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Investigations of Nematode-Destroying Fungi in Central Iowa

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Abstract. Three substrata, dung of *Sylvilagus floridanus,* leaf mulch, and rotting logs, from wooded areas in central Iowa are investigated for nematode-destroying fungi. Fifteen species are newly recorded for Iowa as follows: *Acrostalagmus bactrosporus, Arthrobotrys cladodes* var. *macroides, A. dactyloides, A. robusta, Cystopage lateralis, Dactylaria candida, D. haptotyla, Dactylella bembicoides, D. ellipsospora, Harposporium helicoides, Meristacrum asterospermum, Nematoctonus haptocladus, N. leiosporus, N. robusta,* and *Stylopage hadra.* Eight previously reported species are isolated. A species resembling *Nematoctonus leiosporus* but with shorter spores which were often hooked at the terminal end was found. All three substrata proved highly productive.

Nematode-destroying fungi have attracted increasing attention during the past several decades. They have been explored extensively in both the British Isles (Duddington, 1957) and in Russia (Soprunov, 1958) as a possible method of nematode control. Their prevalence in Iowa remained almost totally uninvestigated until the beginning of the present decade when Norton (1962) reported seventeen species of fungi parasitic on nematodes new to Iowa. Only two previous records of nematode-destroying fungi from Iowa had been reported. Martin (1927) reported *Achlyogeton entophytum* Shenk, which is also known to parasitize nematodes, as occurring on *Cladophora.* Drechsler had reported *Dactylella gephyropaga* Drech. (1937) from Iowa.

Norton sampled predominately soil from the roots of forage legumes (237 samples) with lesser numbers of samples from a bog (7 samples), rotting logs (10 samples), leaf mulch (23 samples), moss and adjacent soil (11 samples), soil (40 samples), river banks (12 samples), and nine miscellaneous samples. Norton recorded 18 species of which 12 were found only in wooded areas even though the majority of his samples were from forage legume fields (237 samples versus 111 samples from wooded areas). Eleven of the 18 species were found in leaf mulch, rotting wood or both, even though these substrata constituted less than 10 percent of his total number of samples.

Norton had one sample of dung from which he isolated one species. Many previous reports have been made of nematode-destroying fungi occurring on dung. The present work consists of further investigation of the presence of nematode-destroying fungi in samples of these substrata from heavily wooded areas in central Iowa.

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Materials and Methods

Wood from rotting logs, leaf mulch with adjacent soil particles and dung pellets of the cottontail rabbit, *Sylvilagus floridanus* Allen, were collected in polyethylene bags. The dung was collected during June of 1966 and the leaf mulch and rotting wood during June of 1967. Samples were collected from heavily wooded areas in central Iowa as listed in Table 1.

Table 1

Collection Sites and Sample Numbers per Site

Collection Sites	Number of Samples per Substrate: Rabbit Dung Pellets	Leaf Mulch Samples	Rotting Log Samples
Dolliver Memorial State Park Webster County		6	6
Emma McCarthy Lee City Park Ames, Story County	75	5	2
Ledges State Park Boone County	41	2	2
Mitigwa Boy Scout Camp Boone County	12		
Pammel Woods, Iowa State University Story County	28		
Pine Lake State Park Hardin County		4	4
Wooded area on the Skunk River 5 miles NEE Ames, Story County	17		
Woodman Hollow State Park Webster County		5	5

Several dung pellets or a few grams of rotting wood or leaf mulch were inoculated on 2 percent water agar plates and incubated at room temperature in plastic containers to prevent excessive evaporation. The plates were examined periodically for 6 to 9 weeks. Sealed lactophenol slides were made of all nematode-destroying fungi observed and cultures of non-endozoic species were maintained on one-half strength corn meal agar (11 grams corn meal agar from Baltimore Biological Laboratories, Baltimore, Maryland, and 10 grams purifies agar from Difco Laboratories, Detroit, Michigan, per liter of water).

All measurements were made from fungi growing on nematode-infested water agar plates. In most instances these were the original plates on which a variety of nematodes had emerged from the inoculum. Certain species of fungi were first grown in pure culture and then inoculated onto nematode-infested water agar to obtain a more standard set of conditions for identification.

Results

A total of 23 species of nematode-destroying fungi were identified

during the present investigation. Eight previously recorded species were found: *Acrostalagmus obovatus, Arthrobotrys conoides, Arthrobotrys musiformis, Arthrobotrys oligospora, Dactylaria brochopaga, Dactylella gephyropaga, Harposporium anguillulae,* and *Meria coniospora.* Fifteen species of nematode-destroying fungi are newly recorded for Iowa. Brief descriptions follow.

