

# Predatory behaviour of trapping fungi against *srf* mutants of *Caenorhabditis elegans* and different plant and animal parasitic nematodes

P. MENDOZA DE GIVES<sup>1, 2, 3\*</sup>, K. G. DAVIES<sup>1</sup>, S. J. CLARK<sup>1</sup> and J. M. BEHNKE<sup>2</sup>

<sup>1</sup> IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK

<sup>2</sup> School of Biological Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, UK

<sup>3</sup> Centro Nacional de Investigaciones Disciplinarias en Parasitología Veterinaria, Instituto Nacional de Investigaciones Forestales y Agropecuarias, Km. 11.5 Carr. Federal Cuernavaca-Cuautla, Col. Progreso, Jiutepec, Estado de Morelos, México. Apdo. Postal 206, CIVAC, 62500 Morelos, México

(Received 30 November 1998; revised 23 January 1999; accepted 23 January 1999)

## SUMMARY

The initial infection process of nematode-trapping fungi is based on an interaction between the trapping structure of the fungus and the surface of the nematode cuticle. A bioassay was designed to investigate the predatory response of several isolates of nematode-trapping fungi against 3 mutants of *Caenorhabditis elegans* (AT6, AT10 and CL261), which have been reported to differ in the reaction of their cuticle to antibodies and lectins. The bioassay was also applied to infective larvae of animal (*Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus axei*) and plant (*Meloidogyne* spp.) parasitic nematodes. Differences in trapping ability were most marked in the first 24 h, and were density dependent. Although the isolate of *Arthrobotrys* responded very rapidly in the first 24 h, *Duddingtonia flagrans* was generally the most effective isolate and *Monacrosporium* responded relatively poorly throughout all experiments. All the fungi tested trapped the *srf* mutants of *C. elegans* more efficiently than the wild type, and there were differences between the different *srf* mutants of *C. elegans*. Differences in trapping ability were also observed between different isolates of *D. flagrans*; similarly, differences in trapping behaviour were observed not only amongst the different species of plant-parasitic nematodes, but also between the sheathed and exsheathed larvae of the animal-parasitic nematodes.

Key words: nematophagous fungi, *Caenorhabditis elegans*, surface mutants, parasitic nematodes.

## INTRODUCTION

The nematode cuticle is a complex structure essential for locomotion, maintenance of morphology and protection against the environment. In parasitic nematodes the cuticle is important in the recognition process by the host immune system of animals, may have a role in the resistance response of plants, and is also a site of attachment by various hyperparasitic bacteria and fungi (Bird & Bird, 1991). Interest in the pathogens of nematodes has become increasingly important in the search for alternative control measures to replace chemical anthelmintics and nematicides.

Fungi have long been known to be parasitic on nematodes (Barron, 1977) and are considered as potential biological control agents for plant (Kerry & Jaffee, 1997) and animal (Wolstrup *et al.* 1996; Llerandi Juárez & Mendoza de Gives, 1998; Mendoza de Gives *et al.* 1998) parasitic nematodes. One group of these fungi, the nematode-trapping fungi, live in soil and develop organs for the capture of nematodes which are killed and then serve as a

source of nutrients for the fungi (Barron, 1977; Duddington, 1951). The presence of nematodes act as a stimulus, inducing the formation of capture organs in nematode-predatory fungi (Bartnicki-Garcia, Eren & Pramer, 1964; Nansen *et al.* 1988). The trapping process is thought to involve a lectin/carbohydrate recognition system between receptors on fungal traps and specific molecules on the nematode surface (Nordbring-Hertz & Mattiasson, 1979; Nordbring-Hertz & Friman, 1982; Rosenzweig & Ackroyd, 1983; Rosenzweig, Premachandran & Pramer, 1985; Tunlid, Jansson & Nordbring-Hertz, 1992). The external surface or cuticle of nematodes serves as a protective barrier against the environment (Politz *et al.* 1990; Bird & Bird, 1991), and constitutes the first site of contact with the natural enemies of nematodes. Spores of the bacterial hyperparasite *Pasteuria penetrans* have been shown to exhibit inter- and intra-host specificity against plant-parasitic nematodes (Sharma & Davies, 1996; Mendoza de Gives *et al.* 1999). Variation in the ability of different nematophagous fungal isolates (Mendoza de Gives *et al.* 1994), and species (Gonzalez Cruz, Mendoza de Gives & Quiroz Romero, 1998), to trap animal-parasitic nematodes has also been reported; a given fungal species may

\* Corresponding author: CENID-PAVET, INIFAP, Apdo. Postal 206, CIVAC, 62500 Morelos, México. Fax: + 52 73 20 55 44. E-mail: mendozap@inifap2.inifap.conacyt.mx

differ in the effectiveness with which it traps different species of nematodes (Nansen *et al.* 1988). Antigenic differences in the cuticle surface between different stages of animal and plant-parasitic nematodes are well recognized (Philipp, Parkhouse & Ogilvie, 1980; Maizels, Meghji & Ogilvie, 1983; Davies & Danks, 1992; Raleigh & Meeusen, 1996) and may well be important in the susceptibility of the nematode to trapping by predatory fungi.

*Caenorhabditis elegans* is a free-living nematode with a fast rate of reproduction which is easily cultured *in vitro* and is therefore a useful laboratory model (Wood, 1988). The production of strains of *C. elegans* with cuticles with different surface properties (*srf* mutants) makes it particularly suitable as a research tool for the study of the nematode cuticle (Link *et al.* 1992; Politz & Philipp, 1992). Among the various mutant strains of *C. elegans* which have been developed, some exhibit altered surface binding by the lectins wheat germ agglutinin (WGA, recognizing *N*-acetyl-glucosamine) and soybean agglutinin (SBA, recognizing *N*-acetyl-galactosamine). Whereas wild-type nematodes bind WGA weakly, and only at the male copulatory bursa and the hermaphrodite vulva, these surface mutants show binding over the whole cuticle (Politz *et al.* 1990; Link *et al.* 1992; Silverman, Blaxter & Link, 1997). The cuticle of the mutant strains appears to have undergone molecular structural changes exposing molecules hidden in the wild-type, thereby altering their properties. However, in other respects, these surface mutant strains of *C. elegans* are not different from wild-type nematodes.

