NEMATODES AS NUTRIENTS FOR SOIL FUNGI

BY

S. SCHENCK and D. PRAMER
Department of Biochemistry and Microbiology, Cook College, Rutgers University, New Brunswick, New Jersey, U.S.A.

Two nematode-trapping fungi, Arthrobotrys conoides and Monacrosporium rutgeriensis, and a non-predacious soil fungus, Rhizoctonia solani, were grown using nematodes as the sole source of nutrient. The test nematode species was Panagrellus redivivus. Colony diameters of all three fungi on water agar, cornmeal agar, and water agar containing nematodes, were not significantly different from each other, although cornmeal and nematode agar supported fungal growth that was the most compact and luxuriant. On “nematode-agar” growth of R. solani was comparable to that on control cornmeal agar. Sporulation and trap formation of A. conoides was greater than on cornmeal agar. With M. rutgeriensis sporulation and trap formation were not increased over than on nematode-agar. Cultures of A. conoides in sterile quartz sand grew faster, sporulated more and produced greater numbers of traps in the presence than in the absence of autoclaved nematodes.

Many species of nematode-trapping fungi have been described (Cooke & Godfrey 1964). They are common in soil and easily isolated. Many will grow on laboratory media and display nutritional patterns similar to those of other Moniliiales (Tarjan 1960; Coscarelli & Pramer 1962). Nematode-trapping fungi are unique, however, in their morphological adaptation to the predacious habit and in their ability to capture and consume prey. Many of these fungi will not form traps in the absence of prey unless supplied with a metabolic product of nematodes, which was designated “nemin”, and has been shown to be peptide in nature (Pramer & Stoll 1959; Kuyama & Pramer 1962; Nordbring-Hertz & Brinck 1974).

Since many nematode-trapping fungi grow and sporulate abundantly as saprophytes, in the absence of prey, their need to evolve and maintain complex and highly specialized anatomical and physiological systems for processes of predation can be questioned. Possibly these features provide the fungi with competitive advantage as they forage for food in soils where the nutrient supply is low and frequently inadequate. Nevertheless, the presence of prey in cultures of five different species of nematode-trapping fungi on cornmeal extract agar did not alter their growth rate as measured by the increase of colony diameter with time (Cooke & Pramer 1968). Fungi-stasis of nematode-trapping species in soil was reversed by the addition of prey (Eren & Pramer 1968), however, and in sterilized soil the growth and sporulation of the predacious fungus Arthrobotrys conoides was greater in the presence of added nematodes. In nonsterile soil the beneficial effects of added nematodes were not apparent, indicating that the indigenous microbial population nullified the suggested competitive advantage the fungi may enjoy because of their capacity for predation (Eren 1965).
The extent to which nematodes serve as a source of nutrients for soil fungi remained to be determined. Experiments were performed, therefore, to establish that nematode-trapping fungi are indeed able to use prey as a sole source of sustenance, and also to compare their capacity to use nematodes as a food source with that of a non-predacious soil fungus.

**MATERIALS AND METHODS**

Pure cultures of the nematode-trapping fungi *Arthrobotrys conoides* Drechsler and *Monacrosporium rutgeriensis* Cooke & Pramer, and the non-predacious fungus *Rhizoctonia solani* Kühn were maintained on cornmeal agar (Difco) and frequently checked for possible contamination. The free-living nematode *Panagrellus redivivus* L., Goodey was grown on an oatmeal-water slurry in petri plates for 14-21 days. The oatmeal culture was placed in eight layers of cheesecloth in a Baermann funnel (Pramer & Schmidt 1964) for 6-8 hr to separate the nematodes from the cereal, and the nematodes were then harvested and rinsed several times in distilled water before use.

Washed nematodes were added to melted water agar, the medium was sterilized at 15 p.s.i for 15 min, and then poured into petri plates. Numbers of nematodes per plate were estimated by making direct counts of worms in three separate areas of 1.0 cm² each. The average number/cm² multiplied by the total area of the agar (58 cm²) indicated that the plates each contained 30,000 to 50,000 nematodes. Control plates were prepared from water agar or cornmeal agar with no nematodes added. Individual plates of the various media were inoculated with an agar plug (4 mm diameter) cut from the growing edge of a fungal culture and incubated for 6 days, when the diameters of the fungal colonies were measured. One day later spore and trap counts were made. The average numbers of each were determined per six microscope fields that were selected randomly. Each study using nematode agar was repeated at least six and as many as ten times, and standard errors were used to judge the significance of differences between means. The production of thick-walled sclerotial cells was used as a measure of growth of *R. solani*, which does not sporulate or form traps.

Washed nematodes were suspended in distilled water, killed by autoclaving, and added to petri plates, each containing 60 g of sterile Ottawa quartz sand (Fisher Scientific Co.). The suspension was adjusted so each plate received 80,000 to 90,000 nematodes and sufficient water to bring the moisture content of the sand to 75% of water holding capacity. Microscopic examination revealed the nematodes to be randomly dispersed throughout the sand plates. Control plates were prepared without nematodes. The center of the surface of individual plates was inoculated with an agar plug (4 mm diameter) cut from the growing edge of a culture of *A. conoides*. The plates were incubated five days at room temperature, and then replicated on cornmeal agar as described by Stotzky (1965). Two replications of each sand culture were made, and the growth on agar provided a means of measuring the extent to which the fungus developed radially in the sand.