GROWTH RELATIONSHIPS BETWEEN *APHELENCHUS AVENAE* AND TWO SPECIES OF NEMATOPHAGOUS FUNGI

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ABSTRACT. — Comparable populations of the mycophagous nematode, *Aphelenchus avenae* Bastian, were cultured for seven days upon agar cultures with two species of nematophagous fungi. In the presence of nematodes the optimum growth temperature of the fungi was usually increased 5°C and fungus colony diameters were greater than controls. Low numbers of living nematodes were recovered from fungus-sown plates after seven days at 15°C and 20°C, but at 25°C and 30°C the numbers of nematodes recovered exceeded the original inoculum. Nematode numbers at the lower temperatures suggested that nematode trapping was higher than nematode reproduction, eggs laid by the nematodes were incapable of hatching, or inhibitory fungal metabolites are stable at lower temperatures and unstable at higher temperatures.

Experimental cultures of nematode-trapping fungi have included primarily either bacterium-feeding or plant-parasitic nematode species. Mycophagous nematodes have been used only recently in such cultures even though they are probably as widely distributed in nature as are the nematophagous fungi. Recent investigations (Cayrol, 1967; Cooke and Pramer, 1968; Feder, 1963; Hechler, 1963; Monoson, 1968a) have included a study of the relationships between nematophagous fungi and mycophagous nematodes.

The purpose of the present study was to determine what effects varying numbers of nematodes have on fungus growth and what effects the fungi have on nematode populations when both are grown together.

MATERIALS AND METHODS

Two species of nematophagous Moniliales were chosen. The fungi used in this study were two adhesive, network-forming species, *Arthrobotrys oligospora* Fres. and *A. musiformis* Drechs. Stock cultures of the fungi were maintained on a medium containing 20 g Difco corn-meal agar (CMA) in 1000 ml water at room temperature. The nematode *Aphelenchus avenae* Bastian was maintained on *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker, and Larson which grew on a medium containing 10 g Difco potato-dextrose agar (PDA) and 15 g agar in 1000 ml water.

Three media were used in this study: 2% Difco CMA, one-quarter-strength Difco PDA (Monoson, 1968a), and one-fifth-strength V-8 agar (V-8) composed of 200 ml V-8 juice® and 20 g agar in 1000 ml water. No attempt was made to adjust the pH's of the media.

Fungus inoculations were according to the technique described by Monoson (1968a). Each of the two fungi was cultured on the three agar media for a period of four days. Fungus plugs, 7 mm diam., were cut from these four-day-old cultures and placed in the center of 9 cm petri plates that contained the same agar medium. Nematodes were extracted from stock cultures according to the following technique (Monoson,

1968b): Nematodes were separated from agar stock cultures by pouring 10-15 ml of sterile water over the surface of a plate. The nematodes readily moved into the surface water and were unable to re-enter the agar. A simple nematode extraction apparatus was formed by placing four layers of type 900-S-Kimwipe® tissues between two plastic funnel tops which rested in a Syracuse dish. The nematode extraction apparatus had been sterilized before hand by autoclaving for 15 minutes at 20 psi. Nematodes were extracted over a 1-hour-period and serially diluted to either 100 or 400 individuals per milliliter of water. Inoculation of either 100 or 400 nematodes into each experimental fungus culture was accomplished by using a sterile measuring dispenser or a calibrated 1 ml duplicating pipette. Each culture was replicated three times and appropriate controls containing fungus alone were used.

Nematophagous fungus cultures containing *A. avenae* were maintained for seven days at 15°, 20°, 25°, or 30°C. Observations and measurements of cultures began 24 hr after inoculation and continued at 24-hr intervals. The radial diameter of colonies was measured to the nearest 0.5 mm.

Upon completion of each test the agar contents of a petri dish were cut into approximately 0.5 cm squares and homoginized in a Waring blendor for 8 sec. The homogenate was poured into a nematode recovery apparatus (Monoson, 1968b) and counts of the living nematodes were made after 24 hr.

Results

Average colony growth of the two fungi in radial diameter in the presence and absence of *A. avenae* are shown in Figure 1. *A. oligospora* and *A. musiformis* had maximum growth at 25°C in the presence of the nematode. *A. musiformis* grew more at 25° than at 20°C on CMA. The growth of *A. oligospora* on PDA with *A. avenae* was as great at 20° as at 25°C. Slight growth differences were noted for both fungi on CMA in the presence of the nematode at 20° and 25°C but were not considered significant.

Neither of the fungi repelled the nematode although in two instances less than 35% of the total number introduced was recovered from an

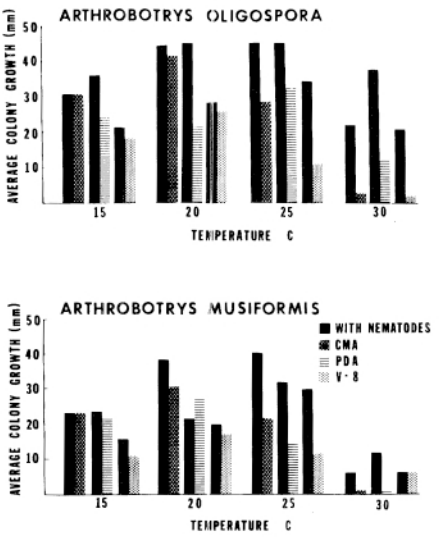

FIGURE 1. Average colony growth of two species of nematophagous fungi on three agar media after seven days of growth with and without *Aphelenchus avenae*.