Quantitative, comparative studies of the interactions between nematodes which differ in their cuticular surface properties and nematode-trapping fungi provide a route to a better understanding of the interactions involved. In this paper *C. elegans* is used as a model in a bioassay to investigate the trapping efficacy of different nematophagous fungi against wild-type and surface mutants of *C. elegans*, as well as plant and animal-parasitic nematodes. We first describe experiments which established the optimum conditions for monitoring quantitatively the capture of *C. elegans* (wild type) by 4 isolates of nematophagous fungi and then, under those optimal conditions, we used surface mutants of *C. elegans* to test the hypothesis that molecular changes in the surface cuticular structures of the nematodes radically alter their susceptibility to capture by the fungi. We also test whether or not the sheath of the infective stages of animal-parasitic nematodes affects the ability of these fungi to trap nematodes.

## MATERIALS AND METHODS

### Fungi

Four predatory Hyphomycetes selected for their ability to produce adhesive 3-dimensional traps,

were used; strains of *Arthrobotrys* sp. (FTHO-3) and *Duddingtonia flagrans* (FTHO-8) were both isolated from sheep faecal material in Mexico; another isolate of *D. flagrans* (PF) was kindly provided by Dr Pelloile, INRA, France; and 1 strain of *Monacrosporium* sp., (R6) isolated from a soil sample in UK. Fungi were routinely subcultured on Petri dishes containing corn meal agar (CMA) at room temperature (25–30 °C).

### Nematodes

*C. elegans* populations (Table 1) were maintained and produced in the laboratory, either on nematode growth medium (NGM) plates to which *E. coli* OP50 had been applied (Wood, 1988) or, to obtain a large quantity of nematodes, plates were supplied with 0.5 g of peanut butter. Before use, the nematodes were washed from the cultures by centrifugation in either M9 buffer (Wood, 1988) or sterile distilled water. Suspensions of nematodes were centrifuged and decanted 3 times to remove extraneous agar and bacteria. Experiments were done with larval stages (L1, L2 and dauer larvae); adults were excluded by using a 125 µm mesh sieve which only allowed the larvae to pass through. Previous experiments had shown that oviposition and hatching of new larvae complicated enumeration (unpublished data). Nematodes were suspended in a standard volume, and the number of nematodes was calculated by counting the nematodes present in 20 aliquots of 5 µl. Populations of infective larvae of 3 different Trichostrongylids from 2 different sources were used (Table 1). These nematodes were washed in 25% sucrose, to remove contaminating detritus, and rinsed 3 times in sterile distilled water to eliminate the sucrose residues. They were finally resuspended in sterile water for use in experiments. Exsheathed larvae were obtained by adding 50 µl of hydrochloric acid (2 M) to 50 ml of a suspension of infective 3rd-stage larvae for 20 sec periods until the majority of larvae exsheathed, followed by rinsing 3 times in distilled water (Boisvenue *et al.* 1983). Root-knot nematode egg masses were collected from tomato roots routinely infected with root-knot nematodes and maintained in a glasshouse at 25 °C. Second-stage larvae were hatched from egg masses by placing them on a small tray in tap water at room temperature (Hooper, 1986). Larvae were separated, washed in M9 medium 3 times and finally, resuspended in sterile water and counted using the technique described by Fenwick (1951).

### Motility of *srf* mutants of *C. elegans*

In order to ascertain that motility of the different nematode treatments was not responsible for differences in capture a motility assay was performed. Three *srf* mutants of *C. elegans* and a wild type (N2) were tested in plastic Petri dishes (6.0 cm

Table 1. Nematode populations, strains, genotypes and their source

Nematode	Strain	Genotype	Source*
<i>Caenorhabditis elegans</i>	AT6	srf-2 (yj262)I	Dr T. Stiernagle (CGC)
	AT10	srf-3 (yj10)IV	
	CL261	srf-5 (ct115)X	
	N2	Wild-type	
<i>Haemonchus contortus</i>	N.A.	N.A.	Dr E. Munn (BI)
<i>Teladorsagia (Ostertagia) circumcincta</i>	N.A.	N.A.	Dr R. Coop (MI)
<i>Trichostrongylus axei</i>	N.A.	N.A.	Dr R. Coop (MI)
<i>Meloidogyne incognita</i>	Race 2	N.A.	NCSU
	Race 1	N.A.	NCSU

\* CGC, *Caenorhabditis* Genetics Center, Minnesota, USA; BI, Babraham Institute, Cambridge, UK; MI, Moredun Institute, Dundee, UK, NCSU, North Carolina State University, USA; N.A., not available.

Table 2. Average trap formation score ( $n = 3$ ) for 4 nematophagous fungi and 2 populations of *Caenorhabditis elegans* on CMA plates after 24 h of incubation at 25 °C at different nematode densities

Fungus	<i>Caenorhabditis elegans</i> *			
	Srf mutant AT6 trap production		Wild-type N2 trap production	
	Low nematode <sup>(a)</sup> density	High nematode <sup>(b)</sup> density	Low nematode <sup>(a)</sup> density	High nematode <sup>(b)</sup> density
<i>Arthrobotrys</i> sp. (FTHO-3)	2	3	1	3
<i>D. flagrans</i> (FTHO-8)	3	3	3	3
<i>D. flagrans</i> (PF)	3	3	3	3
<i>Monacrosporium</i> sp (R6)	1·3 <sup>(c)</sup>	1 <sup>(d)</sup>	1 <sup>(c)</sup>	1·3 <sup>(d)</sup>

\* (L1, L2 and Dauer larvae), 1 = 1–20 traps, 2 = 21–40 traps, 3 = > 40 traps, <sup>(a)</sup> ≤ 200 nematodes per plate, <sup>(b)</sup> ≥ 200 nematodes per plate, <sup>(c)</sup> ≤ 300 nematodes per plate, <sup>(d)</sup> ≥ 300 nematodes per plate.

diameter) containing corn meal agar with 3 replicate dishes per population of nematodes. Two concentric rings at 2 and 3 cm from the centre of the plates were drawn on the base using a marker pen to delimit 2 migrating areas (Area 1 and Area 2, respectively). One drop (20 µl) of an aqueous suspension containing 200 larvae was deposited in the centre of each plate and then incubated at 25–30 °C. The numbers of nematodes present in areas 1 and 2 were recorded at 30 min, 1 h, 2 h and 3 h after inoculation. Numbers of nematodes in each area at each time were analysed using repeated measures ANOVA (Greenhouse & Geisser, 1959) following transformation to logarithms (base 10) after adding 1 to each count to adjust for zeros.

