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The influence of temperature, nutrition, light and the growth time of the mycelium on capture and infection of *Meloidogyne hapla* by *Arthrobotrys oligospora*

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Summary – An isolate of *Arthrobotrys oligospora* (CBS 289.82) captured the second-stage juveniles (J2) of a population of *Meloidogyne hapla* by attachment to its hyphae. The effects of temperature, substrate, light and ageing of fungal mycelium on the ability to capture nematodes was studied in the laboratory. Firm binding of the J2 of *M. hapla* to hyphae of *A. oligospora* (CBS 289.82) occurred within 1 h irrespective of temperature (between 5 to 30 °C) and irrespective of differences in nematode mobility. Subsequent development of ring structures (comparable to adhesive networks) around the nematode, however, was significantly slower at the lower temperatures (5 to 10 °C) than at the higher temperatures (15 to 30 °C). The colonization of the nematode by trophic hyphae was clearly affected by temperature. At temperatures below 15 °C development of trophic hyphae was significantly reduced in comparison to higher temperatures. Furthermore, the nutritional conditions tested did not correlate to the efficacy in nematode-hypha attachment, however the subsequent development of adhesive networks was delayed on water agar. The present results provide evidence that the trapping ability in the isolate tested continues over the whole test period of more than 70 days. Light dit not influence the ability of the fungus to capture nematodes. Our results suggest that the formation of an adhesive hypha is less temperature and nutrient dependent than the development of the morphologically more complex adhesive networks. This observation suggests that the range of conditions under which this isolate of *A. oligospora* captures nematodes is wider than in isolates where adhesive networks are the only capture devices, which makes this isolate interesting for evaluation as a possible control agent.

Résumé – ***Action de la température, du milieu nutritif, de la lumière et de la durée de la végétation du mycélium sur la capture et l'infestation de* Meloidogyne hapla *par une souche d'*Arthrobotrys oligospora *formant des hyphes collantes*** – Un isolat d'*Arthrobotrys oligospora* (CBS 289.82) capture les juvéniles de deuxième stade (J2) d'une population de *Meloidogyne hapla* par adhérence à ses hyphes. L'action de la température, du substrat, de la lumière et du vieillissement du mycélium sur l'aptitude à la capture des nématodes a été étudiée en laboratoire. Une liaison ferme entre J2 de *M. hapla* et hyphes d'*A. oligospora* (CBS 289.82) s'établit en moins d'une heure, et ce indépendamment de la température (entre 5 et 30 °C) et des différences dans la motilité du nématode. Le développement ultérieur de structures annulaires – comparables à des anneaux adhésifs – autour du nématode est, par contre, plus lent aux températures basses (5 à 10 °C) qu'aux températures plus élevées (15 à 30 °C). La colonisation du nématode par les hyphes trophiques est nettement affectée par la température. Aux températures inférieures à 15 °C, le développement de ces hyphes trophiques est significativement plus lent qu'aux températures plus élevées (15 à 30 °C). De plus, les conditions nutritives testées ne sont pas corrélées à l'attachement entre hyphes et nématode, encore que le développement ultérieur des réseaux adhésifs soit retardé sur le milieu eau-agar. Les résultats observés démontrent que la capacité de piégeage de l'isolat testé persiste pendant toute la période de l'expérience, soit plus de soixante-dix jours. La lumière n'a aucune influence sur l'aptitude du champignon à capturer les nématodes. Les résultats obtenus suggèrent que la température et les conditions nutritives influent moins sur la formation des hyphes adhésives que sur le développement ultérieur des réseaux adhésifs, beaucoup plus complexes morphologiquement. Ces observations laissent penser que la gamme des conditions dans lesquelles cet isolat d'*A. oligopora* peut capturer les nématodes est plus large que celle des isolats où les réseaux adhésifs constituent le seul système de capture. Cela confirme l'intérêt de cet isolat en vue de son évaluation comme agent possible de lutte biologique.

Key-words : *Arthrobotrys oligospora, Meloidogyne,* adhesive hyphae, temperature, nutrient level, light, ageing, capture, trophic hyphae.

Microorganisms need to be active in soil conditions which prevail in the field and to survive unfavourable conditions, in order to be effective and widely applicable as biological control agents against soil-inhabiting nematodes (Stirling, 1991). In former studies it was clearly demonstrated that *A. oligospora* (CBS 289.82) can attach to second-stage juveniles of *Meloidogyne hapla* and *M. incognita* by morphologically unmodified hyphae without the formation of adhesive networks. The chronological stages of the infection process are attachment nematode-hyphae, development of an adhesive network (generally composed by one ring structure if juveniles of *Meloidogyne* spp. are added), penetration and the formation of an infection bulb and subsequently the devel-

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opment of trophic hyphae throughout the nematode body.

