Species of Phytophthora **and** Pythium **as Nematode-destroying Fungi**

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Abstract: Pythium monospermum,, P. aphanidermatum, and *Phytophthora palmivora* were found to be capable of destroying certain nonstylet-bearing nematodes through endozoic parasitism by hyphae from ingested zoospores. Hyphae of *P. monospermum* parasitized nematode eggs but could not capture or otherwise prey upon living nematodes. We suggest that endoparasitism of free-living nematodes may be common among Oomycetes in nature. *Key words: Pythium monospermum, Pythium aphanidermatum, Phytophthora palmivora,* endozoic parasitism, nematode control.

The literature of mycology has few, if any, references to either descriptive or experimental research on nematode parasitization by an Oomycete. Duddington (5) mentioned having seen a species of *Phytophthora* in a dead nematode. He did not describe the fungus but suggested it may have been identical with a fungus, described as *Pythium anguillulae,* that Sadeheck had found in vinegar eels.

In the examination of nematodes isolated from garden soil at Macdonald Campus at McGill University, protoplasm was seen streaming toward the apices of short hyphal branches that originated from a dead nematode. Within minutes the hyphal tips bulged out to form numerous zoosporangium-like vesicles. After a short period of differentiation, zoospores were discharged in all directions. Eventually a pure culture of this fungus was established, and it was found to be an Oomycete. The question then arose, do other Oomycetes parasitize nematodes in this manner? In order to determine the answer, *Pythium aphanidermatum* (Edson) Fitz. and *Phytophthora palmivora* Butler, two well known plant-pathogenic fungi, were selected for experimentation and for comparison with the unknown Oomycete.

MATERIALS AND METHODS

A fungus-infected nematode was transferred to 2% water-agar in a petri dish and incubated for 24–48 h at room temperature (23–28 C). A hyphal tip growing from a dead nematode was excised and transferred to half-strength cornmeal agar (Difco Laboratories, Detroit, Michigan) on which a pure culture was established.

The isolated fungus was grown on half-strength cornmeal agar at room temperature for 2 wk before morphological studies were made under a light microscope. The fungus was identified with keys proposed by Hendrix and Papp (6), Middleton (7), and Waterhouse (8).

To induce production of zoospores by the fungus from the dead nematode, the fungus was first cultured at room temperature on hemp-seed broth. A portion of the resulting mycelial mat was transferred to sterile pond water which was cooled for 24 h at 5 C and then brought to room temperature. This treatment induced the formation of zoosporangia, from which many zoospores were discharged. *Phytophthora palmivora* produced zoospores when it was grown for 7 d on V–8 juice-agar at room temperature, then flooded with sterile distilled water, and shaken vigorously for 30 s. Zoospores were collected by passing the fluid through a 43-μm screen. To induce zoospore production by *Phythium aphanidermatum,* the fungus was first grown for 7 d on yeast-extract at room temperature. Mycelium-bearing agar blocks cut form the periphery of the resulting colonies were floated in 10 ml of distilled water in a petri dish and incubated for 48 h at 10 C. This treatment usually induced the formation of inflated filamentous sporangia, but zoospores were not produced until some root segments of *Vigna* sp. were added and the culture chilled for 24 h at 5 C and then returned to room temperature.

Two inoculation methods were used for studying the mode of parasitism on nematodes by *P. aphanidermatum, Phytophthora palmivora,* and the fungus from the dead nematode. In the first method, either stylet

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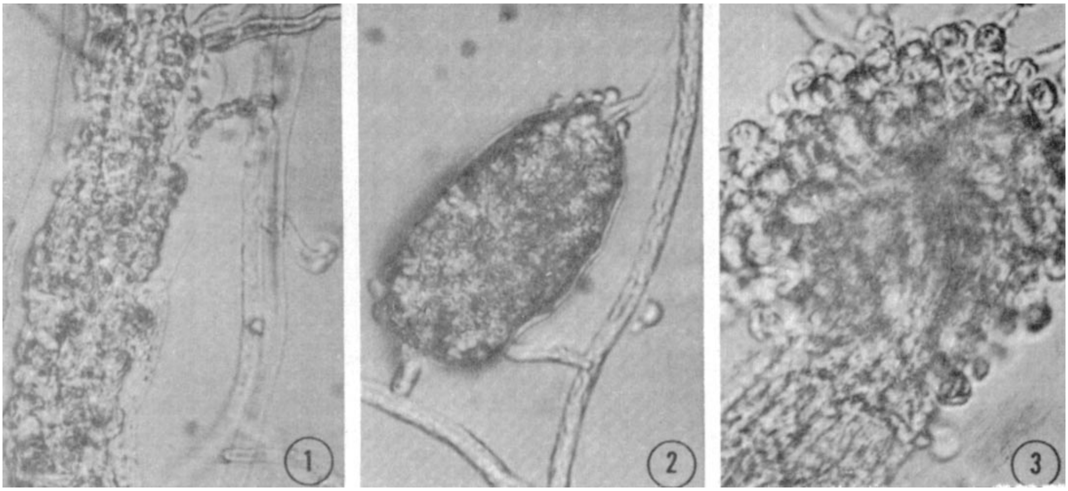
bearing nematodes (*Alphelenchus avenae* Bastian, *Meloidogyne hapla* Chitwood) or free-living nematodes (species of *Cephalobus, Plectus,* or *Rhabditis*) were added to zoospore suspensions of the fungi. In the second method, the nematodes were added to the fungus cultures as they grew on half-strength cornmeal agar. Infected nematodes were transferred to either water or lactophenol on glass slides for viewing under a light microscope. Appropriate lighting and camera equipment were used for time-lapse cinematography.

