NEMATODE-TRAPPING FUNGI

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Many species of flowering plants are known to be carnivorous, capturing and digesting small animal prey. These plants, including the Venus' flytrap, sundews, bladderworts, and pitcher plants, usually grow in soils that are deficient in useable nitrogen. The nitrogen-containing products of animal digestion are probably valuable nutritional supplements for these insectivorous plants.

Less well-known are the carnivorous fungi. Fungi that entrap (Fig. 1) and consume nematodes, or roundworms, are found in the soil and in fresh and salt waters. In fact, over 150 species of nematode-destroying fungi have been described.

Most of the nematode-destroying fungi belong to the Deuteromycetes or the Imperfect Fungi, those which have either a poorly understood or no known sexual stage. Nematode-destroyers are also found in the Oomycetes (water molds), the Chytrids, the Zygomycetes, and the Basidiomycetes. Recently, 10 species of mushrooms were found to be carnivorous on nematodes. These mushrooms commonly grow on decaying wood, an environment low in nitrogen. It is

believed that nematode consumption supplements the low nitrogen level in their environment in a manner similar to the mode of nutrition of higher carnivorous plants.

NEMATODES

Nematodes are extremely abundant and occur in about every type of habitat (Fig. 2). Most of those living in the soil and in water are very small (0.1 to 1.0 mm). They are so abundant that a spadeful of soil or a bucketful of pond water may contain up to one million. Some nematodes cause serious animal diseases, such as trichinosis and roundworm and hookworm infections. Nematodes also cause millions of dollars in crop damage annually, but most nematodes are free-living and play a vital role in soil aeration and organic decomposition.

Nematodes are secretive animals with limited behavioral patterns. Just under their outer tough cuticle is a layer of longitudinal muscle. These muscles and the stiff cuticle limit their range of movement. The worms usually thrash about in a random, inefficient manner. However, for their size, they are powerful and enormously active. When you consider



FIGURE 1 Arthrobotrys conoides, the nematode-trapping fungus, showing the constricting rings that ensuare the nematodes.



FIGURE 2 Rhabditis, a common soil-inhabiting nematode.

the delicate nature of fungal hyphae, it is remarkable that the hyphae can trap and hold these worms until escape is impossible.

PREDATORS AND PARASITES

The fungi that are predaceous on nematodes are, by and large, soil inhabitants. They grow and reproduce in a characteristic manner until exposed to nematodes or nematode extracts. Evidently, the change induced in fungal structure is stimulated by one or more substances collectively called nemin which are secreted by the nematodes. Nemin appears to contain several amino acids (valine, leucine, and isoleucine) and specific peptides of low molecular weights.

The carnivorous fungi are either endoparasites or predators, or both. The endoparasitic fungi do not form an extensive mycelium outside the host. They exist in the soil as conidia (asexual spores) which either become attached to the nematode's mouthparts or are ingested. The spores then germinate inside the gut wall, and mycelium develops throughout the host's body. Only the reproductive structures, the conidiophores and the conidia, penetrate the cuticle and develop outside the nematode's body.

TRAPPING DEVICES

The predatory forms produce extensive mycelium and, at intervals along their hyphae, various trapping devices such as adhesive knobs (Dactylaria sp.), lateral branches (Monacrosporium sp.), or nets that produce a sticky substance to which the nematode becomes attached. Once the nematode becomes trapped, these fungi then produce additional hyphae that penetrate the animal's body.

Still other predaceous fungi produce either constricting rings (Arthrobotrys conoides) or nonconstricting rings (Dactylaria candida). In the latter, the worm becomes wedged inside the ring and cannot disengage itself. These rings are easily detached from the fungus by the worm's thrashing about. However, these detached rings are still capable of penetrating and killing the worm.

Penetration of the host by the predatory fungus is by a haustorium, an absorbing organ produced on a hypha. Once inside the body of the nematode, the fungus swells up to produce a globe-shaped vesicle which has been given various baleful names (mortiferous excrescence, infection bulb, post-infection



FIGURE 3 Rhabditis with hyphae throughout its body.

bulb). From this bulb, hyphae develop in both directions and consume the host's contents (Fig. 3).

The most dramatic trap is the constricting ring found in *Arthrobotrys*, *Dactylaria*, and *Monacrosporium*. This ring consists of three curved cells at the end of a short stalk extending from a hypha.

RING MECHANISM

The mechanism of ring closure is a fascinating one. The inner surface of the ring is sensitive to rubbing. A fine glass rod inserted through the ring and followed by gentle friction triggers closure. Other stimuli, such as a stream of dry air or heat, are also effective. The enlargement of the cells comprising the ring is accompanied by their vacuolation and by the stretching of the inside wall of the ring.



FIGURE 4 Conidia (asexual spores) of Arthrobotrys conoides form on unbranched, aerial conidiophores, while the hyphae penetrate the agar.

The stimulated cell constricts first, followed by constriction of the other two cells of the ring. Ring closure requires about 0.1 second. Volume changes within these cells are about threefold. Several physiological changes probably occur during closure. These include:

1. Changes in membrane permeability allowing rapid water uptake.

2. Water uptake over the surface of the ring.

Changes in microfibrils making up the inner wall which allow this wall to become thinner. The inner wall of Dactylaria brochophaga has four layers, two of which rupture as the wall expands.

4. Changes in osmotic concentration allowing rapid uptake of water. Possibly the polymers within the cell are hydrolyzed, thus increasing osmotic concentration of cytoplasm and allowing water uptake until osmotic balance is restored.