Little is known about the influence of abiotic and biotic conditions such as temperature, light, substrate and the age of the mycelium, on the formation of the adhesive hyphae in nematophagous fungi and on subsequent capture and infection of nematodes. Gronvold (1989) found a significant effect of temperature on the adhesive network development in *Arthrobotrys oligospora* (ATCC 24927) : mycelium did not respond to juveniles or responded only slowly with the development of adhesive networks at temperatures below 15 °C. Also *Dactylella* spp. captured larger proportions of nematodes between 20 and 24 °C than at lower temperatures (Feder, 1963). The question arises if adhesive hyphae are active in a broader temperature range than adhesive networks. Generally nutrients available in the environment of the nematophagous fungus have effects on its metabolism and its morphogenesis (Blackburn & Hayes, 1966; Hayes & Blackburn, 1966). *In vitro* studies on the nutrition of nematophagous fungi, including *A. oligospora,* showed that mycelial growth and formation of nematode-induced adhesive networks are differently influenced by the composition of the media and extensive mycelial growth is not always correlated with high predacity. Few adhesive networks are developed in *A. oligospora* (ATCC 24927) on water agar (Nordbring-Hertz, 1977), whereas vegetative hyphae develop normally (Soprunov, 1966) and the question raise if the predacity of adhesive hyphae forming fungi is affected by composition of the media.

Morphological responses to light have been described for many fungi (Leach, 1971). Gronvold (1989) reported that light suppresses development of adhesive networks in *A. oligospora* (ATCC 24927), whereas Olthof and Estey (1965) did not observe any influence of light on the vegetative growth of another isolate of this fungus. Loss of virulence of old cultures of nematode-capturing fungi was observed by Couch (1937) and Feder (1963) but details on the age of the fungal colony were not given. Such loss of virulence by nematode-capturing fungi is of special significance because it may limit their usefulness for nematode control. More recent work by Heintz (1978) showed that ageing of mycelium of *A. dactyloides* and *A. cladodes* resulted in a reduction of the ability to capture nematodes. Loss of adhesiveness of adhesive networks of *Dactylella megalospora* was found within seven days (Esser *et al.,* 1991), while in adhesive networks of *A. oligospora* (ATCC 24927) this occurred within seven weeks, depending on temperature (Gronvold, 1989).

In the present paper the hypothesis has been tested that : 1) attachment of *Meloidogyne* spp. to adhesive hyphae of *Arthrobotrys oligospora* (CBS 289.82) is less temperature-dependent than the development of the adhesive networks themselves; 2) adhesive hyphae can be developed on a nutrient lower culture medium than adhesive networks; 3) light suppresses the formation of adhesive hyphae less than that of adhesive networks and 4) ageing of the fungal mycelium results in a reduction of attachment of nematodes to adhesive hyphae and subsequent ring structure formation and infection of nematodes.

Materials and methods

Organisms

Arthrobotrys oligospora strain CBS 289.82 was cultured on corn meal agar (Oxoid, CMA 1:1, 1.5 %) in Petri-dishes (diameter 8.8 cm) at 25 ± 1 °C, and transferred monthly to fresh medium. In experiments described below, generally the fungus was transferred by taking individual 4-mm plugs from the periphery of the actively growing stock culture. These were placed upside down in small Petri-dishes (Lux, diameter 44 mm) on CMA 1:10 and removed a few days after incubation. The Petri-dishes used for microscopical observation have a hole in the bottom covered by a coverglass glued to it, thus facilitating microscopic observations with an inverted microscope (Axiovert 10 equipped with an enhanced video system).

Meloidogyne hapla Chitwood originally isolated from rose plants, was obtained from the DLO-Centre for Plant Breeding and Reproduction Research, Wageningen. Since 1988, this nematode species were maintained continuously on tomato plants (*Lycopersicon esculentum* Mill. cv. Moneymaker) in riversand at a temperature of 20 °C in a greenhouse. Newly hatched second-stage juveniles were obtained by incubating egg masses on a 50 µm sieve in water for 2 days at 20 °C. The outer surface of 2 day-old juveniles of *Meloidogyne* spp. was sterilized in a mixture of 0.02 % (w/v) ethoxy-ethylmercury chloride (Aretan) and 0.1 % (w/v) streptomycin sulphate for about 2 h in a 10 ml conical centrifuge tube and subsequently washed three times in sterile water (s'Jacob & van Bezooyen, 1984). The juveniles had been acclimatized to the various test temperatures for two days.

Capture ability of the fungus and subsequent infection of nematodes were assessed after addition of a drop containing about 50 axenic second-stage juveniles of *Meloidogyne hapla* to each fungal colony.

All experiments were performed with *A. oligospora* (CBS 289.82) unless indicated otherwise.

Temperature experiments

The conditions during incubation and testing in the various temperature experiments are summarized (Table 1). Twenty-eight or 42 day-old cultures of *A. oligospora* grown at 25 °C (Experiments 1 and 2, respectively) were placed at each of the following constant temperatures, 48 h prior to the addition of nematodes : 5, 10, 15, 20, 25, 30 °C (and also 35 °C in Experiment 2) and tested at those temperatures. In Experiment 3, 78 day-old fungal cultures (grown for 42 days at 25 °C

and subsequently 36 days at 5, 10 or 15 °C) were tested while in Experiment 4 and 5 the cultures were grown and tested at 5, 10 and 15 °C (Table 1).

Table 1. *Experimental conditions of temperature experiments 1 to 5.*

	Time of culturing fungus before experiment (days)	Temperature during culturing before experiment (°C)	Test temperature (°C)
Exp. 1	28	25	5, 10, 15, 20, 25, 30
Exp. 2	42	25	5, 10, 15, 20, 25, 30, 35
Exp. 3	42	25	
	+ 36	5, 10, 15	5, 10, 15
Exp. 4	42	5, 10, 15	5, 10, 15
Exp. 5	28	15	

In experiments 1, 2 and 3 observations on numbers of juveniles captured by hyphae and on those surrounded by adhesive networks and filled with trophic hyphae started at day 1 and were repeated several times during one week. As in interactions with *Meloidogyne* spp. juveniles this network consisted of only one adhesive ring, the term « ring structures » has been used. Nevertheless this ring structure is the most simple form of an adhesive network as found in other species of the *Dactylaria*-complex.

