Nematode-Trapping Fungi as Mycopathogens

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Portion of a thesis submitted by the senior author to McGill University, in partial fulfillment of the requirements for the Ph.D. degree.

The authors are grateful to N. A. Croll for permitting the use of an electron microscope; J. F. Peterson, A. F. Yang, L. G. Soon, and J. Smith for technical assistance and advice on electron microscopy; and to P. H. B. Talbot for tentative identification of 
Marruchota varians.

Accepted for publication 10 March 1978.

ABSTRACT


Arthrobotrys oligospora, A. robusta, and A. superba, three well known nematode-trapping fungi, were found to be pathogenic to Marruchota varians, Rhizoctonia solani, and a species of Geotrichum by induced, specialized coil-form hyphae. Light- and electron-microscopic views of the host-pathogen interface showed that the coils squeezed the host hyphae and degraded the host cell walls, causing them to collapse and eventually to lyse.

Additional key words: mycoparasitism, mycopathology.

Barnett and Binder (2) reviewed in some detail five species of mycoparasites which contact their hosts by specialized short branches that varied in shape from curved or hooked, fingerlike appendages (15), to wedged clamps (10), hold-fasts (12), or buffer cells (3). It has been hypothesized that these variously shaped contact cells function to increase the permeability of host cell membranes and to absorb the released nutrients (3). However, when Calcarisporium parasiticum Barnett parasitizes Physalospora obtusa (Schw.) Cke., a portion of the appressed wall at the host-parasite interface is dissolved, creating a pore through which nutrients are taken from the host by the parasite (6). A study of the contact mycoparasitic relationship between Alternaria tenuis Ness. and Gonatobrytos simplex Corda indicated that cytoplasmic continuity between host and parasite was via plasmodesmata, and that such structures served as avenues for the transfer of nutrients and growth regulators (7).

While exploring possibilities for the biological control of plant parasitic nematodes, it was discovered that the nematode-trapping fungi, Arthrobotrys oligospora (Fres.) Drechsler, A. robusta Duddington, and A. superba Corda, were pathogenic to certain other fungi in a manner that appeared to be different from previously described examples of mycoparasitism or mycopathogenicity. A study of this relationship is the major topic of this paper.

MATERIALS AND METHODS

Arthrobotrys oligospora, A. robusta, and A. superba, growing on malt extract agar, were individually cultured at room temperature, on half-strength corn-meal agar (Difco) in standard 90-mm-diameter petri dishes. Three 2-mm-diameter portions of a potential host colony and three of the parasite, were placed in two lines of three in opposing rows, approximately 4 cm apart, on half-strength corn-meal agar. They were periodically examined through a light microscope for evidence of pathogenicity. For transmission electron microscopy, small mycelia-bearing agar blocks were excised from areas where pathogenicity was evident. These blocks were fixed with 2.5% glutaraldehyde in 0.05 M phosphate buffer, pH 7.0, for 2 hr and following several rinses in that buffer, were postfixed with 1% chilled OsO4 for 1 hr in the same buffer. The samples were washed twice in distilled water, dehydrated in a graded alcohol series, and embedded in low-viscosity epoxy resin (13). Ultrathin sections were cut with a glass knife on a Reichert Om-U2 ultramicrotome and mounted on collodion-coated, carbon-stabilized Athen grids. The sections were stained with uranyl acetate and lead citrate (11) and examined with a Zeiss EM 9 electron microscope operated at 50 kV.

RESULTS

When Arthrobotrys oligospora, A. robusta, and A. superba were individually cultured with a mycoparasitic species of Geotrichum Link reciprocal pathogenic relationships occurred. The hyphae of the nematode-trapping fungi were partially lysed by the Geotrichum sp. at the juncture of the two colonies. However, an unusual and unexpected event occurred within the overrun colonies of predacious fungi; some of the hyphae of the Geotrichum sp. were tightly clasped by coils of hyphae produced by the nematode-trapping fungi. In a subsequent host range survey, it was also found that Marruchota varians Boul. and Rhizoctonia solani Kühn

00032-949X/78/00 2286-03 00/00
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were similarly attacked by all three nematode-trapping fungi (Table 1). The first visible reaction of the predacious fungi to species that elicited this coiling response was the formation of short hook-like protuberances at certain points of contact with the host hypha. These protuberances elongated and sometimes grew along the axis of the host for a short distance before encircling it with one to ten hyphal coils. The coils were at acute or

right angles to their parent hyphae, depending on the contact angle, and the extending direction of the host relative to the pathogen. Examples of these coiling hyphae, are illustrated in Fig. 1-4. Throughout these studies, no evidence was found to indicate that the host cell wall had been penetrated.