*Effect of incubation time on fungal trapping ability*

The 4 fungi previously described were grown from plugs of agar placed in the centre of 3·5 cm Petri dishes containing CMA and allowed to grow for 10 days at 25 °C. A set of CMA plates without any

fungus was used as control. Samples of 25 µl of a suspension containing approximately 5000 nematodes of *C. elegans* wild type N2 in M9 solution were added to each Petri dish. All plates were incubated at 25 °C. Nematodes were recovered by washing and rinsing the surfaces of plates after 12, 24, 36, 48 and 60 h post-incubation. Each treatment was replicated 5 times. The total number of recovered nematodes was estimated by counting the number of specimens present in five 5 µl aliquots using a stereomicroscope (25 and 40 ×). The percentage reduction in each nematode population by the action of the fungi (after a given incubation time) was estimated by comparing the numbers of nematodes in treated and untreated plates using the following formula:

$$\% \text{ Reduction} = \frac{X_c - X_t}{X_c} \times 100,$$

where:  $X_c$  = mean number of nematodes recovered from control plates ( $n = 5$ ), and  $X_t$  = mean number of nematodes recovered from treated plates ( $n = 5$ ).

The percentage reductions for each fungus were compared at each incubation time using ANOVA.

#### *Effect of nematode density on trap formation and trapping ability*

The effect of nematode density on the trap formation and trapping ability in the different fungal isolates was assessed against 2 *C. elegans* strains (AT6 and N2) by adding nematodes to CMA plates (6 for each fungus/nematode combination) at densities ranging between 84 and 477. Trapped and untrapped nematodes were counted after 24 h at 25 °C using a microscope (25 and 40×). Trap formation was scored on a 3-point scale. The percentage of trapped nematodes was calculated for each plate, transformed to logits ( $\log_e(p/(1-p))$ , where  $p = (r+0.5)/(n+1)$ ) and plotted against the density of nematodes for that plate. The results were analysed by a parallel model regression analysis (Weisberg, 1985) using the statistical package GENSTAT 5 (Genstat 5 Committee, 1993).

#### *Comparison of the trapping of 4 strains of C. elegans and animal and plant-parasitic nematodes by Arthrobotrys sp and D. flagrans*

The trapping ability of 2 nematode-trapping fungi isolates, *Arthrobotrys* sp. (FTHO-3) and *D. flagrans* (FTHO-8), which displayed a good record of trapping ability, were evaluated against a wild-type (N2) and 3 different *srf* mutants of *C. elegans* (AT6, AT10 and CL261). Fungi were cultured as previously described. This experiment was conducted using approximately 200 nematodes (min = 109, max = 349, mean = 203) per plate (5 replicates per treatment) and trapping was evaluated after 24 h at 25 °C. The experiment was repeated (4 replicates per treatment) using sheathed and exsheathed L3 larvae Trichostrongylids including *Haemonchus contortus*, *Teladorsagia (Ostertagia) circumcincta* and *Trichostrongylus axei*, and the plant-parasitic nematodes *Meloidogyne incognita* and *M. arenaria* (Table 1), which differ in the reaction of their cuticles to a polyclonal antibody (Davies & Danks, 1992). The proportion of trapped nematodes in each case was analysed using logistic regression, accounting for overdispersion where necessary using Williams' procedure (Williams, 1982).

## RESULTS

### *Motility of srf mutants of C. elegans*

A significant interaction between nematode population and time was observed ( $F_{5-1, 28.4} = 3.45$ ,  $P < 0.05$ ). After 30 min a greater number of nematodes of

the wild-type (mean = 1.25, log scale) and the AT10 (mean = 0.90) populations were found migrating from the centre compared to populations of AT6 (mean = 0.40) and CL261 (mean = 0.45) which remained relatively concentrated in the middle of the plates. However, by 1 h after inoculation, migration of CL261 had increased substantially (mean = 1.40) compared to the other populations (AT6 = 0.843, AT10 = 1.243, N2 = 1.274). After 1 h, migration continued to increase but only at a similarly low rate for each population and reached means of 0.995, 1.306, 1.413 and 1.516 for AT6, AT10, CL261 and N2, respectively.

### *Trap formation in nematophagous fungi*

There was little difference in overall trap formation by the fungi (Table 2; total scores of 58 and 55 for AT6 and N2, respectively). However, the *srf* mutant AT6 produced a slightly stronger response in terms of trap formation in *Arthrobotrys* sp. compared to the wild type at low densities (< 200 nematodes per plate). Trap formation was not affected by the nematode population at higher nematode densities. Both isolates of *D. flagrans* consistently produced a large number of traps irrespective of the nematode and its density. *Monacrosporium* produced the least traps in all plates.

### *Effect of incubation time on fungal trapping ability*

The predatory activity of all the fungi tested against the wild-type *C. elegans* increased with time so that by 60 h more than 95% were trapped when 5000 nematodes were added per plate (Table 3). However, differences in trapping ability were observed between the different fungi within the first 12 h ( $F_{3, 16} = 8.55$ ,  $P = 0.001$ ). *Arthrobotrys* sp. had the most rapid trapping response against this nematode recording more than 77% of nematodes trapped after 12 h. *D. flagrans* (PF) trapped 45% of nematodes by this time. In contrast, *D. flagrans* (FTHO-8) and *Monacrosporium* sp. had the slowest trapping responses with only 23% and 3%, respectively, of nematodes being trapped within 12 h. No statistical differences among the fungi existed after 24 h or 36 h, but *Monacrosporium* sp. was significantly less efficient after 48 h ( $F_{3, 16} = 5.86$ ,  $P = 0.007$ ), whilst *D. flagrans* PF was slightly but still significantly less efficient after 60 h ( $F_{3, 15} = 3.98$ ,  $P = 0.029$ ).

### *Trapping ability of nematode-trapping fungi against C. elegans srf mutants*

The trapping percentages of *Arthrobotrys* FTHO-3, *D. flagrans* FTHO-8 and PF, and *Monacrosporium* sp. against the wild type and the *srf* mutant AT6 are shown in Fig. 1. The effect of nematode density on

Table 3. Mean ( $n = 5$ ) percentage reduction in the number of nematodes (*Caenorhabditis elegans* N2 wild-type) by action of 4 nematode-trapping fungi at 25 °C in CMA plates after different incubation periods

(Nematode density approximately 5000 specimens per plate. Nematode population included L1, L2 and dauer larvae.)