Numbers were expressed as a percentage of the total number of nematodes counted immediately after the start of the experiment (at the start of the experiments the number of dead nematodes was nihil). In Experiment 4 observations on capture started 6 h after addition of the juveniles. In Experiment 5 observations commenced 1 h following addition of the juveniles.

At the end of each experiment the mycelial mat was examined for any morphological changes.

The proportion of nematodes captured by hyphae, surrounded by ring structures and filled with trophic hyphae were analyzed with a Generalized Linear Model (GLM) for binomial data (McCullagh & Nelder, 1989), leading to an analysis of deviance for quantal data and subjected to a Student's t-test for pairwise comparison of treatments on a logit-scale. This was only possible if the mean proportions were not equal to 0 or 1. For these means significance was obtained by considering confidence intervals. Analysis were carried out with Genstat (1987). All statistical tests were performed with a significance level of 0.05. In addition to the variables mentioned above, in Experiment 1 and 2 the colonization of each nematode was also determined at regular intervals (day 1, 2, 5, 8, 12, 15, 21, 27, 36, 44, 63) and expressed as a percentage of body length filled with trophic hyphae. Five classes were distinguished : 0 (no visible mycelium within body), 0-25, 25-50, 50-75 and 75-100 % body length with hyphae. The products of the total percentage counts in each of the groups and the midpoints of these groups was summed to give a percentage estimate of the body length filled with trophic hyphae. The technique is based on the assumption that the presence of trophic hyphae is indicative of colonization. T_{50} and T_{95} (days after inoculation when 50 and 95 % of the nematode body length was filled with trophic hyphae) were calculated under the assumption of logistic growth increase of trophic hyphae filled body length. T_{50} and T_{95} were estimated for each Petri-dish (containing about 50 nematodes) and subsequently analyzed by analysis of variance followed by Student's t-test for pairwise comparison of treatments. Because both experiments showed the same trends results are given for only one experiment.

Effect of temperature on activity of second-stage juveniles of *M. hapla*

Nematode activity was measured in order to analyze whether any temperature effects on the capture of nematodes was due to the ability of the fungus to capture juveniles of *M. hapla* or to the activity of the juveniles.

Fifty axenic second-stage juveniles of *M. hapla* (from the same batch as in Experiment 2) were inoculated in the centre of a Petri-dish with CMA 1:10, 1.5 % agar. Two hours after introduction, nematode mobility was assessed by counting the number of juveniles in four concentric zones of 5 mm from the inoculation point. Tests were performed at 5°, 10°, 15°, 20°, 25°, 30° and 35 °C.

For each temperature an average mobility was calculated using the following formula :

$$M = \sum_{i=0}^{i=3} f.Di$$

f = fraction of nematodes present in area i
Di = average distance (mm) from centre for area i

$i = 0$	$0 < r \leq 5$ mm	$Di = 5$ mm
$i = 1$	$5 < r \leq 10$ mm	$Di = 7.5$ mm
$i = 2$	$10 < r \leq 15$ mm	$Di = 12.5$ mm
$i = 3$	$15 < r \leq$ rim Petri-dish	$Di = 17.5$ mm

The nematode mobility for different temperatures was analyzed by using one-way ANOVA, followed by a Student's t-test for comparison of means.

Nutrient experiment

The fungus was grown on different substrates : water agar 1.5 %, corn meal agar (CMA, 1:10) and a low nutrient mineral salts medium with or without 200 µg thiamin/liter and 5 µg biotin/liter (LNM + and LNM- respectively, Nordbring-Hertz, 1973). Seven day and 28 day-old fungal cultures of *A. oligospora* grown at 25 °C, were inoculated with 50 axenic second-stage juveniles of *M. hapla* at 25 °C. Observations on number of attached and infected nematodes were made 1 and 6 h and 1, 2, 6 and 16 days after addition of nematodes. The average number of ring structures around the attached nematodes was counted one day after the start of the

experiment. Each treatment consisted of three replicates and the experiment was repeated three times.

The number of captured nematodes, nematodes surrounded with ring structures and nematodes with trophic hyphae were analyzed as described for the temperature experiments.

In order to analyze if differences in capture of nematodes were due to a higher frequency of nematodes encountering hyphae and or ring structures, mycelium growth, density and number of spontaneous ring structures (freely formed and not surrounding nematodes) were determined. Average mycelial growth rate (mm/day) was recorded as the average distance from the inoculated agar piece to the outer rim of the growing hyphae (at day 3 and 5 in ten replicates). The hyphal density, expressed as the area of mycelium mm^2 per 100 mm^2 was measured in two areas (each 50 mm^2) in each of 10 Petri-dishes, using the image analyser GOP-302 (Context Vision). The advantage of the used hardware was, that beside conventional grey level thresholding, structures could be detected by texture analysis. The actual detection of the hyphae was done with a line detection operation, which generated an output image in which the grey level intensity represented the amount of estimated line energy. By thresholding a certain grey level range in the output image, a selection of the most dominant lines could be made. So by variation of the lower threshold level a selection could be made of the hyphae on the surface or the hyphae growing through the agar. Because hyphae grown in the agar were a minor part of the total amount and did not influence the total density significantly, data are only given for those hyphae growing on the agar.