PHYCOMYCETES

Cystopage lateralis Drech. (1941a)

Nematode capture by adhesive mycelium. Mycelium sparce, aseptate, hyaline, capturing nematodes by adhesion to morphologically unmodified hyphae, producing a yellowish adhesive at the point of contact; haustorial fiilaments 1.5-2.5 microns wide, invading the nematode and assimilating the fleshy contents; chylamydospores produced laterally, sessile, or following evacuation of the narrow basal portion surmounting an empty pedicel, colorless, 20-50 by 13-25 microns, varying in shape from globose to elongate-ellipsoidal or elongate-obovoid.

Isolated from wood, Pine Lake State Park.

Meristracrum asterospermum Drech. (1940)

Endozoic. Vegetative hyphae hyaline, mostly simple, 50-400 by 5-10 microns, breaking into hyphal bodies 25-80 microns long, rounding out at both ends and becoming up to 15 microns in width; each hyphal body giving rise to a single spiraling, aerial conidiophore up to 350 microns in length and 5.5 microns wide; later the proximal end becoming void of cytoplasm, the distal end swelling to a width of up to 12 microns, becoming divided into 3-13 cells by basipetal deposition of transverse crosswalls at intervals of 4-12 microns; each cell then giving rise to a single sessile, one-celled, obovoid conidium, 6-12 by 5-8 microns.

Isolated from wood, Pine Lake State Park; leaf mulch, Ledges State Park; leaf mulch, Woodman Hollow State Park; dung, Emma McCarthy Lee City Park, Ames.

Stylopage hadra Drech. (1935)

Nematode capture by adhesion to morphologically unmodified hyphae. Mycelium sparce, hyaline, aseptate, 3-5 microns wide, holding nematodes by a yellowish adhesive produced at the point of contact, giving rise to an orbicular protuberance within the adhesive, infective hyphae arising from the orbicular protuberance; infective hyphae 2-3 microns wide, extending to the ends of the nematode; conidiophores up to 350 microns long, 4-5 microns wide near the base, tapering to 3-4 microns near the apex where a single conidium is produced or after repeated elongation of the conidiophore, up to four conidia; conidia one-celled, obovoid, 25-45 by 12-18 microns, filled with densely granular cytoplasm.

Isolated from leaf mulch, Emma McCarthy Lee City Park, Ames; leaf mulch, Pine Lake State Park.

FUNGI IMPERFECTI

Acrostalagmus bactrosporus Drech. (1941b)

Endozoic. Mycelium hyaline, branched, septate, 1.5-2 microns wide; condiophores erect, non-branching, 2-3 microns wide and up to 180 microns in length, bearing whorls of usually 2-5 conidiophorous branches 15-21 microns long, tapering from 2-3 microns near the base to less than one micron near the tip, bearing clusters of conidia; conidia one-celled, 2-5 by 1.5-3 microns, bacilliform.

Isolated from leaf mulch, Emma McCarthy Lee City Park, Ames.

The Iowa material differs slightly from that described by Drechsler in that the conidiophores were not seen to branch and the conidia of certain specimens exceeded the dimensions of 2-3 by 1.3-1.6 microns as described by Drechsler. The bacilliform shape of the conidia would eliminate it from being any other previously described species of Acrostalagmus.

Arthrobotrys cladodes var. *macroides* Drech. (1944)

Nematode capture by sticky networks. Mycelium hyaline, septate, 2-5 microns wide, giving rise to loops and bails 30-40 microns outside diameter with a greatest hyphal width of 6 microns, frequently compounded into complex networks; conidiophores erect, simple or branched, 120-300 microns in length, tapering from 4-5 microns near the base to 2-2.5 microns near the apex, bearing a single head of conidia on a slightly inflated irregular apex, or following branching several such heads; conidia elongate-ellipsoid to elongate-obovoid, two-celled, 13-22 by 5-8 microns, having the crosswall above, at, or below the middle.

Isolated from dung and leaf mulch, Emma McCarthy Lee City Park, Ames; dung, Ledges State Park; dung, Pammel Woods, Iowa State University; leaf mulch, Pine Lake State Park.

Arthrobotrys dactyloides Drech. (1937)

Nematode capture by constricting rings. Mycelium hyaline, septate, 3-5 microns wide, producing constricting rings 24-32 microns outside diameter, 15-20 microns inside diameter, composed of three arcuate cells, supported by a short heavy stalk 5-10 by 4-6 microns; conidiophores erect, 180-320 microns in length, tapering from 5-7 microns near the base to 3-4 microns near the apex, bearing 3-11 conidia in loose capitate arrangement; conidia two-celled, elongate, straight or slightly curved, rounded distally, tapering at the base, 33-46 by 6-9 microns.

Isolated from wood, Ledges State Park.