Fungus	<i>Arthrobotrys</i> sp. (FTHO-3)	<i>D. flagrans</i> (FTHO-8)	<i>D. flagrans</i> (PF)	<i>Monacrosporium</i> sp. (R-6)	S.E.D.*
Time (h)					
12	77.3	22.7	45.3	2.9	15.45
24	86.7	82.8	86.4	70.2	9.03
36	85.0	87.4	88.8	64.7	9.86
48	97.4	93.2	96.0	74.8	6.15
60	99.2	97.4	95.8	98.4	1.05

\* Standard error of the difference between means.

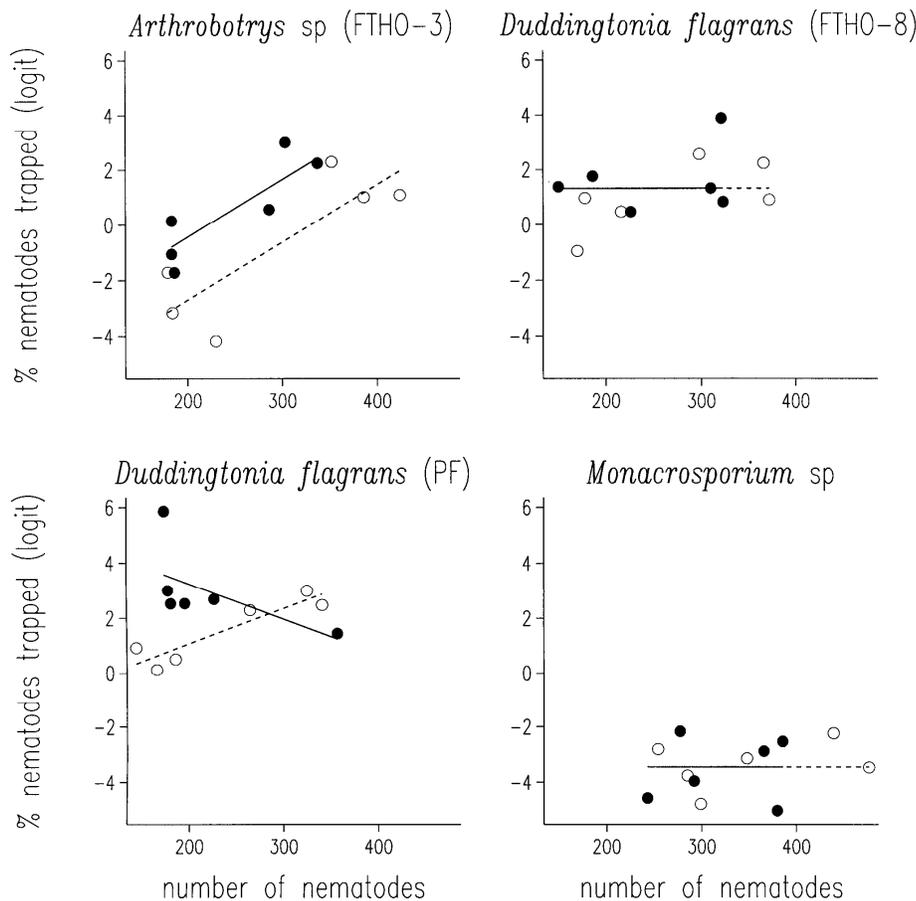


Fig. 1. Trapping proportions (after logistic transformation) of a mixture of stages (L1, L2 and dauer larvae) of 2 populations of *Caenorhabditis elegans*, wild-type N2 and AT6 *srf* mutant by 4 nematode-trapping fungi after 24 h on CMA plates at 25 °C at different nematode densities. (○---○) and (●—●) Regression lines for N2 and AT6, respectively.

trapping ability was dependent on the fungal isolate and the nematode population. For example, whilst a marked increase in trapping percentage with nematode density was seen for the 2 nematodes for the *Arthrobotrys* isolate, overall AT6 was trapped more efficiently ( $F_{1,8} = 7.80, P = 0.023$ ). The *D. flagrans* PF isolate behaved very differently against the 2 nematodes both in terms of the change in trapping percentage with density ( $F_{1,8} = 9.39, P = 0.015$ ) and

the overall level of trapping ( $F_{1,8} = 6.48, P = 0.034$ ). There was no difference in trapping of the wild-type and *srf* mutant of *C. elegans* by the other 2 fungi, *D. flagrans* FTHO-8 and *Monacrosporium*, and for neither of these fungi was there evidence of density dependence in the trapping percentage. However, these 2 fungi trapped very different percentages of nematodes overall.

*Arthrobotrys* sp. showed a very low activity against

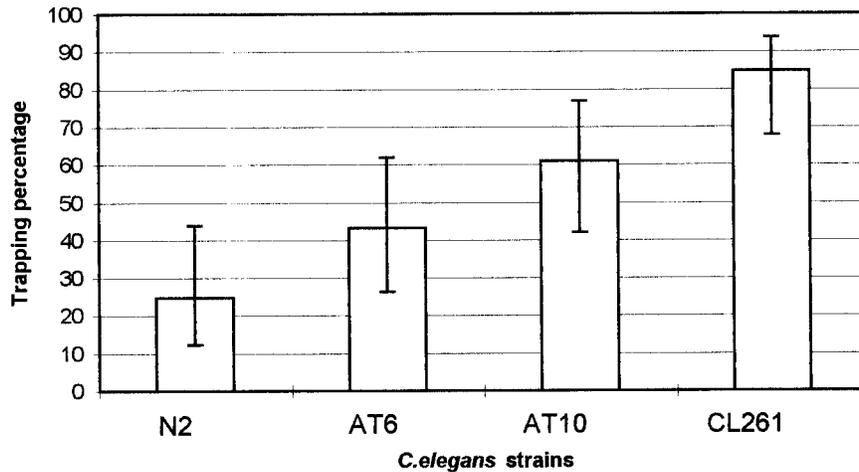


Fig. 2. Mean percentage of wild-type (N2) and different surface mutants of *Caenorhabditis elegans* trapped by *Duddingtonia flagrans* FTHO-8 after 24 h on CMA plates at 25 °C. Bars represent 95% confidence intervals around the mean ( $n = 5$ ).