The areas in which the hyphae density was measured were selected by a computer controlled scanning stage on equal distance (1 cm) left and right from the initial inoculation point with the plug.

For each nutrient level in 28 day-old fungal colonies the hyphal density was estimated. Ring structure formation was observed one day before and one day after addition of nematodes, by counting the number of ring structures in 20 fields (at 200 × magnification). The effects of the growth time of the fungal mycelium on the capture and infection of nematodes were conducted as parts of the experiments on the temperature and nutrient level by using fungal cultures varying in growth time.

LIGHT EXPERIMENT

Twenty-eight days old cultures of *A. oligospora* were inoculated with 50 axenic second-stage juveniles of *M. hapla* and incubated at 25 °C. Three Petri-dishes were placed in the dark and three Petri-dishes were incubated under constant artificial light (Philips Pls lamp 11 Watt, wavelength 310-765 nm, 8.6 J/cm^2/h). Numbers of nematodes captured by hyphae, surrounded by ring structures and filled with trophic hyphae were counted at regular intervals (day 1, 2, 4, 7, 9, 11, 14, 23). The number of ring structures surrounding juveniles was counted 3 and 13 days after the start of the experiment. On each Petri-dish ten randomly selected fields of sight were examined (at 200 × magnification). Only completely closed ring structures were enumerated.

In one experiment two isolates of *A. oligospora* (ATCC 24927 and CBS 115.81) have been included under the same experimental conditions.

Results

TEMPERATURE

Effect of temperature on nematode-hypha attachment

At the first observation 24 h after inoculation of the nematodes, all second-stage juveniles of *M. hapla* were captured by the hyphae of 28 day-old cultures of *A. oligospora* at all temperatures below 35 °C (Experiment 1 and 2, Fig. 1). Nematodes became attached at any part of the body. At 35 °C, hyphae failed to attach to the nematodes.

Mycelium of *A. oligospora* grown under suboptimal temperatures for vegetative growth (Experiment 3 and 4, Fig. 1), did not show any decline in nematode attachment compared to hyphae grown at the optimum temperature for vegetative growth (Experiments 1 and 2, Fig. 1). In experiment 4, all nematodes were captured at the first observation after six hours, irrespective of the temperatures tested. At day 1 the development of the ring structures was started in all temperatures tested but was significantly lower at 5 °C (Fig. 1).

Immediate observations one hour after the start of Experiment 5 showed that all nematodes were captured at the temperatures tested.

Effect of temperature on ring structure development

Subsequent development of ring structures around the nematodes differed significantly between tested temperatures during the first six days after capture (Experiment 1, Fig. 1). At 5 and 10 °C only 1 and 2 % of the nematodes became surrounded, at 15 °C the number of nematodes surrounded by ring structures was 15 % and at 20, 25 and 30 °C this number was significantly higher. At day 2 these differences were still significant although less pronounced. At 5 and 10 °C the number of surrounded nematodes reached only 20 %, at 15 °C this number reached about 60 % and at the three highest temperatures tested, the proportion of nematodes surrounded by ring structures reached almost 100 %. At day 8 at all temperatures the ring structure development was 90 % or higher.

In the second temperature experiment the development of ring structures around nematodes progressed more rapidly (Fig. 1). After one day, almost all nematodes were surround by ring structures at 15 °C, whereas at 5 and 10 °C these numbers were less than 40 %. At days 7 and 8 all nematodes were surrounded at all temperatures ≤ 30 °C even when the mycelium was grown at low temperatures (Experiment 3 and 4; Fig. 1).