RESULTS AND DISCUSSIONS

The formation of zoosporangia and the discharge of zoospores from hyphae that grew from a naturally infected nematode indicated that this endozoic fungus was an Oomycete. A subculture of the fungus, in axenic liquid culture, formed filamentous sporangia, produced evacuation vesicles, and discharged zoospores in a manner identical to those of the fugus growing from the naturally infected nematode. As a sporangium matured, protoplasm flowed rather rapidly to its apex, which then extended to form a spherical vesicle. The protoplasm within the vesicle trembled vigorously and differentiated into zoospores within 5 min. The crowded, moving zoospores soon broke the fragile wall of the vesicle, from which they scattered in all directions. The zoospores were reniform to ovate and laterally biflagellate. After encysting, the zoospores germinated by means of germ tubes.

Filamentous sporangium-like hyphae were formed on an agar medium but did not discharge zoospores even when irrigated with water. Sexual reproduction by this fungus was characterized by a fusion of distinctive antheridia and oogonia. There was usually only one antheridium per oogonium, but rarely there were two. The globose oogonia were smooth walled, terminal, and intercalary; each was about 19–23 μm d. Each oogonium contained only one oospore. The antheridia were usually clavate at points of contact with oogonia. Young hyphae of this fungus were without septa, and they commonly branched at right angles to the parent hypha. Septate hyphae occasionally formed bundles of coils. Microsclerotium-like lateral outgrowths were seen occasionally. The general morphology of the fungus isolated from the nematode cadaver indicated that it was a strain of *Pythium monospermum* Pringsheim, which, according to Middleton (7), was originally described as a saprohyte on insect cadavers in water.

P. monospermum did not produce any specialized trapping devices in response to the presence of nematodes in the solid agar medium, nor did it secrete adhesive substances in the manner of such phycomyceteous nematophagous fungi as *Stylopage hadra* Drechlser or *Cystopage lateralis*

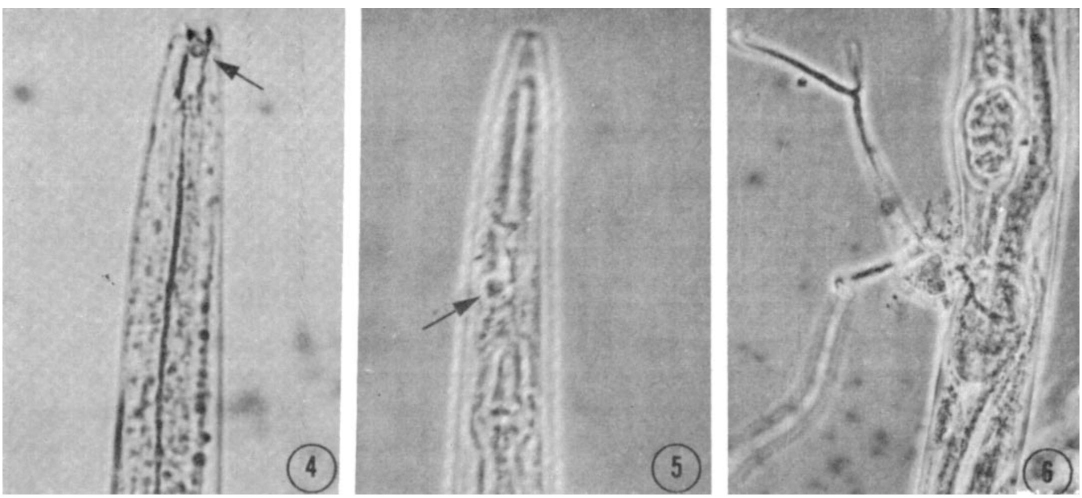


Figs. 1–3. Invasion and consumption of nematodes, and an egg, by *Phythium monospermum*. 1) Nematodes filled with the hyphae of the fungus. ×660. 2) A nematode egg, infected and deformed. ×800. 3) Zoospores clustered around the head of an infected nematode. ×600.