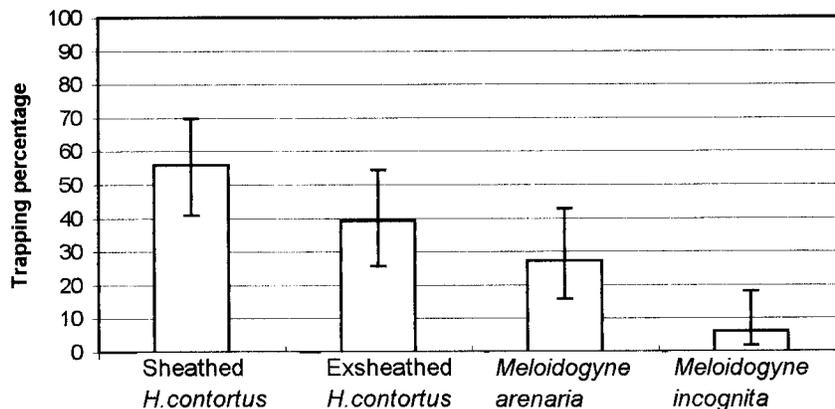


Fig. 3. Mean percentage of sheathed and exsheathed infective *Haemonchus contortus* and *Meloidogyne* spp. trapped by *Duddingtonia flagrans* (FTHO-8) after 24 h at 25 °C on CMA. Bars represent 95% confidence intervals around the mean ( $n = 3$ ).

the wild-type and *srf* mutants of *C. elegans*, after 24 h at 25 °C recording the following trapping percentages: 0.67 (0, 25.89), 4.32 (0.8, 19.11), 4.63 (1.0, 9.44) and 25.97 (13.10, 44.95) against N2, AT6, AT10 and CL261, respectively. The trapping percentages of *D. flagrans* (FTHO-8) against the wild-type and the 3 *srf* mutants are shown in Fig. 2. The wild-type *C. elegans* (N2) was less efficiently captured by *D. flagrans* (FTHO-8) than any of the *srf* mutants; there were also differences in the percentage of trapped nematodes between each of the *srf* mutants with increased susceptibility to capture as follows: AT6 < AT10 < CL261.

#### Cuticle variation and trapping ability on plant and animal parasites

*D. flagrans* FTHO-8 was consistently more successful in trapping animal and plant-parasitic nematodes than the *Arthrobotrys* FTHO-3 isolate. In fact, *Arthrobotrys* trapped less than 2% of the nematodes

under study so only data for *D. flagrans* FTHO-8 were analysed here. The trapping behaviour of *D. flagrans* FTHO-8 depended on the species of nematode tested ( $\chi^2_3 = 45.13$ ,  $P < 0.001$ ); fewer root-knot nematodes, (*Meloidogyne* spp.) were trapped than *H. contortus*; and fewer *M. incognita* were trapped than *M. arenaria* (Fig. 3). Although the percentages of sheathed infective larvae of *H. contortus* and *T. axei* trapped by *D. flagrans* FTHO-8 were higher when compared with their corresponding exsheathed larvae (Figs 3 and 4), no statistical difference was found between them. However, sheathed *T. circumcincta* larvae were more efficiently trapped than exsheathed larvae by both fungi (Fig. 4) Overall, *T. circumcincta* was more effectively trapped than *T. axei* ( $\chi^2_1 = 36.30$ ,  $P < 0.001$ ), *D. flagrans* FTHO-8 trapped a higher percentage of larvae than *Arthrobotrys* sp. FTHO-3 ( $\chi^2_1 = 5.88$ ,  $P < 0.05$ ) and sheathed larvae were more effectively trapped than exsheathed larvae ( $\chi^2_1 = 28.1$ ,  $P < 0.001$ ).

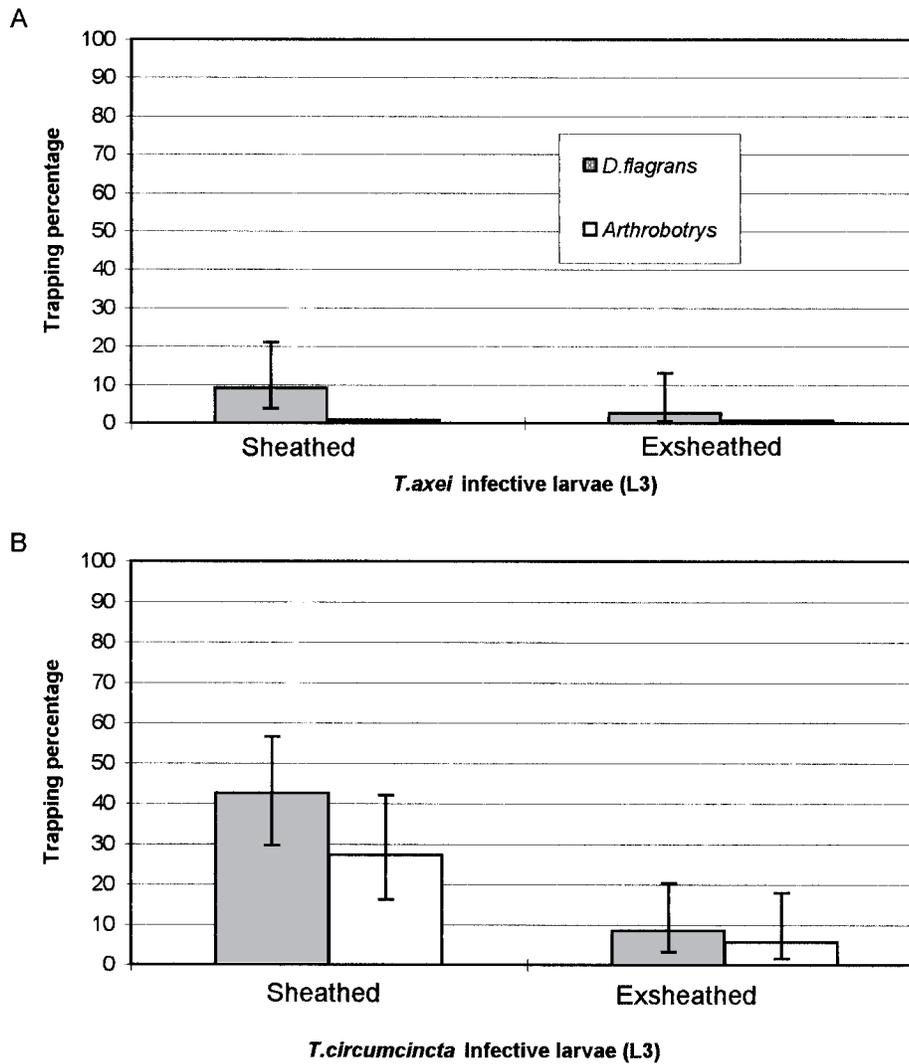


Fig. 4. Mean percentage of *Trichostrongylus axei* (A) and *Teladorsagia circumcincta* (B) infective larvae trapped by *Duddingtonia flagrans* (FTHO-8) and *Arthrobotrys* sp. (FTHO-3) after 24 h on CMA plates at 25 °C. Bars represent 95% confidence intervals around the mean ( $n = 4$ ).