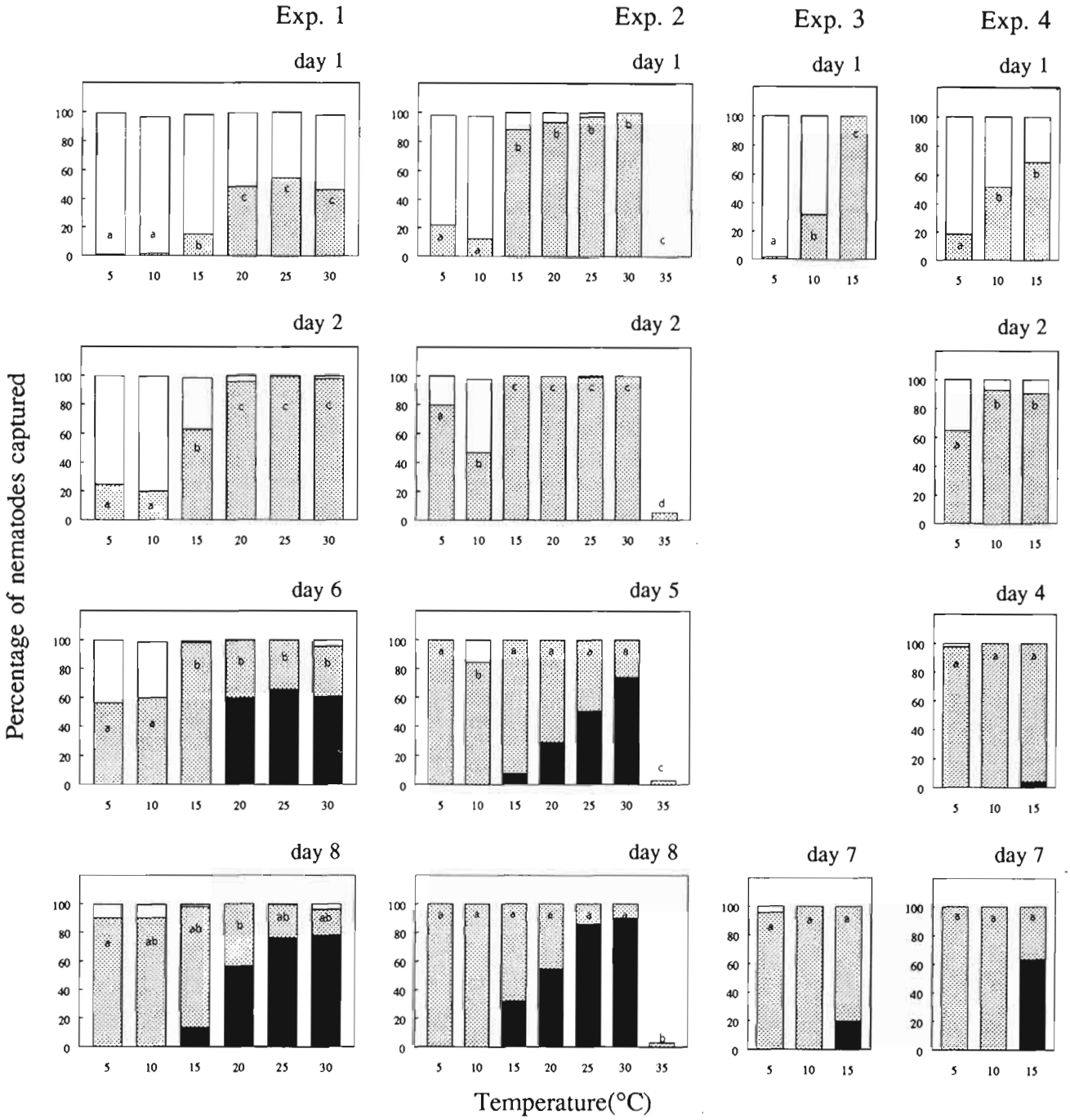

Fig. 1. *Ability of* Arthrobotrys oligospora *(CBS 289.82) to capture and infect second-stage juveniles of* Meloidogyne hapla *at different temperatures. – Exp. 1 : carried out with 28 day-old cultures at 25 °C followed by 2 days at the test temperatures; Exp. 2 as Exp. 1 but with 42 day-old cultures. Exp. 3 carried out with a culture grown for 42 days at 25 °C and followed by 36 days at the test temperatures; Exp. 4 carried out with a cultures grown for 42 days at the experimental temperatures. (Open bar : % of nematodes captured by adhesive hyphae; dotted bar : % of nematodes captures by adhesive hyphae and surrounded by ring structures; filled bar : % of nematodes captured by adhesive hyphae, surrounded by ring structures and at least filled with trophic hyphae. Different letters indicate significant differences between means of % of juveniles surrounded by ring structures within each experiment and each day; $P < 0.05$).*

Effect of temperature on trophic hyphae development

Development of trophic hyphae in the nematode body (Fig. 2) was significantly influenced by temperature. This resulted in significant differences in the number of days after which 50 % or 95 % of the nematode body length was filled with hyphae (Fig. 3, Table 2). In mycelium of *A. oligospora,* grown or kept under suboptimal conditions for vegetative growth (Experiment 3 and 4), development of trophic hyphae was not altered in comparison to the earlier experiments (Fig. 1).

Forty-two day-old cultures of *A. oligospora* did not show a decline in capture and infection of nematodes in comparison to 28 day old cultures (Experiment 2 and 1, respectively; Fig. 1).

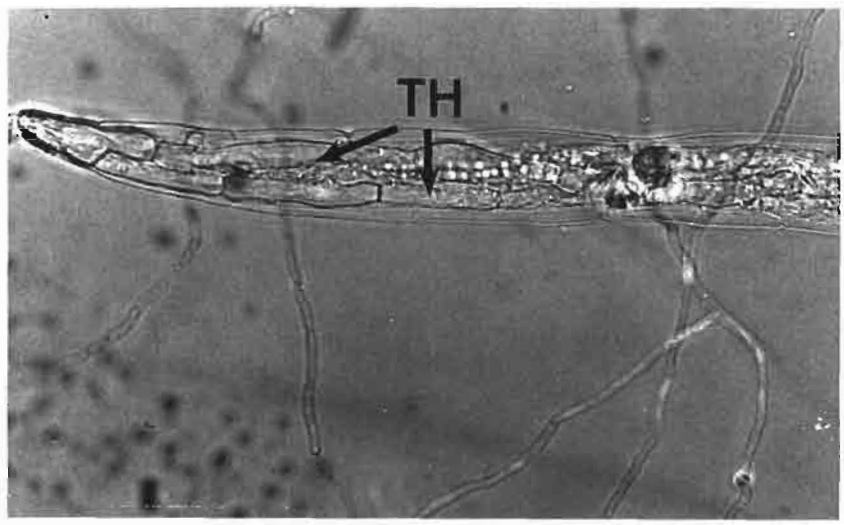

Fig. 2. *Trophic hyphae (TH) of* Arthrobotrys oligospora *(CBS 289.82) in a typical wave form in second-stage juveniles of* Meloidogyne hapla.

