Attraction of Nematodes to Living Mycelium of Nematophagous Fungi

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Methods were designed to detect attraction and repulsion of nematodes by fungi and determine the attraction intensity of different fungi. Of 23 fungi tested, 15 attracted the bacteria-feeding nematode *Panagrellus redivivus*. Of the 14 nematophagous fungi tested, ten attracted and one repelled nematodes, whereas three were neutral. Among nine non-nematophagous fungi, five attracted nematodes. In general, the attraction intensity increased with increasing dependence of the fungi on nematodes for nutrients.

INTRODUCTION

The accumulation of both plant-parasitic and non-parasitic nematodes around plant roots and fungal and bacterial colonies is well known. Several factors, such as temperature, electrical potential, carbon dioxide and various organic and inorganic substances, have been thought to be involved (Croll, 1970; Green, 1971).

The attraction of nematodes to fungi has been investigated primarily to show host-finding mechanisms of fungus-feeding nematodes (e.g. Townshend, 1964; Klink *et al.*, 1970). The only study on attraction of nematodes to living mycelium of nematophagous fungi is presented in a note by Field & Webster (1977). Monoson *et al.* (1973) studied the attraction of *Aphelenchus avenae*, a fungus-feeding nematode, to culture filtrates of a nematophagous fungus. Balan & Gerber (1972) and Balan *et al.* (1974, 1976) demonstrated attraction of the bacteria-feeding nematode *Panagrellus redivivus* to culture filtrates of several nematophagous fungi with different types of trapping devices.

Since accumulation of plant-parasitic nematodes in the rhizosphere might facilitate root invasion not only by nematodes, but also by fungi, bacteria and viruses (Powell, 1971), studies of microbe–nematode interrelationships may have significant practical implications. The purpose of the present investigation was to study the attraction of the bacteria-feeding nematode *P. redivivus* to living mycelium of different types of nematophagous fungi. Methods were developed to detect nematode attraction or repulsion, and to determine the attraction intensity.

METHODS

Fungi. Fungi were maintained at 20 °C in 9 cm diam. Petri dishes on corn meal agar (Difco) diluted 10-fold (CMA 1:10) and were subcultured once a month. When used in the attraction tests, fungi were allowed to grow almost to the edges of the plates. This usually took 1 to 3 weeks, depending on the species.

Of the 14 species or strains of nematophagous fungi investigated (see Table 2), 13 belonged to the nematode-trapping group and one was an endozoic parasite. Eight of the former group produced traps of the sticky network type and were comparatively fast-growing (group 1), the mycelium reaching the edge of the dish within 1 to 2 weeks. Only three of these produced traps spontaneously on CMA 1:10: *Arthrobotrys oligospora* (strain Drechsler), *Arthrobotrys superba* and *Arthrobotrys arthrobotryoides*. The other five fast-growing fungi were tested as mycelium without traps. Five nematode-trapping fungi (group 2) with different types of traps were slow-growing (2 to 3 weeks). They produced traps spontaneously: *Monacrosporium ellipsosporum*

and *Dactylaria candida* (sticky knobs); *Monacrosporium cionopagum* (sticky branches); *Dactylaria gracilis* and *Arthrobotrys dactyloides* (constricting rings). The endozoic parasite *Harposporium anguillulae* (group 3) with conidia as infectious agents grew extremely slowly in axenic culture.

The nine non-nematophagous fungi tested (see Table 2) were common soil fungi. One of these, *Verticillium dahliae*, was a severe plant pathogen.

Nematodes. The bacteria-feeding nematode *Panagrellus redivivus* was cultivated axenically at 20 °C in 50 ml Erlenmeyer flasks, each containing 4.3 ml 4% (w/v) Soya-peptone (Oxoid) and 100 mg Bacto-liver (Difco) (Rothstein & Cook, 1966), and subcultured weekly. For attraction experiments, nematodes from a 7 to 10 d-old culture were harvested and washed aseptically to remove the nutrient solution and solid liver extract. The nematode suspension was transferred to a centrifuge tube and 5 to 10 ml water were added. Centrifugation (approx. 3000 g, 1 min) gave a nematode layer above the liver extract. The nematodes were removed with a Pasteur pipette, and the washing and centrifugation procedure was repeated 5 to 6 times. The final nematode suspension was diluted to the desired density, usually about 4000 nematodes ml⁻¹.

Attraction assay. The assay was used to determine if the fungi attracted or repelled the nematodes and was based mainly on the methods described by Townshend (1964) and Klink *et al.* (1970). Discs (1 cm diam.) were cut with a cork borer from the edge of a colony growing on CMA 1:10 and placed in two quadrants (I, III) on fresh CMA 1:10 plates, 1 cm from the edge of the plate. Control discs of CMA 1:10 without fungi were placed in the other two quadrants (II, IV). The plates were incubated for 24 h so that a concentration gradient of substances diffusing into the medium was established. A drop of a nematode suspension (containing about 100 specimens) was placed in the middle of the plate and, after another 24 h, the numbers of nematodes in, on or under the discs were counted using a stereomicroscope.