DISCUSSION

Differences found in the motility of the *C. elegans* population tested did not reflect any relation with the trapping efficiency or in the trap formation by the nematode-trapping fungi. The data reported here clearly demonstrate that the nature of the cuticle is important in determining the ability of trapping fungi to capture nematodes. The different fungi used responded at different rates to the presence of the N2 wild-type *C. elegans* during the first 24 h whereas all fungi were able to capture this nematode with an equally high efficiency by 60 h. The differences in the ability to capture this nematode were not only observed amongst fungi from different genera, but even between the isolates of *D. flagrans*. This suggests that the molecular recognition in the fungal traps of the different genera, species and even isolates of trapping fungi plays an important role in the capture efficiency of nematodes and interacts with the molecular nature of the nematode cuticle.

Different abilities to capture other species of nematodes have been found with trapping fungi. Morgan *et al.* (1997) found that *Arthrobotrys oligospora*, *D. flagrans* and *Monacrosporium megalosporum* reduced the population of *Heligmosomoides polygyrus* 3rd-stage infective larvae by less than 5% after 1 day of interaction and by about 55, 70 and 50%, respectively, by the fourth day. Comparable results were obtained by Galper *et al.* (1995) who made a comparison of the *in vitro* predatory activity of a group of nematode-trapping fungi including *Monacrosporium* sp. and 2 isolates of *A. oligospora* against wild-type *C. elegans*; more than 90% of nematodes were trapped after 24 h. Other fungal isolates have been found to differ in their ability to capture animal-parasitic nematodes; the *in vitro* trapping ability of different species of fungi against *Trichostrongylus axei* and *Ostertagia ostertagi* at different times was evaluated by Pandey (1973) who found *Arthrobotrys* spp. had trapped 70% of the larvae of both nematodes after 3 days. The lowest trapping

effectiveness after 3 days was shown by some species of *Monacrosporium* including the following records: *M. bembicoides*, 20%; *M. cionopaga*, 47%; and *M. ellipsospora*, 9%. The low and slow trapping activity characteristic of *Monacrosporium* sp. in the present experiment is comparable with these results.

Predatory behaviour in *A. oligospora*, *D. flagrans* and *M. megalosporum* against *H. polygyrus* has been reported to be nematode density-dependent, with an increase in the predatory activity as larval density rises (Morgan *et al.* 1997). Moreover, nematode density was found to be important in the capture or infection of *Meloidogyne javanica* larvae by nematophagous fungi in the soil microcosms, where *Hirsutella rhossiliensis* showed the greatest density-dependent parasitism, followed by *M. ellipsosporum* and *Arthrobotrys dactyloides*; the least dependent was found in *A. oligospora* and *M. cionopagum* (Jaffee, Tedford & Muldoon, 1993). In preliminary tests, a large density of nematodes (5000 per plate) promoted a high trapping activity in all the fungi under study, and these studies suggested that trapping behaviour was density dependent and less effective at low nematode densities. In the present study, we found clear differences in the capture of N2 wild-type by *Arthrobotrys* when the nematode density increased, but this was not consistent. Increasing the density of the wild-type *C. elegans* produced a strong effect on the trapping response of *Arthrobotrys* sp. and *D. flagrans* PF, however, this effect was not seen in *D. flagrans* FTHO-8 and *Monacrosporium* sp. We also found that at a low nematode density the *Arthrobotrys* and the 2 *D. flagrans* isolates showed more efficient predatory activity against the AT6 strain than the N2 wild-type. The reason for these differences is unclear but almost certainly involves the interactions between the surface polymers on the traps and the glycocalyx of the nematode cuticle which is thought to involve a lectin-carbohydrate recognition process (Tunlid *et al.* 1991; Tunlid, Johansson & Nordbring-Hertz, 1992). Other processes which involve the reorganization of the surface polymer layer and release of enzymes, which in turn lead to the firm binding of the nematode, may also be influential (Tunlid *et al.* 1992). All 3 *srf* mutants of *C. elegans* tested here were more efficiently captured than wild-type N2 by *D. flagrans*. Politz *et al.* (1990) have suggested that nematodes have hidden antigenic determinants which, if unmasked by mutagenesis, can be revealed in the cuticle surface. This work suggests that these hidden antigens make the *srf* mutant nematodes more susceptible to the fungal traps than the wild type.

Throughout all the experiments with plant and animal-parasitic nematodes *D. flagrans* was more effective in capturing nematodes than *Arthrobotrys*, where, apart from *T. circumcincta*, the number of trapped nematodes was never more than 6%. In

particular, the fact that *D. flagrans* trapped *M. arenaria* more efficiently than *M. incognita* suggests that the latter could also possess some masking structures on its cuticle which hide the molecular receptors involved in the trapping process, but which are not present on *M. arenaria*. The observation that *Arthrobotrys* sp. showed a very low activity against *M. arenaria* and no detectable trapping of *M. incognita*, when compared to *D. flagrans*, is unclear. However, experiments with *C. elegans* wild-type suggested that at low nematode densities *Arthrobotrys* produced a smaller number of traps than *D. flagrans*, and this may be due, either to the inability of *Arthrobotrys* to recognize the elicitors which stimulate trap production, or to far fewer elicitors being produced by these nematodes. The failure of *D. flagrans* to capture *Meloidogyne* species reported by Belder & Jansen (1994) compared with our results suggest that some antigenic variation could be present in different isolates of root-knot nematodes, or even in different isolates of the same species of a nematode-trapping fungus, which could result in a different trapping efficiency. The sheath of infective Trichostrongylids is regarded as a protective layer against the environment, so it is surprising that sheathed *T. circumcincta* were more easily trapped than exsheathed larvae. Stage-specific surface molecules in *H. contortus* have been identified by using monoclonal antibodies; antibodies recognize epitopes which are present in the surface of 2nd-stage larvae and in the protective sheath of the 3rd-stage larvae but absent from the L3 cuticle itself (Raleigh, Brandon & Meeusen, 1996). Such differences could be responsible for a different trapping efficiency in the fungi in the present investigation. Similar results were found by Wharton & Murray (1990) in the capture of sheathed and exsheathed *Trichostrongylus colubriformis* by *A. oligospora*; removal of the sheath prevented the capture of infective larvae. In contrast, Timper & Kaya (1989) reported that the L2 cuticle in entomogenous nematodes can be protected from infection by endoparasitic fungi, *H. rhossiliensis* and *Drechmeria coniospora*; they found that entomogenous nematodes without L2 cuticle were susceptible to infection by these fungi. The infective stages of some animal-parasitic nematodes, for example *H. contortus*, do not lose their sheath until they have entered into their respective hosts (Summerville, 1954). In fact, the sheath plays an important role in resistance of *H. contortus* infective larvae to proteolytic digestion within the digestive tract (Fetterer & Rhoads, 1996), thereby endowing this stage with the ability to be infective. Entomopathogenic nematodes such as Steinernematids have been shown to escape nematode trapping fungi by slipping out of their 2nd-stage cuticle (Poinar & Jansson, 1986). The interaction between the fungus and the nematode cuticle is clearly important and can substantially alter the ability of a fungus to