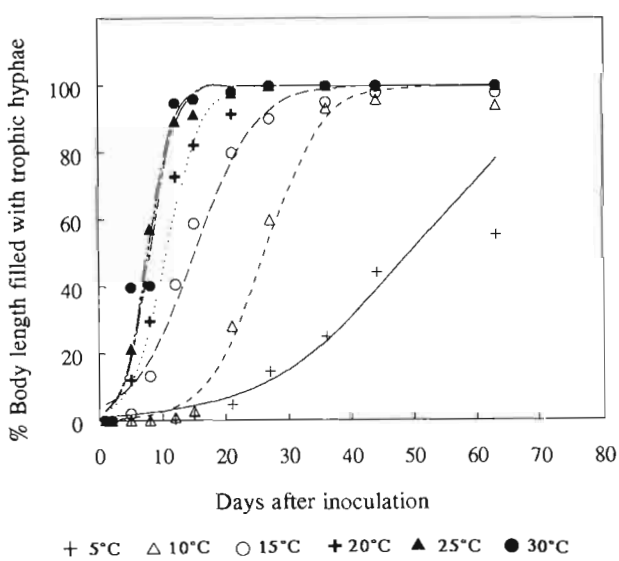

Fig. 3. *Development of trophic hyphae of* Arthrobotrys oligospora *(CBS 289.82) inside* Meloidogyne hapla *at different temperatures. Trophic hyphae development was quantified through estimation of the percentage of the nematode body length filled with trophic hyphae. Observed values are in markers, calculated values (Fieller-procedure in Genstat 5) in lines.*

Table 2. *Development of trophic hyphae of* Arthrobotrys oligospora *(CBS 289.82) in second-stage juveniles of* Meloidogyne hapla *at different temperatures, expressed in days after inoculation when 50 % and 95 % of the nematode body length was filled with trophic hyphae (T_{50} and T_{95}).*

Temperature (°C)	T_{50}		T_{95}	
5	47.6 ± 3.8[1]	a[2]	77.2 ± 8.9	a
10	24.8 ± 1.0	b	35.4 ± 4.7	b
15	15.1 ± 1.8	c	28.3 ± 2.8	bc
20	10.6 ± 0.8	cd	18.5 ± 0.3	cd
25	7.9 ± 0.3	d	13.4 ± 1.1	d
30	7.8 ± 0.9	d	13.5 ± 0.7	d
35	∝	n.t.	∞	n.t.

[1] Mean s.e.
[2] T_{50} and T_{95} were estimated for each Petri-dish and subsequently tested by analysis of variance followed by Student's t-test for pairwise comparison of treatments. Different letters indicate significant differences between means in the column ($P < 0.05$).
n.t. : not tested.

Capture ability of *A. oligospora* and subsequent infection by trophic hyphae did not show any decline after keeping mycelium at low temperatures during a prolonged period of time (Experiment 3, 78 days : 42 days

at 25 °C and subsequently 36 days at 5, 10 or 15 °C; Experiment 4, 42 days at 5, 10, 15 °C). In this respect there was no significant difference with Experiment 1. In none of the experiments morphologically aberrant hyphae were found in the time course of the experiments.

Nematode mobility in response to temperature

All tested juveniles of *M. hapla* dispersed over the agar. The nematode activity is shown in Fig. 4. Mobility differed significantly between the temperatures tested : at 25 °C the highest mobility was reached, 7.3 mm h^{-1}, while at 5 °C a mobility of 2.2 mm h^{-1} was observed.

EFFECT OF GROWTH SUBSTRATES ON CAPTURE ABILITY

The number of nematodes surrounded by ring structures and number of nematodes with trophic hyphae was affected by the different substrates (Fig. 5). Within one hour following addition of nematodes, all second-stage juveniles of *M. hapla* were captured by the hyphae of *A. oligospora,* irrespective of differences in hyphal density on the different media (Table 3). One day after the start of the experiment a higher percentage of nematodes was surrounded by ring structures on LNM + (76 %) as compared to LNM- and CMA 1:10 (61.0 and 63 %, respectively), while a significantly fewer juveniles were surrounded by ring structures (39 %) on WA. After 2 days this percentage increased to 84 % on WA and to about 97 % on the other substrates. The number of ring structures around the nematodes was significantly larger on LNM + and LNM –, than either on water agar or corn meal agar (Table 3).

At day 6 and 14 the development of trophic hyphae in the interior of the nematode body reached about 55 % and 85 % in LNM –, CMA and LNM +. On WA these percentages were significantly lower : in only 25 % and 50 % of the nematodes trophic hyphae developed.

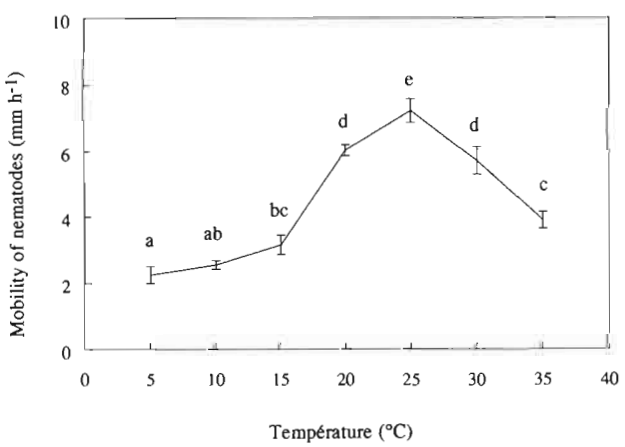

Fig. 4. *Mobility of second-stage juveniles of* Meloidogyne hapla *on CMA (1 : 10, 1.5 % agar) at different temperatures. (Different letters indicate significant differences between means. Analysis of variance followed by a Student's test; P < 0.05).*