Arthrobotrys robusta Duddington (1951)

Nematode capture by sticky networks. Mycelium hyaline, septate, 3-6 microns wide, bearing bails, loops and simple networks; loops 25-35 microns outside diameter with a greatest hyphal width of 8 microns; conidiophores 200-325 microns in length; tapering from 5-6 microns near the base to 2-4 microns near the slightly inflated apex, beaing a cluster of conidia; conidia oblong-pyriform, two-celled, 18-28 by 8-11 microns, the cells nearly equal or the distal cell slightly larger.

Isolated from leaf mulch, Dolliver Memorial State Park; dung, Emma McCarthy Lee City Park, Ames; dung, Pammel Woods, Iowa State University; leaf mulch and wood, Pine Lake State Park.

Dactylaria candida (Nees) Sacc. (Drech., 1937)

Nematode capture by sticky knobs and non-constricting rings. Mycelium hyaline, septate, 1.6-2.3 microns wide, usually giving rise to stalked adhesive knobs 7-10 microns long by 5-8 microns wide before giving rise to non-constricting rings; rings 16-23 microns outside diameter, borne on stalks 10-30 microns long; conidiophores erect, 150-400 microns in length, tapering from 3-4 microns near the base to 1-2 microns near the apex, bearing 2-6 conidia singly on spicules up to 10 microns in length; conidia 4-6 celled, spindle-shaped, 31-51 by 8-11 microns.

Isolated from dung, Pammel Woods, Iowa State University.

Dactylaria haptotyla Drech. (1950)

Nematode capture by adhesive knobs. Mycelium sparce, septate, 2-3.5 microns wide, giving rise to stalked adhesive knobs; adhesive knobs spherical to prolate, 6-8 to 6-10 microns, borne on stalks 1.5-2.5 by 7-20 microns; conidiophores 150-300 microns in length, tapering from 3-4 microns near the base to 2 microns near the apex, bearing 1-6 conidia on short branches in loose capitate arrangement; conidia hyaline, spindle-shaped, distally rounded, truncate at the base, five-celled, 37-45 by 6-9 microns, the center cell largest.

Isolated from leaf mulch, Pine Lake State Park.

Dactylella bembicoides Drech. (1937)

Nematode capture by constricting loops. Mycelium septate, 2-5 microns wide, bearing constricting rings consisting of three arcuate cells borne on short 1-2-celled stalks 3-10 microns in length; 25-35 microns outside diameter, 15-25 microns inside diameter; conidiophores rarely branched, 200-450 microns in length, 5-7 microns wide near base, tapering to 2-3 microns near the apex, bearing a single conidium; conidia hyaline, top-shaped, 38-50 by 15-22 microns, mostly four-celled with the third cell comprising slightly over one-half the total length.

Isolated from wood, Dolliver Memorial State Park.

Dactylella ellipsospora Grove (Drech., 1937)

Nematode capture by adhesive knobs. Mycelium sparce, septate, hyaline, 2-4 microns wide, bearing stalked adhesive knobs; adhesive knobs spherical to prolate, 5-8 by 5-10 microns, borne on unicellular stalks 5-15 microns long by 2-4 microns wide; conidiophores erect 150-325 microns long, bearing a single conidium; conidia hyaline, spindle-shaped, mostly five-celled, 38-50 by 10-14 microns, middle cell usually by far the largest, occasionally three-celled, then measuring 22-25 by 8-10 microns.

Isolated from leaf mulch and wood, Dolliver Memorial State Park.

Harposporium helicoides Drech. (1941b)

Endozoic. Assimilative hyphae septate, hyaline, 2.5-4 microns wide, branched, often running the entire length of the nematode; conidiophores external, hyaline, septate, 60-300 microns long, bearing globose phialides 3-5 microns in diameter along their entire length, but often more abundantly near the apex; phialides occasionally divided once, usually simple, giving rise to a single conidium; conidia hyaline, filiform, one-celled, 23-50 by 0.8-1.5 microns, helicoid, straight or hooked, tapering to a blunt apex, bearing a basal mucous droplet.

Isolated from leaf mulch and wood, Dolliver Memorial State Park; leaf mulch and dung, Ledges State Park; wood, Pine Lake State Park; wood, Woodman Hollow State Park.

Nematoctonus haptocladus Drech. (1946)

Endozoic. Mycelium hyaline, 1.5-2.5 microns wide, branching, giving rise to external, septate, hyphae 1.8-2.7 microns wide, of indefinite length, bearing clamp-connections; trapping nematodes on knobbed branches; branches 26-80 microns long, up to 5 microns wide; terminal dumbbell-shaped cell 4-5 by 2-3 microns, usually surrounded by a drop of adhesive liquid; conidia borne on short spicules, usually on aerial hyphae; conidia cylindrical or elongate-ellipsoidal, straight or slightly curved, 10-18 by 3-6 microns.