The bars are arranged in pairs. The solid black is the left-hand member of the pair, and gives the average colony growth in the presence of the nematode with an initial inoculum of 100 individuals. The right-hand member of the pair gives the average colony growth in the absence of the nematode, and is appropriately shaded to designate the medium. (see key).

experimental culture (Table 1). All of the nematodes inoculated into a dish settled in the fungus plug after 12 hr. The nematodes moved freely in and around the inoculum plug prior to the 12 hr but were not observed to leave a colony once they began feeding on the hyphae. Fungal satellite colonies were never formed through the transportation of spores on the bodies of nematodes. At no time were immobile nematodes observed other than those which had been captured by a fungus.

Cultures were evaluated on the basis of nematodes recovered alive. Low numbers of nematodes were recovered from all the media maintained at 15° and 20°C, but at 25° and 30°C numbers were much higher than used as inoculum (Table 1). The number of live nematodes recovered was divided by the total area of the colony, in square centimeters,

TABLE 1.—Total numbers of *Aphelenchus avenae* recovered per plate* after being cultured seven days with two nematophagous fungi on various agar media.

Temperature	No. Nematodes Added	No. Nematodes Recovered					
		Arthrobotrys Oligospora			*Arthrobotrys Musiformis*		
		CMA	PDA	V-8	CMA	PDA	V-8
15° C	100	52	32	28	56	42	47
15° C	400	56	124	44	74	111	41
20° C	100	74	69	42	56	54	79
20° C	400	61	295	56	219	181	32
25° C	100	231	130	126	835	238	87
25° C	400	1500	445	254	1600	406	139
30° C	100	890	296	690	2600	793	834
30° C	400	5000	919	661	3900	339	427

* Numbers recorded are averages of three replicates in each case.

to yield the number of nematodes present per square centimeter of fungus (Table 2).

DISCUSSION

The addition of *A. avenae* to fungus cultures usually resulted in more fungus growth compared to cultures without the nematode. Cultures that contained 100 nematodes as inoculum most often produced the optimum fungus growth in this study.

Monoson (1968a) reported that *A. avenae* was captured very effectively by these two fungi after four days of maintenance at 15°, 20°, 25°, and 30°C on 2% CMA, one-quarter-strength PDA, and one-fifth-strength V-8. In the present study, the low numbers of living nematodes recovered at 15° and 20°C indicated that nematode trapping might have occurred after the seven days of these tests. The data also suggested that nematode trapping was higher than nematode reproduction or that eggs laid by the nematodes were incapable of hatching.

Cooke and Pramer (1968) reported that nematode-trapping fungi displayed no predaceous activity until

TABLE 2.—Numbers of *Aphelenchus avenae* per cm² recovered after seven days of growth with two nematophagous fungi on various agar media.*

Temperature	No. Nematodes Added	*Arthrobotrys Oligospora*			*Arthrobotrys Musiformis*		
		CMA	PDA	V-8	CMA	PDA	V-8
15° C.	100	1	1	2	3	2	6
15° C.	400	2	3	3	5	6	6
20° C.	100	1	1	2	1	4	6
20° C.	400	1	5	2	7	7	2
25° C.	100	4	2	3	17	7	3
25° C.	400	24	7	4	39	22	7
30° C.	100	60	6	48	2300	202	629
30° C.	400	602	328	52	9750	484	237

* Average of three replicates.

they had completely colonized the agar substrate. In addition, they stated that the retardation of predaceous activity was due to the concentration of nematodes in a zone at or beyond the colony margin too juvenile to capture nematodes. It was of interest to note that neither of these situations were observed in the present study.

Temperature affected the amount of fungal growth in the same way both with and without nematodes (Figure 1). With the exception of *A. oligospora* on PDA fungus cultures that contained nematodes had an optimum temperature for growth five degrees above that of the controls.

Large amounts of fungus growth and high numbers of live nematodes were recovered at either 25° or 30°C on the three media. Released nematode metabolic products may have caused the stimulated growth of the fungi due to alteration of the agar media. Small shifts in the pH's of the media were noted but not enough data were available to ascribe specific effects to pH shifts.

Monoson (1968a) reported that 20-50 nematodes were recovered from CMA cultures of *A. oligospora* and *A. musiformis* after four days at 25°C when the inoculum level was 100 nematodes. Almost identical numbers of live nematodes were recovered under those same environmental conditions when the inoculum level was 400 individuals. In the present study, similar fungus cultures on CMA at 25°C with an inoculum level of 100 nematodes contained 231, 835, and 373 nematodes after seven days. These numbers were considered low when compared with those produced on *P. terrestris* (a non-predaceous fungus) after a similar maintenance period at 25°C. The number of nematodes expected on *P. terrestris* cultures after seven days was either equalled or surpassed, for the most part, during a similar period of time on *A. oligospora* and *A. musiformis*, at the two higher temperatures.

The large nematode numbers could be interpreted as being due to: nonfunctioning of the trapping mechanism after a seven-day period at high temperatures—i.e., 25° and 30°C, (2) increase of nematode reproduction over the rate of nematode trapping, or (3) decrease in generation

time of the egg to the larval and adult stages because of cultural conditions. Microscopic observation of whether or not a trap still functioned after seven days was considered impossible because of the amount of fungal growth.

Acknowledgments

Thanks are due to Dr. Helen C. Hechler for providing the nematode culture; Drs. R. C. Cooke, W. A. Feder, and A. C. Tarjan for providing the fungus cultures.

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Manuscript received January 13, 1970