EFFECT OF LIGHT ON CAPTURE ABILITY

As the results of all treatments in each of three experiments on the influence of light (8.6 Joule/cm^2/h, 310-765 nm) on attachment of juveniles to hyphae, development of ring structures and trophic hyphae were alike, detailed results are not given. Light did not influence the number of nematodes captured by *A. oligospora* by adhesive hyphae, nor did have any significant effect on the number of ring structures induced or the development of trophic hyphae in nematodes. At day 1 all nematodes already attached to the hyphae, while over 60 % of nematodes had been surrounded by ring structures. The number of ring structures surrounding juveniles (at 3 and 13 days after start of the experiments), was not influenced significantly by light (at day 3:3.3 and 3.6 and at day 13, 3.6 and 3.7 respectively). The first trophic hyphae were observed at day 4, irrespective light or dark regime and their growth was similar in both treatments.

Discussion

Part of research on nematode-capturing fungi has focused on the transition from saprophytic to predacious behaviour and factors that induce trap formation. For many species trap structure development appears conditioned by environmental factors. The observation that nematode-fungus attachment is not only realized by complex capture structures but can also be accomplished by not visibly differentiated vegetative hyphae (Den Belder & Jansen, unpubl.) raised the question whether adhesive hyphae are active in a temperature range or under nutrient conditions different from those that induce formation of adhesive networks.

The present work demonstrated that while temperatures between 5 and 30 °C clearly affected ring structure development, attachment of nematodes to hyphae of *A. oligospora* (CBS 289.82) was not affected. All active second-stage juveniles of *M. hapla* were attached to hyphae within a very short time after addition to fungal colonies, irrespective of the temperature even though the nematode mobility at 5 °C is one third of that at 25 °C. At the first observation, one hour after the addition of the nematodes, all juveniles observed were already attached to the hyphae at 5, 10 as well at 15 °C. This confirms earlier observations at 25 °C in which 28 out of 30 juveniles became attached to hyphae within 45 min (Den Belder & Jansen, unpubl.). At temperatures occurring in the field in temperate regions this fungal isolate and the juveniles of this nematode species are both sufficiently active to ensure capture if contact occurs.

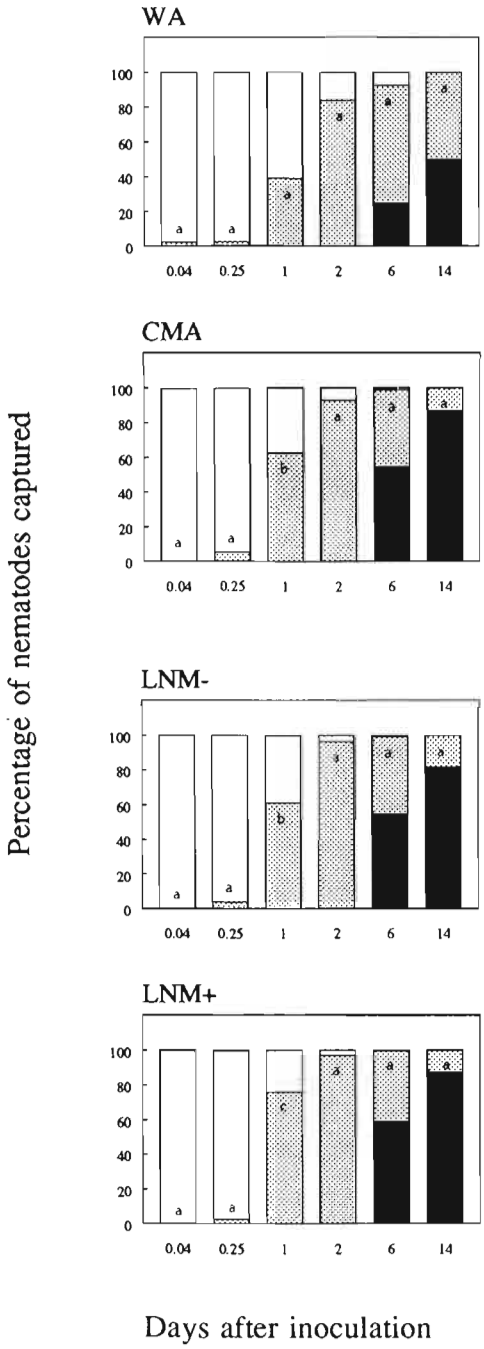

Fig. 5. *Ability of* Arthrobotrys oligospora *(CBS 289.82) to capture and infect second-stage juveniles of* Meloidogyne hapla *on different substrates; WA : water agar 1.5 %; CMA : corn meal agar 1 : 10, LNM + and LNM – : low nutrient mineral salts media respectively with and without 200 µg/liter thiamin and 5 µgr biotin/liter (Open bar : % of nematodes captured by adhesive hyphae; dotted bar : % of nematodes captured by adhesive hyphae and surrounded by ring structures; filled bar : % of nematodes captured by adhesive hyphae, surrounded by ring structures and filled at least partly with trophic hyphae. Different letters indicate significant differences between means of % of juveniles surrounded by ring structures within each day; P < 0.05).*