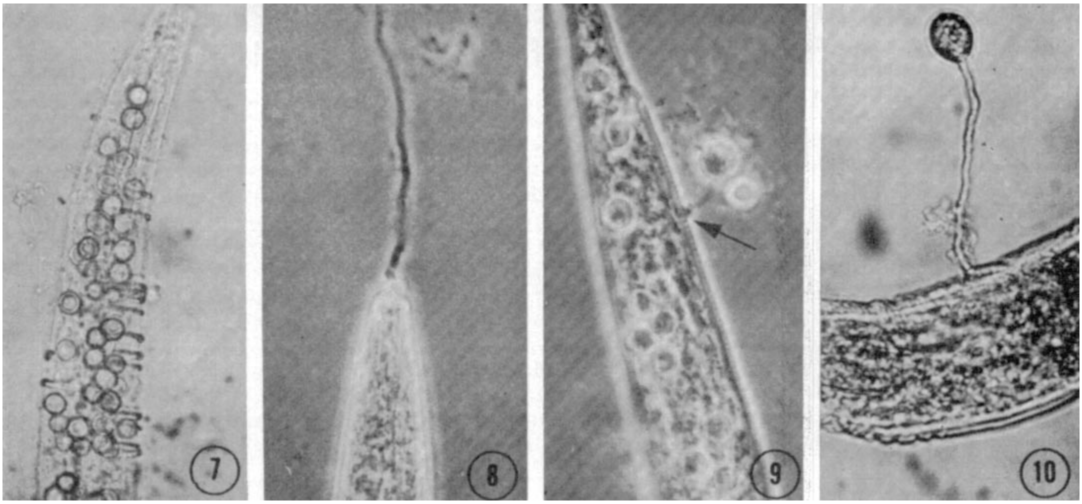
Drechsler (4). Hyphae of *P. monospermum* did not attach themselves to adult or juvenile nematodes even when the inoculated petri dishes were kept at low temperatures (5–15 C) to make the nematodes very sluggish. On the other hand, dead nematodes of all the test species were penetrated and consumed by this fungus (Fig. 1). *P. monospermum* did parasitize apparently viable nematode eggs by unspecialized hyphae. The infected eggs were eventually consumed and destroyed (Fig. 2). Barron (2) commented that very few fungi are known to specialize as parasites of nematode eggs. Although *P. monospermum* could not ensnare nematodes by trapping or by adhesion, it could parasitize species of free-living nematodes from the inside if its zoospores were swallowed. The ingested zoospores, some of which may have been swallowed as cysts, were not digested by the nematodes; instead, they encysted, germinated, and initiated infection. Some zoospores lodged and germinated in the stoma of the host nematode, whereas others germinated in the esophagus. Trophic hyphae usually grew throughout the body of the nematode (Figs. 3, 4, 5, 6), and zoosporangia formed and zoospores were released within 48 h after the zoospores or cysts had germinated, thus completing the cycle.

The mode of parasitism of *Phytophthora palmivora* and *Pythium aphanidermatum* was found to be the same as that of *P. monspermum*. Whether the ingested zoospores of *P. aphanidermatum* lodged and germinated in the stoma (Fig. 4) or in the esophagus (Fig. 5), infection hyphae soon grew throughout the infected nematode and protruded out of the body cavity, as illustrated in Fig. 6. Nematodes that were lightly infected remained motile for several hours, but as infection increased they gradually became nonmotile and soon died. Hundreds of zoospores or cysts of *P. palmivora* were commonly seen in individual nematodes (Fig. 7). Hyphae sometimes grew through the stoma of the infected nematode and extended to the surrounding medium (Fig. 8), and occasionally there was a secondary invasion of a dead or moribund nematode by zoospores that developed from attached sporangia (Figs. 9, 10). It may be noteworthy that the internal infection hyphae of *P. aphanidermatum* and *P. palmivora* did not develop to the extent of those of *P. monospermum* during the same infection time.

Also seen in Fig. 7 were spherical bodies whose identity and function were unknown to Capstick et al. (3) who reported seeing them on the cuticle of infected nematodes isolated from forest litter. We assumed them to be encysted zoospores because of their morphological similarity to ones that were known to be cysts. Another species of *Pythium*, probably *P. dissotocum* Drechsler (7), also had the same



Figs. 4–6. Free-living nematodes infected with *Pythium aphanidermatum*. 4) Ingested zoospore located in the buccal cavity of a nematode (arrow). ×840. 5) Ingested zoospore germinating in the esophagus of a nematode (arrow). ×625. 6) Hyphae protruding from from an infected nematode. ×550.



Figs. 7–10. Free-living nematodes infected with *Phytophthora palmivora*. 7) Ingested zoospores or their cysts in the body of a nematode. ×670. 8) Hapha from an ingested zoospore protruding from the stoma of a nematode. ×720. 9) A zoosporangium extending from an infected nematode (arrow). The spherical structures inside the nematode are cysts and zoospores. ×890. 10) An enlarged view of a zoosporangium that grew from inside a nematode. ×920.

mode of parasitism as the above mentioned fungi (personal observations).

None of the stylet-bearing nematodes were parasitized during these trials.

DISCUSSION

The tested species of *Phytophthora* and *Pythium* parasitized nematodes by a method similar to that described by Barron (1) for *Harposporium anguillulae* Lohde and *H. helicoides* Drechsler. These fungi are unlikely to be of use in biological control of plant-parasitic nematodes because practically all such nematodes have a stylet or spear-like mouthpart with a lumen too narrow for ingesting zoospores.

Our observations suggest that endoparasitism of nematodes by the zoospores of Oomycetes may be a common phenomenon, and one is tempted to speculate that endozoic parasitism may have some significance for the survival of these fungi.

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