capture nematodes, particularly at low densities and in the first 24 h of interaction. This observation has implications for the selection of isolates to be deployed in the management of nematodes.

This work was supported by IACR-Rothamsted and the Consejo Nacional de Ciencia y Tecnología, México. Authors thank Dr Theresa Stiernagle, *Caenorhabditis* Genetics Center, Minnesota, USA, for supplying the isolates of *C. elegans*. The *Caenorhabditis* Genetics Center is funded by the NIH National Center for Research Resources (NCRR). We also thank Dr Ed Munn from the Babraham Institute, Cambridge, UK, and Dr Bob Coop from the Moredun Institute, Dundee, UK, for supplying the animal-parasitic nematodes, and North Carolina State University for supplying the *Meloidogyne* sp. IACR-Rothamsted receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the United Kingdom.

## REFERENCES

- BARRON, G. L. (1977). The nematode destroying fungi. *Topics in Mycobiology*, no. 1, pp. 1–140. Canadian Biological Publications Ltd, Canada.
- BARTNICKI-GARCIA, S., EREN, J. & PRAMER, D. (1964). Carbon dioxide dependent morphogenesis in *Arthrobotrys conoidea*. *Nature, London* **204**, 804.
- BELDER, D. E. & JANSEN, E. (1994). Capture of plant-parasitic nematodes by an adhesive hyphae forming isolate of *Arthrobotrys oligospora* and some other nematode-trapping fungi. *Nematologica* **40**, 423–437.
- BIRD, A. F. & BIRD, J. (1991). *The Structure of Nematodes*, 2nd Edn. Academic Press, San Diego.
- BOISVENUE, R. J., BRANDT, M. C., GALLOWAY, R. B. & HENDRIX, J. C. (1983). *In vitro* activity of various anthelmintic compounds against *Haemonchus contortus* larvae. *Veterinary Parasitology* **13**, 341–347.
- DAVIES, K. G. & DANKS, C. (1992). Interspecific differences in the nematode surface coat between *Meloidogyne incognita* and *M. arenaria* related to the adhesion of the bacterium *Pasteuria penetrans*. *Parasitology* **105**, 475–480.
- DUDDINGTON, C. L. (1951). The ecology of predacious fungi. I. Preliminary survey. *Transactions of the British Mycological Society* **34**, 322–331.
- FENWICK, D. W. (1951). A new modification of the McMaster slide for use in potato-root eelworm investigations. *Journal of Helminthology* **25**, 173–176.
- FETTERER, R. H. & RHOADS, M. L. (1996). The role of the sheath in resistance of *Haemonchus contortus* infective-stage larvae to proteolytic digestion. *Veterinary Parasitology* **64**, 267–276.
- GALPER, S., EDEN, M. L., STIRLING, G. R. & SMITH, J. L. (1995). Simple screening method for assessing the predacious activity of nematode-trapping fungi. *Nematologica* **41**, 130–140.
- GENSTAT 5 COMMITTEE (1993). *Genstat 5 Release 3 Reference Manual*. Clarendon Press, Oxford.
- GONZALEZ CRUZ, M. E., MENDOZA DE GIVES, P. & QUIROZ ROMERO, H. (1998). Comparison of the trapping ability of *Arthrobotrys robusta* and *Monacrosporium gephyropagum* on infective larvae of *Strongyloides papillosus*. *Journal of Helminthology* **72**, 209–214.
- GREENHOUSE, S. W. & GEISSER, S. (1959). Methods in the analysis of profile data. *Psychometrika* **24**, 95–112.
- HOOPER, D. J. (1986). Extraction of nematodes from plant material. In *Laboratory Methods for Work with Plant and Soil Nematodes* (ed. Southey, J. F.), pp. 51–58. Ministry of Agriculture, Fisheries and Food, London, HMSO.
- JAFFEE, A. B., TEDFORD, C. E. & MULDOON, E. A. (1993). Test for density-dependent parasitism of nematodes by nematode-trapping and endoparasitic fungi. *Biological Control* **3**, 329–336.
- KERRY, B. & JAFFEE, B. A. (1997). Fungi as biological control agents for plant parasitic nematodes. In *The Mycota IV, Environmental and Microbial Relationships* (ed. Wicklow, D. T. & Soderstrom, B.), pp. 203–218. Springer-Verlag, Berlin and Heidelberg.
- LINK, C. D., SILVERMAN, M. A., BREEN, M. & WATT, K. E. (1992). Characterization of *Caenorhabditis elegans* lectin-binding mutants. *Genetics* **131**, 867–881.
- LLERANDI JUAREZ, R. D. & MENDOZA DE GIVES, P. (1998). Resistance of chlamydospores to the digestive processes of sheep in Mexico. *Journal of Helminthology* **8**, 155–158.
- MAIZELS, R. M., MEGHJI, M. & OGILVIE, B. M. (1983). Restricted sets of parasite antigens from the surface of different stages and sexes of the nematode parasite *Nippostrongylus brasiliensis*. *Immunology* **38**, 107–121.
- MENDOZA DE GIVES, P., DAVIES, K. G., MORGAN, M. & BEHNKE, J. M. (1999). Attachment test of *Pasteuria penetrans* to the cuticle of plant and animal parasitic nematodes, free-living nematodes and *srf* mutants of *Caenorhabditis elegans*. *Journal of Helminthology* **73**, 67–71.
- MENDOZA DE GIVES, P., FLORES-CRESPO, J., HERRERA-RODRIGUEZ, D., LIEBANO-HERNANDEZ, E., VAZQUEZ-PRATZ, V. M. & ONTIVEROS-FERNANDEZ, G. E. (1998). Biological control of *Haemonchus contortus* infective larvae in ovine faeces by administering an oral suspension of *Duddingtonia flagrans* chlamydospores to sheep. *Journal of Helminthology* **72**, 343–347.
- MENDOZA DE GIVES, P., ZAVALA, M. E., HERRERA, R. D. & QUIROZ, R. H. (1994). *In vitro* trapping capability of *Arthrobotrys* spp. on infective larvae of *Haemonchus contortus* and *Nacobbus aberrans*. *Journal of Helminthology* **68**, 223–229.
- MORGAN, M., BEHNKE, J. M., LUCAS, A. J. & PEBERDY, F. J. (1997). *In vitro* assessment of the influence of nutrition, temperature and larval density on trapping of the infective larvae of *Heligmosomoides polygyrus* by *Arthrobotrys oligospora*, *Duddingtonia flagrans* and *Monacrosporium megalosporum*. *Parasitology* **115**, 303–310.
- NANSEN, P., GRONVOLD, J., HENRIKSEN, S. A. & WOLSTRUP, J. (1988). Interactions between the predacious fungus *Arthrobotrys oligospora* and third-stage larvae of a series of animal parasitic nematodes. *Veterinary Parasitology* **26**, 329–337.
- NORDBRING-HERTZ, B. & FRIMAN, E. (1982). A recognition mechanism in the adhesion of nematodes to nematode-trapping fungi. In *Lectins-Biology, Biochemistry, Clinical Biochemistry*, Vol. II. pp. 83–90. Walter de Gruyter & Co., Berlin.
- NORDBRING-HERTZ, B. & MATTIASSON, B. (1979). Action of a nematode-trapping fungus shows lectin-mediated host-microorganism interaction. *Nature, London* **281**, 477–479.