The temperature independent capture of nematodes by hyphae appears quite exceptional in comparison with fungi that capture nematodes through more complex structures only. Several authors reported that the nematode capturing fungi they studied, including other isolates of *A. oligospora,* did not respond to nematodes at low temperatures (Soprunov, 1966; Cayrol & Brun, 1975). Studies on the induction of adhesive networks in *A. oligospora* (ATCC 24927) showed a total failure at 5, 30 and 35 °C (Gronvold, 1989). Our isolate of *A. oligospora* also developed adhesive networks over a wider temperature range than isolates that have adhesive networks as the only capture device such as in isolate *A. oligospora* ATCC 24927 (Gronvold, 1989) or *A. superba* (Cayrol & Brun, 1975). Nevertheless at temperatures below 15 °C the development was significantly slower than at 15 to 30 °C.

Our results show that colonization of the nematode body by trophic hyphae was clearly affected by temperature. At temperatures below 15 °C development of trophic hyphae was very slow as compared to higher temperatures. Hence, under common soil temperatures of temperate regions colonization of this nematode species would be a very slow process.

The present investigation demonstrated that both hyphae of *A. oligospora* (CBS 289.82), developing on water agar and those growing on low nutrient salt media or corn meal agar, are able to capture all *M. hapla* juveniles present within one hour. Under nutritional conditions ranging from simple to more complex, the rate of nematode-hypha attachment did not appear to be influenced. A critical point or range in nutrients determining the development of capture devices, as suggested by Olthof and Estey (1966), was not observed for the attachment to hyphae grown on the media tested.

The delay in ring structure development on the water agar in comparison with other media, was similar to results obtained with several other isolates of *A. oligospora* (ATCC 24927) (Nordbring-Hertz, 1968; Jansson & Nordbring-Hertz, 1980). This observation is not in agreement with the hypothesis that capture structure development in nematophagous fungi would be increased when few energy sources are available (Cooke, 1962 *a, b*). Apparently the fungus requires at least some nutrients from the medium to form adhesive networks or initiate formation of trophic hyphae.

Light, whether continuous or alternating with darkness, had little effect on mycelial growth of *A. oligospora, A. conoides* and *A. brochopaga* (Olthof & Estey, 1965). Our results showed that light did not affect nematode-hypha attachment or ring structure development in *A. oligospora* (CBS 289.82).

It has already been shown (Den Belder & Jansen, unpubl.) that very young mycelium (24 h) was able to attach to nematodes and to develop ring structures around them. The present results showed that the trapping ability of hyphae of isolate CBS 289.82 was com-

Table 3. *Growth rate, mycelial density and formation of ring structures in* Arthrobotrys oligospora *(CBS 289.82) on different substrates before and after addition of second-stage juveniles of* Meloidogyne hapla.

Medium		Vegetative hyphae		Number of ring structures		
	pH	Growth[1]	Density[2]	Before addition of *M. hapla*[3]	After addition of *M. hapla*[3]	Number of ring structures around *M. hapla*[4]
1.5 % water agar	5.5	2.7 ± 0.1 *a*[5]	23.2 ± 1.5[5] *a*	5	4	2.8 ± 1.3[5] *a*
LNM-[6]	6.5	3.1 ± 0.1 *b*	19.9 ± 2.8 *b*	3	2	4.0 ± 1.8 *b*
LNM+ [6]	6.5	3.4 ± 0.1 *c*	20.7 ± 2.6 *bc*	2	2	4.6 ± 1.6 *b*
CMA 1:10	5.5	2.9 ± 0.1 *ab*	21.5 ± 1.5 *c*	1	1	2.5 ± 1.4 *a*

[1] Radial growth : mm/day.
[2] Mycelial density : mm² mycelium per 100 mm² agar in 28 day-old fungal colonies.
[3] Number of ring structures in 20 fields of sight (at 200 × magnification).
[4] Number of ring structures around 10 nematodes per Petri-dish one day after addition of the nematodes.
[5] Mean ± s.e. Different letters indicate significant differences (Student's t-test, $P < 0.05$).
[6] Low nutrient mineral salts medium with (+) and without (–) thiamin and biotin.

parable to younger colonies, even when the hyphae were kept for 36 days at 5 °C and few hyphae were formed. Loss of adhesiveness as found for *Dactylella megalospora* after 7 days (Esser *et al.,* 1991) or a reduction of adhesiveness after several weeks as found in *A. oligospora* (Gronvold, 1989) was never found. In colonies in which nematodes mainly encountered aged hyphae (mycelium, developed during 42 days at 25 °C and subsequently kept for 36 days at 5 °C) any change in attachment efficacy or development of trophic hyphae was found in comparison to mycelium grown for 28 days at 25 °C. Development of conidiophores or complex three-dimensional adhesive networks developing on the ring structure surrounding the nematodes as observed by Poinar and Jansson (1986), Nordbring-Hertz *et al.* (1987) and Jaffee *et al.* (1992) after the contents of the nematode were absorbed never occurred in the tested isolate.

Under adverse temperatures that do not favour adhesive network development or vegetative growth, or under poor nutritional conditions for adhesive network development, our isolate tested shows the capacity to capture nematodes with adhesive hyphae. This clearly illustrates lower demands on temperature and nutrition than needed for ring structure formation. This implies that the range of circumstances for this fungus to capture nematodes and to be active as a control agent, may be much broader for fungi in which adhesive networks are the only capturing devices.

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