The test plates had to be dried for at least 2 to 3 d before use, otherwise the nematodes aggregated in the middle of the plate when the nematode suspension water disappeared. This also happened if too many nematodes (e.g., more than 200) were added to the plate. Dilute corn meal agar provided a thin mycelial mat, thus facilitating microscopic observations of both mycelium and nematodes. The assay was carried out in the dark at room temperature (20 to 22 °C). Each assay usually consisted of six replicates. A χ^2 test was performed on the values from control discs and discs with fungus.

Attraction intensity assay. The same procedure was followed as described above, except that the numbers of nematodes accumulating under each disc were counted hourly. To avoid capture of nematodes by the trap-bearing nematophagous fungi, the assay was not run for more than 6 h. When attracting fungi were used, there was a linear increase in the number of attracted nematodes with time. The slope of this line was used as a measure of the attraction intensity.

Ultraviolet (u.v.) irradiation. After 24 h incubation, some assay plates with fungal discs were irradiated with u.v. light (254 nm, 2 W, 25 cm, 20 min) to kill the fungi. Immediately after the u.v. treatment, nematodes were added and the attraction assay was completed.

RESULTS

Determination of attraction and repulsion

A typical result of an attraction test with the nematophagous fungus *Arthrobotrys musiformis* is shown in Table 1. On average, 75% of the nematodes were found under the discs of attracting fungi in quadrants I and III after 24 h.

Of the 14 nematophagous fungi tested, 10 attracted *P. redivivus* (Table 2). Only one fungus, *A. arthrobotryoides*, with spontaneously formed traps, repelled the nematodes. *Arthrobotrys dactyloides* and *D. gracilis*, both with constricting ring traps, neither attracted nor repelled the nematodes. The same result was found with *A. conoides*, which did not form traps spontaneously. All three strains of *A. oligospora* showed moderate attraction, irrespective of the presence or absence of spontaneously formed traps.

Of the nine non-nematophagous fungi tested, five attracted and one repelled the nematodes (Table 2).

Determination of attraction intensity

The three groups of nematophagous fungi were tested for their attraction intensity. The endozoic parasite (*H. anguillulae*) always showed the highest attraction intensity (slope > 6). Group 2, with the slow-growing attracting fungi *M. ellipso sporum*, *M. cionopagum* and *D. candida*, showed a somewhat lower attraction (slope 3 to 6), while the fast-growing species (group 1) had the lowest attraction intensity (slope < 3). The non-nematophagous fungi tested (*Trichothecium roseum*, *Verticillium dahliae*, *Mortierella parvispora* and *Penicillium*

Table 1. *Attraction of the nematode Panagrellus redivivus to the nematophagous fungus Arthrobotrys musiformis*

The results show the numbers of nematodes accumulated in, on or under each disc after 24 h.

Disc quadrant no.	Plate no.					
	1	2	3	4	5	6
I	30	37	42	31	46	37
II	17	10	6	4	6	18
III	34	25	45	37	51	50
IV	8	16	19	8	13	13

Mean and standard error: discs with fungi (I, III): 38.8 ± 2.4

control discs (II, IV): 11.5 ± 1.5

χ^2 value: 92.2 ($P_{0.001} = 20.5$, $n = 6$)

Table 2. *Attraction of the nematode Panagrellus redivivus to different fungi on CMA 1:10*

Nematophagous fungi	Spon- taneous formation of traps	Attraction/ repulsion*	Attraction/ repulsion after u.v.†
Group 1			
<i>Arthrobotrys oligospora</i> Fres. ATCC 24927	—	+	0
<i>A. oligospora</i> Fres. (strain Drechsler)	+	+	0
<i>A. oligospora</i> Fres. (strain Swart) CBS 106.49	—	+	ND
<i>A. musiformis</i> (Drechsler)	—	+	0
<i>A. conoides</i> Drechsler CBS 109.52	—	0	0
<i>A. arthrobotryoides</i> (Berl.) Lindau CBS 118.54	+	—	—
<i>A. entomopaga</i> Drechsler CBS 168.75	—	+	0
<i>A. superba</i> Corda QM 1688, NCC	+	+	0
Group 2			
<i>Monacrosporium ellipso sporum</i> (Grove) R. C. Cooke & Dickinson CBS 225.54	+	+	+
<i>M. cionopagum</i> (Drechsler) Subram. CBS 228.52	+	+	+
<i>Dactylaria candida</i> (Nees) Sacc. CBS 220.54	+	+	+
<i>D. gracilis</i> Duddington CBS 225.52	+	0	0
<i>A. dactyloides</i> Drechsler CBS 109.37	+	0	0
Group 3			
<i>Harposporium anguillulae</i> Lohde emend. Zopf		+	+
Non-nematophagous fungi			
<i>Trichothecium roseum</i> (Pers.) Link ex S. F. Gray		+	0
<i>Verticillium dahliae</i> Kleb.		+	0
<i>V. bulbiliosum</i> W. Gams & Malla		0	ND
<i>Trichoderma viride</i> Pers. ex S. F. Gray		+	ND
<i>Trichoderma polysporum</i> (Link ex Pers.) Rifai		—	ND
<i>Mortierella parvispora</i> Linnemann		+	0
<i>Mortierella nana</i> Linnemann		0	ND
<i>Penicillium spinulosum</i> Thom		+	0
<i>Tolypocladium inflatum</i> W. Gams		0	ND

ND, Not determined.