- PANDEY, V. S. (1973). Predatory activity of nematode-trapping fungi against the larvae of *Trichostrongylus axei* and *Ostertagia ostertagi*: A possible method of biological control. *Journal of Helminthology* **43**, 35–48.
- PHILIPP, M., PARKHOUSE, R. M. E. & OGILVIE, B. M. (1980). Changing proteins on the surface of a parasitic nematode. *Nature, London* **287**, 538–540.
- POINAR, G. O. J. & JANSSON, H. B. (1986). Infection of *Neoplectana* and *Heterohabditis* (Rhabditida: Nematoda) with the predatory fungi *Monacrosporium ellipsosporum* and *Arthrobotrys oligospora* Moniliales: Deuteromycetes. *Revue de Nematologie* **9**, 241–244.
- POLITZ, S. M. & PHILIPP, M. (1992). *Caenorhabditis elegans* as a model for parasitic nematodes: A focus on the cuticle. *Parasitology Today* **8**, 6–12.
- POLITZ, S. M., PHILIPP, M., ESTEVEZ, M., O'BRIEN, J. P. & CHIN, J. K. (1990). Genes that can be mutated to unmask hidden antigenic determinants in the cuticle of the nematode *Caenorhabditis elegans*. *Proceedings of the National Academy of Sciences, USA* **87**, 2901–2905.
- RALEIGH, J. M., BRANDON, R. M. & MEEUSEN, E. N. T. (1996). Stage-specific expression of surface molecules by the larval stages of *Haemonchus contortus*. *Parasite Immunology* **18**, 125–132.
- RALEIGH, J. M. & MEEUSEN, E. N. T. (1996). Developmentally regulated expression of a *Haemonchus contortus* surface antigen. *International Journal for Parasitology* **26**, 673–675.
- ROSENZWEIG, D. W. & ACKROYD, D. (1983). Binding characteristics of lectins involved in the trapping of nematodes by fungi. *Applied and Environmental Microbiology* **46**, 1093–1096.
- ROSENZWEIG, D. W., PREMACHANDRAN, D. & PRAMER, D. (1985). Role of trap lectins in the specificity of nematode capture by fungi. *Canadian Journal of Microbiology* **31**, 693–695.
- SHARMA, S. B. & DAVIES, K. G. (1996). Comparison of two sympatric *Pasteuria* populations isolated from a tropical vertisol soil. *World Journal of Microbiology and Biotechnology* **12**, 361–366.
- SILVERMAN, M. A., BLAXTER, L. M. & LINK, D. C. (1997). Biochemical analysis of *Caenorhabditis elegans* surface mutants. *Journal of Nematology* **29**, 296–305.
- SUMMERVILLE, R. I. (1954). The second ecdysis of infective nematode larvae. *Nature, London* **174**, 751–752.
- TIMPER, P. & KAYA, K. H. (1989). Role of the second-stage cuticle of entomogenous nematodes in preventing infection by nematophagous fungi. *Journal of Invertebrate Pathology* **54**, 314–321.
- TUNLID, A., JANSSON, H. B. & NORDBRING-HERTZ, B. (1992). Fungal attachment to nematodes. *Mycological Research* **96**, 401–412.
- TUNLID, A., JOHANSSON, T. & NORDBRING-HERTZ, B. (1991). Surface polymers of nematode-trapping fungus *Arthrobotrys oligospora*. *Journal of General Microbiology* **137**, 1231–1240.
- WEISBERG, S. (1985). *Applied Linear Regression*. Wiley, New York.
- WHARTON, D. A. & MURRAY, D. S. (1990). Carbohydrate/lectin interactions between the nematophagous fungus, *Arthrobotrys oligospora*, and the infective juveniles of *Trichostrongylus colubriformis* (Nematoda). *Parasitology* **101**, 101–106.
- WILLIAMS, D. A. (1982). Extra-binomial variation in logistic linear models. *Applied Statistics* **31**, 144–148.
- WOLSTRUP, J., NANSEN, P., GRONVOLD, J., HENRIKSEN, S. A. & LARSEN, M. (1996). Toward practical biological control of parasitic nematodes in domestic animals. *Journal of Nematology* **28**, 129–132.
- WOOD, B. W. (1988). The nematode *Caenorhabditis elegans*. Cold Spring Harbor Laboratory, New York, USA.