Fig. 5-6. Longitudinal sections of hyphae of *Rhizoctonia solani* encircled by the coil-form hypha of *Arthrobactrum superba*. 5) An intrahypha extending from a Septum (×6,500). 6) Constricted host hypha with papillae (Pa). Zymogen inclusions (Z) in the coiled hypha (Co) of the parasite (×6,300).
However, several pathogenic effects were clearly visible. For instance, when *R. solani* was the host fungus, its clasped hyphae usually lost protoplasm, gradually became more transparent, and eventually was lysed. In some instances, *R. solani* produced an intraphyhal hypha in response to the effect of the coils (Fig. 2, 5).

Transmission electron microscopy of a near median longitudinal section of a hypha of *R. solani* clasped by coils of *A. superba* showed that the intraphyhal hypha originated from the adjacent hyphal cell of *R. solani* and not from the pathogen (Fig. 5). Most views of this host-pathogen relationship showed that the host hyphae were first constricted and that this was followed by obvious changes in their protoplasm. Electron-dense amorphous material, commonly in the form of papillae-like deposits, accumulated on the inner surface of the host cell wall (Fig. 6). The interior of the coils of the pathogen often revealed the presence of what are presumed to be zymogen inclusions and an electron-dense layer at the periphery of the protoplast of each coil (Fig. 6). Eventually the protoplasm of the host, in the region of the coils, completely disappeared and portions of its cell wall were dissolved.

Although the deleterious effect of these nematode-trapping fungi on certain host fungi was very evident, they did not destroy their hosts as rapidly as did other mycoparasites studied by the senior author (14). Usually it took 30–40 days to destroy one-third to half of a host colony, such as *R. solani*, on half-strength corn-meal agar, a medium which favored their pathogenicity. On other media (e.g., malt extract or Czapek’s solution agar) the deleterious effect of these pathogens on their hosts was often negligible.

**DISCUSSION**

*Arthrobotrys oligospora*, *A. robusta*, and *A. superba* were, for the first time, found to be playing roles as pathogens of fungi as well as nematodes. They can prey on nematodes by adhesive hyphal network traps (5), or on certain fungi by specialized hyphal coils. Both the nematode-trapping fungi and the fungus-coiling devices are induced structures which usually do not form in an axenic culture. It has been shown that network traps are produced after nematodes (5), or substances such as simple peptides (8), nemin (9), or products of animal origin (5) are added to their cultures. Thus, the morphogenesis of the trapping-devices seems to be related to some exogenous substance(s). Likewise, all three species produced specialized coils of hyphae only when in contact with their fungal hosts. Their production of coils appeared to be initiated by a specific trophic reaction similar to the positive tropism reported for the haustorial mycoparasite, *Piptocephalis virginiana* Leadbeater and Mercer (1).

It was particularly interesting to see these nematode-trapping fungi counterattack the normally mycoparasitic *Geotrichum* sp., in what may be termed a type of reverse mycoparasitism (12).

During the pathogenic process, portions of the appressed walls of the hosts were dissolved, while the walls of the coil-form hyphae in contact with the hosts remained intact; therefore, the cytoplasm of pathogen and host never intermixed. In this respect, the pathogenicity of these three fungi differs from the mycoparasitism of *Parasitella simplex* Bainier, *Chaetocladium brefedi* var. *macrosporum* V.T. & Le Mon (4), and *Calcarisporium parasiticum* (6), and those examples studied by Hoch (6, 7) none of which produced necrotic or diletaceous responses in the cytoplasm of the parasitized hosts.

The formation of almost identical coiling structures by the three fungi in response to the same fungal hosts, is probably a reflection of the close phylogenetic relationship between *A. oligospora*, *A. robusta*, and *A. superba*.

**LITERATURE CITED**