5. Rearrangement of membranes. The rapid increase in cell volume and surface area necessitates a concomitant rearrangement of cell membrane materials within the cell. In fact, a network of cell-membrane-bound materials has been detected subjacent to the cell membrane in Arthrobotrys dactyloides. In expanded rings, a more usual type of plasma membrane has been found, suggesting that the cell-membrane-bound materials had contributed to formation of the enlarged cell membrane.

Contact with the rings is not entirely due to chance, but may be a result of chemotactic movement of nematodes toward the fungus. If nematodes are placed equidistant between two agar cubes of a predaceous fungus (one having been induced to form traps, and the other not), a significantly higher number of nematodes will move toward the cube with traps.

The capture of the nematode is quickly followed by its death, although it may struggle violently for a time. In the case of constricting rings, the constriction of the nematode's body may cause death. There is evidence that toxins may also be produced by predaceous fungi. Arthrobotrys dactyloides has been shown to produce a nematotoxin, the active ingredient being ammonia.

Nematotoxins are produced by conidia in some of the endoparasitic fungi as well. The



PIGURE 5 Rhabditis caught by the constricting rings of Arthrobotrys conoides.

conidia adhere to the cuticle of the animal and secretion of the toxin may immobilize the host animal until hyphae can penetrate its body.



FIGURE 6 Arthrobotrys conoides exhibits increased sporulation at the site of nematode consumption.

FUNGAL SPORULATION

Under natural conditions, fungal sporulation is often induced by a decrease in the level of available food supply. The reduction in food supply which leads to sporulation is preceded by a period of intensive feeding when the food supply is abundant.

For example, Arthrobotrys conoides in culture produces a moderate number of conidia around the periphery of the petri dish. This fungus is a rather innocuous looking one producing translucent, colorless hyphae and conidia (Fig. 4). In fact, you must observe the plate against a light source to see any growth at all. Conidia are two-celled and are produced on unbranched, erect conidiophores in a single whorl around the apex. Few to no constricting loops are formed in culture.

Within 24 hours after the culture is inoculated with the nematode *Rhabditis*, constricting rings or lassos are evident along the hyphac (Fig. 1). Within the next 24 hours, *Rhabditis* become entrapped in these lassos (Fig. 5). From 48 to 96 hours after inoculation, the entrapped roundworms cease movement and the animals' bodies show signs of deterioration. By the end of 96 hours, almost all traces of *Rhabditis* have disappeared. *Arthrobotrys conoides* then exhibits massive amounts of sporulation all over the culture, especially at the sites where the nematodes

were trapped and absorbed. Thus, the feeding period seems to trigger increased sporulation (Fig. 6).

Watching this fungus feeding on nematodes under a stereomicroscope is a fascinating laboratory demonstration guaranteed to engage students' attention.

BIOLOGICAL CONTROL

Because some nematodes are serious plant and animal pathogens, some research has focused on controlling parasitic nematodes in the soil by the addition of the nematode-trapping fungi. Although results have been encouraging, no biological control methods have proven to be commercially practical at this time.

FURTHER READING

Alexopoulos, Constantine J. and Mims, Charles W., Introductory Mycology, John Wiley and Sons. New York, 1979.

Barth, Robert H. and Broshears, Robert E., The Invertebrate World, Saunders College Publishing, Philadelphia, 1982.

Bessey, E. A., Morphology and Taxonomy of Fungi. Hafner Press, New York, 1950.

Drechsler, Charles, Some hyphomycetes that prey on freeliving terricolous nematodes, Mycologia, 1939, 29, 447-479

Thorn, R. G. and Barron, G. L., Carnivorous mushrooms. Science, 1984, 224, 76-78.

Webster, John, Introduction to Fungi. Cambridge University Press. Cambridge, 1980.

PEST CONTROL

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A while ago, I wrote to Mr. Joe Mazrimas concerning the elimination of small black flies and maggots which infested my new tropical sundew terrarium. The pests came from plants purchased from Australia. At first, I thought the flies would be a good food source for the plants. Within a month, the surface of the peat moss began to crawl repulsively with plump white maggots.

Joe suggested that I use a pest striphis idea being that the gas vapor would kill the flies and eventually the maggot problem would be cradicated. At that time, I could not find any pest strips in the store because of the season. I bought a non-toxic product instead called Fly Ribbon (Terro), distributed by the Senoret Chemical Co., Kirkwood, MO 63122. The ribbon proved to be effective. It is very sticky, so if you have long hair like I do, care must be taken not to get it stuck to your locks or beard, and of course your plants. I taped it very securely to the back glass of the terrarium (when removed, the brownish glue stavs on the glass and is very unattractive). This fly ribbon killed a multitude of flies while I killed the maggots with tweezers. Between the two methods, it took several months to get rid of them.

My wife, Michele, informed me that a store was selling an Insect Strip by Starbar for \$3.99 (Starbar, Zoecon Corp., 12200 Denton Drive, Dallas, Texas 75234). This agricultural commodity is VERY TOXIC, so care must be taken when handling it.

I also had infestations of several chewing insects from the same plants purchased from Australia, Also, a small brown fly invaded the terrarium. When I used this pest strip, the infestations disappeared within 24 hours. Here are directions on working with the material. First, cut pieces of thick, sturdy plastic on which the smaller piece of strip will go. For a piece of pest strip 2" x 2", cut a piece of plastic about 5" x 5" for easy handling. This will also prevent poison from seeping and contaminating the soil. Next, put on some plastic gloves or thick plastic bags, lay out old newspapers and open the contents, being careful to unwrap only up to the desired amount. Avoid breathing in this vapor and try not to get too close to it. It smells somewhat like perfume. With a razor blade, cut a piece down the width of the bar for small pieces and use the length of the bar for bigger pieces. The strip is very hard and tough, so care must be taken to avoid