* +, Attraction; —, repulsion; 0, neither attraction nor repulsion. All values significant at $P < 0.001$ (χ^2 test, six replicate test plates).

† Attraction/repulsion of fungi killed by u.v.-irradiation prior to addition of nematodes.

Fig. 1

Fig. 2

Fig. 1. Attraction of *Panagrellus redivivus* to nematophagous fungi. The slope of each line indicates the attraction intensity: ○, *Harposporium anguillulae*, group 3, slope 6.5; ●, *Monacrosporium elliposporum*, group 2, slope 5.5; □, *Arthrobotrys superba*, group 1, slope 1.9. Each point is the mean from five replicate plates.

Fig. 2. Attraction intensity curves for some members of groups 1 and 2 of nematophagous fungi. Group 2: *Dactylaria candida* (○), *Monacrosporium elliposporum* (●) and *M. cionopagum* (□); slopes 3.7 to 6.0. Group 1: *Arthrobotrys oligospora* (■) and *A. musiformis* (△); slopes < 2. Each point is the mean from five replicate plates.

spinulosum) also showed low attraction intensities. Figure 1 shows examples of these differing responses.

Results from one experiment with several members of groups 1 and 2 are shown in Fig. 2. The difference in attraction intensity between the two groups is evident. In a parallel experiment, each slow-growing fungus (group 2) was tested against a fast-growing fungus (group 1) on the same plate (e.g. *M. elliposporum* versus *A. oligospora*, *M. cionopagum* versus *A. musiformis* etc.). After 6 h the nematodes under each disc were counted. Once again, members of group 2 attracted more nematodes than those of group 1 ($P < 0.01$, five replicate plates). Although the slow-growing group 2 fungi had traps, which the fast-growing group 1 fungi lacked, very few nematodes were captured during this 6 h period. The higher attraction intensity of the fungi in group 2 could therefore not have been due to capture by the traps.

Effect of u.v.-irradiation

To determine if the attraction/repulsion pattern of the nematophagous fungi shown in Table 2 was confined to living fungi, attraction tests were done after killing the fungi with u.v.-irradiation. After irradiation, group 1 fungi and non-nematophagous fungi no longer attracted nematodes but the slow-growing fungi (groups 2 and 3) still did so (Table 2). Repulsion and neutral responses to the other fungi remained unchanged.

DISCUSSION

The assay used in this investigation allowed us not only to detect attraction or repulsion, but also to determine differences in the attraction intensity of different fungi; in both cases it gave reproducible results.

Field & Webster (1977), studying attraction to living mycelium of five nematophagous

fungi with different types of traps, showed that fungi stimulated to trap formation with horse serum or nematode extract attracted both a *Rhabditis* sp. (bacteria-feeding) and the fungus-feeding *Aphelenchus avenae*, whereas the unstimulated fungi did not. They concluded that attraction was dependent on the presence of traps. We have shown that the presence of traps does not necessarily cause attraction, and one trap-forming fungus, *A. arthrobotryoides*, even repelled the nematodes. Further, the constricting ring forms (*A. dactyloides* and *D. gracilis*) neither repelled nor attracted the nematodes (Table 2). An explanation for these differing results might be that substrate composition affects the attractiveness of the fungi. Addition of proteinaceous trap-inducing materials, such as horse serum or nematode extract, may result in attracting excretion products, not necessarily coupled to trap formation.

When the fungi were killed by u.v.-irradiation before nematodes were added, the attraction of group 1 fungi was completely abolished. The attracting fungi of groups 2 and 3 were still attractive, although somewhat less so, after such treatment. This suggested that a volatile substance, or a small rapidly diffusing compound, continuously produced, was responsible for attraction to the fast-growing fungi. With the fungi of groups 2 and 3, larger or less volatile, slowly diffusing compounds might be responsible for attraction. The non-nematophagous fungi resembled the fast-growing nematophagous fungi in this respect. However, the substances responsible for attraction are unknown.

Cooke (1963) concluded that rapid growth rate and good saprophytic ability were accompanied by lower predacious efficiency. In our study, the attraction intensity was lowest in fungi with moderate or high saprophytic ability (Table 2, group 1 and the non-nematophagous fungi). The u.v.-irradiation tests also showed a similarity between these fungi. Attraction intensity increased with increasing parasitism of the fungi in groups 2 and 3. It therefore appears that the attraction intensity reflects the dependence of the fungi on nematodes for nutrients.

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