0.3 *a*	17.8 ± 1.7 *b*
0 dead + 0 living	+ 50 living	11.0 ± 0.4 *a*		20.0 ± 0.4 *a*	

Different letter indicate significant differences between the means in the columns (Student's t-test, $p > 0.05$).

M. hapla is of decisive importance for the response of the mycelium of *A. oligospora* (CBS 289.82).

Live J2, only able to move the head or the stylet, are principally surrounded by ring structures around the head. Similarly in juveniles attached to adhesive hyphae, ring structures developed in 50 % at the initial attachment site but at the sites the nematodes were vigorously moving (den Belder, unpubl.). Signals triggering ring structure formation include direct contact of the living nematode with the hyphae (Nordbring-Hertz, 1987). A positive correlation between nematode motility and the ability to induce traps in *A. oligospora* (ATCC 24927) was found by Jansson and Nordbring-Hertz (1980). Initiation of ring formation could be the consequence of changes in membrane potential following nematode movement (Nordbring-Hertz, 1977).

Besides physical stimuli, the importance of specific chemical cues in the formation of fungal structures have been illustrated (Charnley, 1989). The lack of a response to nematodes might have been due to the fact the nematode was unrecognizable in the presence of sodium dodecyl sulphate. On the other hand the fungus might have avoided contact with this aggressive detergent. It confirms that morphogenesis in fungi seems to be easily affected by inhibitory concentrations of various chemicals and volatiles (Nordbring-Hertz, 1987).

Dead nematodes with an intact cuticle, were invaded by the fungus after development of a corkscrew-like structure. In contrast, the fungus reacted to nematodes with a broken cuticle by totally overgrowing them with thin vegetative hyphae along the nematode cadaver which was colonized. Growth of vegetative hyphae into intact nematodes as described by Nordbring-Hertz (1968), was never observed.

Recognition of the status of the prey elicits a defined functional and morphological response. When juveniles were irradiated with a non-lethal dose of gamma irradiation, in 30 % of the nematodes a ring structure and a corkscrew-like structure developed. This phenomenon might be explained by the fact that during the course of the experiment the nematode died. Stimuli initially given by the living juvenile might have been absent finally. Whether the fungus responds to chemical or to physical stimuli that might very well differ according to viability of the nematode in a way comparable to that of spores of plant-pathogenic fungi to plants (Wynn, 1976; Hoch *et al.*, 1987; Bourett & Howard, 1990), warrants further research.

The lack of response to nematode treated with very high doses gamma irradiation (100 kGy) clearly illustrates the role of chemical stimuli.

Nematodes filled with trophic hyphae of *A. oligospora* (ATCC 24927) as observed in *Panagrellus redivivus* 24 h after attachment to ring structures (Nordbring-Hertz *et al.*, 1986) or complete consumption of juveniles of *Neoaplectana* or *Heterorhabditis* after three days, conidia emerging from the cadavers were never observed in *M. hapla* (Poinar & Jansson, 1986). Our results showed that the fungus needed ten days to occupy 50 % of the nematode body length.

When living and dead J2 were added together to the fungal colony attachment to the live J2 was not diminished, presenting evidence that the presence of food in the form of dead J2 did not affect attachment. This confirms former results where nutritional conditions ranging from simple to more complex, did not influence nematode attachment (den Belder & Jansen, 1994).

An increased proportion of nematodes with ring structures one day after addition of the live nematodes indicates that the fungus apparently uses the nutrient supply from the dead J2 in the development of ring structures or is triggered/stimulated by the dead nematodes, to produce such structures. This refutes the hypothesis that capture structure development would be increased at low nutrient concentrations (Cooke, 1963 *a, b*). On the contrary, the fungus appeared to be more capable of forming ring structures. This agrees with the finding that the development of ring structures

occurred sooner on corn meal agar than on water agar (den Belder & Jansen, 1994) and also with the observation that an increase in nutrient level of the medium resulted in an increase in coiling frequency of *A. oligospora* (ATCC 24927) around *Rhizoctonia solani* (Persson & Bååth, 1992).

A delay in ring structure development on water agar in comparison with other media (den Belder & Jansen, 1994) might be compensated by the presence of dead nematodes.

The presence of living J2 did not affect the proportion of dead J2 infected through a corkscrew-like structure by *A. oligospora* (CBS 289.82). However, trophic hyphae grew more slowly through dead J2 when live ones were present than when dead J2 were added alone. Our results do not only show that this fungus is able to feed on live and dead nematodes simultaneously, but also that development of trophic hyphae through living J2 occurred sooner than through dead J2. Living nematodes not only form an alternative nutrient source (Blackburn & Hayes, 1966) but they are digested more rapidly by the fungus. This isolate of *A. oligospora* shows, through the adaptation of vegetative hyphae, a placticity to exploit different nutrient sources : the same mycelium can adapt in such a way that living nematodes are attached and subsequently surrounded by ring structures or dead material is penetrated through the formation of a corkscrew-like structure or covered by mycelium so that the nutrients will be used. This isolate of *A. oligospora* does produce different vegetative structures in response to changing conditions encountered by a dynamic mycelium growing under heterogeneous conditions. Thus different parts of the colony may fulfil different functions. There is no evidence in this isolate for a limited saprophytic ability.

Acknowledgements

We thank Dr. C. J. H. Booij, Prof. Dr. L. Brussaard, Dr. J. W. D. M. Henfling, Ing. P. W. T. Maas and Dr. L. J. M. F. den Nijs for their comments on the manuscript, G. van der Wal and W. Bekers for their assistance with the experiments, F. Thiel and K. Hulsteijn for their assistance operating the cryo-SEM, A. Koedam for his help with the photographs. Ing. E. van Remortel and Dr. J. de Bree for their help with the statistical analysis.

A part of his work was supported by the Programme Committee on Agricultural Biotechnology.

References

DEN BELDER, E., BOEKESTEIN, A., VAN ESCH, J. W. J. & THIEL, F. (1993). Low-temperature scanning electron microscopy of fungus-nematode interaction. *Scanning,* 15 : 37-42.

DEN BELDER, E. & JANSEN, E. (1994). The influence of temperature, nutrient quality, light and the growth time of the mycelium on capture and infection of *Meloidogyne hapla* by an adhesive hyphae former *Arthrobotrys oligospora*. *Fundam. appl. Nematol.*, 17 : 57-66.

BLACKBURN, F. & HAYES, W. A. (1966). Studies on the nutrition of *A. oligospora* Fresn. and *A. robusta* Dudd. I. The saprophytic phase. *Ann. appl. Biol.*, 58 : 43-50.

BOURETT, T. M. & HOWARD, R. J. (1990). *In vitro* development of penetration structures in the rice blast fungus *Magnaporthe grisea*. *Can. J. Bot.*, 68 : 329-342.

CHARNLEY, A. K. (1989). Mechanisms in fungal pathogenesis in insects. *In :* Whipps, J. M. & Lumsden, R. D. (Eds). *Biotechnology of fungi for improving plant growth.* Cambridge University Press : 85-125.

COOKE, R. C. (1963 *a*). Ecological characteristics of nematode-trapping Hyphomycetes. I. Preliminary studies. *Ann. appl. Biol.*, 52 : 431-437.

COOKE, R. C. (1963 *b*). The predacious activity of nematode-trapping fungi added to soil. *Ann. appl. Biol.,* 51 : 295-299.

COOKE, R. C. (1977). Fungi that capture microscopic animals. *In :* Cooke, R. C. (Ed.). *The biology of symbiotic fungi.* John Wiley & Sons, London : 15-37.

COOKE, R. C. & WHIPPS, J. M. (1980). The evolution of modes of nutrition in fungi parasitic on terrestrial plants. *Biol. Rev.*, 55 : 341-362.

COOKE, R. C. & WHIPPS, J. M. (1987). Saprotrophy, stress and symbiosis. *In :* Rayner, A. D. M., Brasier, C. M. & Moore, D. (Eds). *Evolutionary biology of the fungi.* Cambridge University Press : 137-148.

DACKMAN, C., OLSSON, S., JANSSON, H. B., LUNDREN, B. & NORDBRING-HERTZ, B. (1987). Quantification of predatory and endoparasitic fungi in soil. *Microb. Ecol.*, 3 : 89-93.

FERMOR, T. R. & WOOD, D. A. (1981). Degradation of bacteria by *Agaricus bisporus* and other fungi, *J. gen. Microbiol.*, 126 : 377-387.

FRITSCH, A. & LYSEK, G. (1989). Nematode-capturing hyphomycetes from soils over xerophytic calcareous rock in Upper Bavaria. *Bot. Acta,* 102 : 270-275.

GRAY, N. F. (1983). Ecology of nematophagous fungi : *Panagrellus redivivus* as the target organism. *Plant and Soil,* 73 : 293-297.

GREEN, C. D. & WEBSTER, J. M. (1965). The effect of ultraviolet radiation on the stem and bulb nematode *Ditylenchus dipsaci* (Kühn). *Nematologica,* 11 : 638-642.

HAYES, W. A. & BLACKBURN, F. (1966). Studies on the nutrition of *Arthrobotrys oligospora* Fres. and *A. robusta* Dudd. II. The predacious phase. *Ann. appl. Biol.*, 58 : 51-60.

HOCH, H. C., STAPLES, R. C., WHITEHEAD, B., COMEAU, J. & WOLF, E. D. (1987). Signalling for growth orientation and cell differentiation by surface topography in *Uromyces*. *Science,* 235 : 1569-1662.

JAFFEE, B. A. & ZEHR, E. I. (1985). Parasitic and saprophytic abilities of the nematode-attacking fungus *Hirsutella rhossiliensis*. *J. Nematol.,* 17 : 341-345.

JAMES, A. W. & NOWAKOWSKI, R. N. (1968). The effect of carbon sources on the trap formation by *Arthrobotrys conoides*. *Can. J. Microbiol.,* 14 : 1260-1261.

JANSSON, H. B. (1982). Predacity by nematophagous fungi and its relation to the attraction of nematodes. *Microb. Ecol.,* 8 : 233-240.

JANSSON, H. B. & NORDBRING-HERTZ, B. (1979). Attraction of nematodes to living mycelium of nematophagous fungi. *J. gen. Microbiol.,* 1121 : 89-93.

JANSSON, H. B. & NORDBRING-HERTZ, B. (1980). Interactions between nematophagous fungi and plant-parasitic nematodes : attraction, induction of trap formation and capture. *Nematologica,* 26 : 383-389.

JATALA (1986). Biological control of plant-parasitic nematodes. *Ann. Rev. Phytopathol.,* 24 : 453-489.

LEWIS, D. H. (1972). Concepts in fungal nutrition and the origin of biotrophy. *Biol. Rev.,* 48 : 261-277.

DE LEY, F. A. A. M. (1992). *The significance of ecology in the development of* Verticillium chlamydosporium *as biological control agent against root-knot nematodes* (Meloidogyne *spp.*). Ph. D. Thesis, Dept. Nematol., Agric. Univ. Wageningen, The Netherlands, 140 p.

LUTTRELL, E. S. (1974). Parasitism of fungi on vascular plants. *Mycologia,* 66 : 1-15.

MYERS, R. F. (1960). The sensitivity of some plant-parasitic nematodes and free-living nematodes to gamma and X-irradiation. *Nematologica,* 5 : 56-63.

MYERS, R. F. & DROPKIN, V. H. (1959). Impracticability of control of plant-parasitic nematodes with ionizing radiations. *Pl. Dis. Reptr,* 43 : 311-313.

NORDBRING-HERTZ, B. (1968). The influence of medium composition and additions of animal origin on the formation of capture organs in *Arthrobotrys oligospora*. *Physiol. Pl. Pathol.,* 21 : 52-65.

NORDBRING-HERTZ, B. (1977). Nematode-induced morphogenesis in the predacious fungus *Arthrobotrys oligospora*. *Nematologica,* 23 : 443-451.

NORDBRING-HERTZ, B. (1987). Ecology and recognition in the nematode-nematophagous fungus system. *In :* Marshall, K. C. (Ed.). *Advances in microbial ecology,* New York, Plenum Press : 81-114.

NORDBRING-HERTZ, B., STÅLHAMMAR-CARLEMALM, M. (1978). Capture of nematodes by *Arthrobotrys oligospora,* an electron microscope study. *Can. J. Bot.,* 56 : 1297-1307.

NORDBRING-HERTZ, B., ZUNKE, U., WYSS, U. & VEENHUIS, M. (1986). Trap formation and capture of nematodes by *Arthrobotrys oligospora*. Film (C 1662), Institut für Wissenschaftlichen Film, Göttingen, Germany.

POINAR, G. O. & JANSSON, H. B. (1986). Infection of *Neoaplectana* and *Heterorhabditis* (Rhabditida : Nematoda) with the predatory fungi, *Monacrosporium ellipsosporum* and *Arthrobotrys oligospora* (Moniliales; Deuteromycetes). *Revue Nématol.,* 9 : 241-244.

PERSSON, Y. & BÅÅTH, E. (1992). Quantification of mycoparasitism by the nematode-trapping fungus *Arthrobotrys oligospora* on *Rhizoctonia solani* and the influence of nutrient levels. *FEMS Microbiol. Ecol.,* 101 : 11-16.

PRAMER, D. (1964). Nematode-trapping fungi. *Science,* 144 : 382-388.

QUINN, M. A. (1987). The influence of saprophytic competition on nematode predation by nematode-trapping fungi. *J. Invert. Pathol.,* 49 : 170-174.

REDDIGARI, S. R., JANSMA, P. L., PREMACHANDRAN, D. & HUSSEY, R. S. (1986). Cuticular collagenous proteins of second-stage juveniles and adult females of *Meloidogyne incognita :* isolation and partial characterization. *J. Nematol.,* 18 : 294-302.

SCHLEGEL, G. S. (1986). *General microbiology*. Sixth Ed, Cambridge University Press. 587 p.

WYNN, W. K. (1976). Appresorium formation over stomates by the bean rust fungus : response to surface contact stimulus. *Phytopathology,* 66 : 136-146.