Isolated from dung, Ledges State Park.

Nematoctonus leiosporus Drech. (1941b)

Endozoic. Mycelium hyaline, 2-3 microns wide, branching, giving rise to external conidiophores; conidiophores up to 1.5 mm. long, at first aerial, later bending over and at least the distal portion becoming prostrate; conidia borne on spicules 3-7 by 1.5-2 microns; spicules sometimes arising from clamp connections; conidia hyaline, digitiform, one-celled, 20-26 by 2-4 microns.

Isolated from leaf mulch, Pine Lake State Park; leaf mulch, Woodman Hollow State Park.

Nematoctonus robustus Jones (1964)

Endozoic. Assimilative hyphae hyaline, 2.5-3.5 microns wide, giv-

ing rise to external septate hyphae 2.5-3.5 microns wide, of indefinite length, bearing clamp connections; trapping nematodes by stalked, adhesive, dumbbell-shaped knobs; knobs 7-10 by 2.5-3.5 microns, usually surrounded by a drop of adhesive liquid; stalks 2-7 microns in length; conidia borne of short spicules on prostrate or erect hyphae; conidia cylindrical to oblong, curved, 6-11 by 3-5 microns.

Isolated from dung, Ledges State Park.

Nematoctonus sp.

An isolate of *Nematoctonus sp.* was obtained from rotting logs from Emma McCarthy Lee City Park, Ames, which resembles *Nematoctonus leiosporus* in most respects except those of spore size and shape. This isolate produced spores which were spindle-shaped to digitiform, 12-22 by 4-6 microns. Not only were the spores shorter and thicker, but many of the shorter ones tapered abruptly at the distal end and bent over sharply 1-2 microns from the tip to form a small hooked tip about 1 micron in diameter at the point of flexure. When grown in pure culture this isolate produced spores 10-15 by 4-6 microns, most of which possessed the characteristic hooked tip.

Note on *Meria coniospora* Drech. (1941b)

Drechsler reports the spores as being 4-7 microns long by 1.8-2.5 microns wide near the base, sometimes bearing a terminal knob. Norton (1962) reports one sample which appeared to be an elongate form of *Meria coniospora* as having dimensions of 6-9 by 1.5-2 microns (average 7.8 by 1.5 microns). The present material produced mainly spores in the 4-7 by 1.8-2.5 range, but lacking terminal knobs, with 5-10 percent of the spores being 6-9 microns long with terminal knobs.

Remarks

All three substrata proved highly productive as indicated in Table 2. Only a few samples failed to yield any nematode-destroying fungi. These were usually overrun early with other species of fungi. The present investigation would seem to indicate a relative abundance of nematode-destroying fungi in wooded areas of central Iowa. All areas from which leaf mulch and rotting logs were both sampled yielded eight or more species while the two areas from which all three substrata were sampled yielded 10 and 11 species, respectively. The rabbit dung from Pammel Woods, Iowa State University, yielded five species. Nematode-destroying fungi are probably more abundant than indicated in Table 2 since certain species of *Arthrobotrys* and *Dactylella* develop rapidly and overrun the plates before slower growing species have opportunity to develop. It seems that much work remains to be done to achieve a reasonable survey of the presence and abundance of nematode-destroying fungi in central Iowa.

Table 2

Sites and Substrata from Which Individual Species Were Isolated

Species Isolated	Substrata and Site of Isolation		
	Dung	Leaf Mulch	Rotting Logs
Acrostalagmus bactrosporus		E	
Acrostalagmus obovatus		D	
Arthrobotrys cladodes var. *macroides*	ELP	EI	
Arthrobotrys conoides	P	DELW	EID
Arthrobotrys dactyloides			L
Arthrobotrys musiformis		E	
Arthrobotrys oligospora	LM	EILW	DI
Arthrobotrys robusta	EP	DI	I
Cystopage lateralis			I
Dactylaria brochopaga		DI	DLW
Dactylaria candida	P		
Dactylaria haptotyla		I	
Dactylella bembicoides			D
Dactylella ellipsospora		D	D
Dactylella gephyropaga		DLW	DL
Harposporium anguillulae	LPS	DLW	EDIW
Harposporium helicoides	L	DL	DIW
Meria coniospora		I	I
Meristacrum asterospermum	E	LW	I
Nematoctonus haptocladus	L		
Nematoctonus leiopsorus		IW	
Nematoctonus robustus	L		
Nematoctonus sp.			E
Stylopage hadra		EI	

D—Dolliver Memorial State Park.
E—Emma McCarthy Lee City Park.
I—Pine Lake State Park.
L—Ledges State Park.
M—Mitigwa Boy Scout Camp
P—Pammel Woods, Iowa State University.
S—Skunk River Site.
W—Woodman Hollow State Park.

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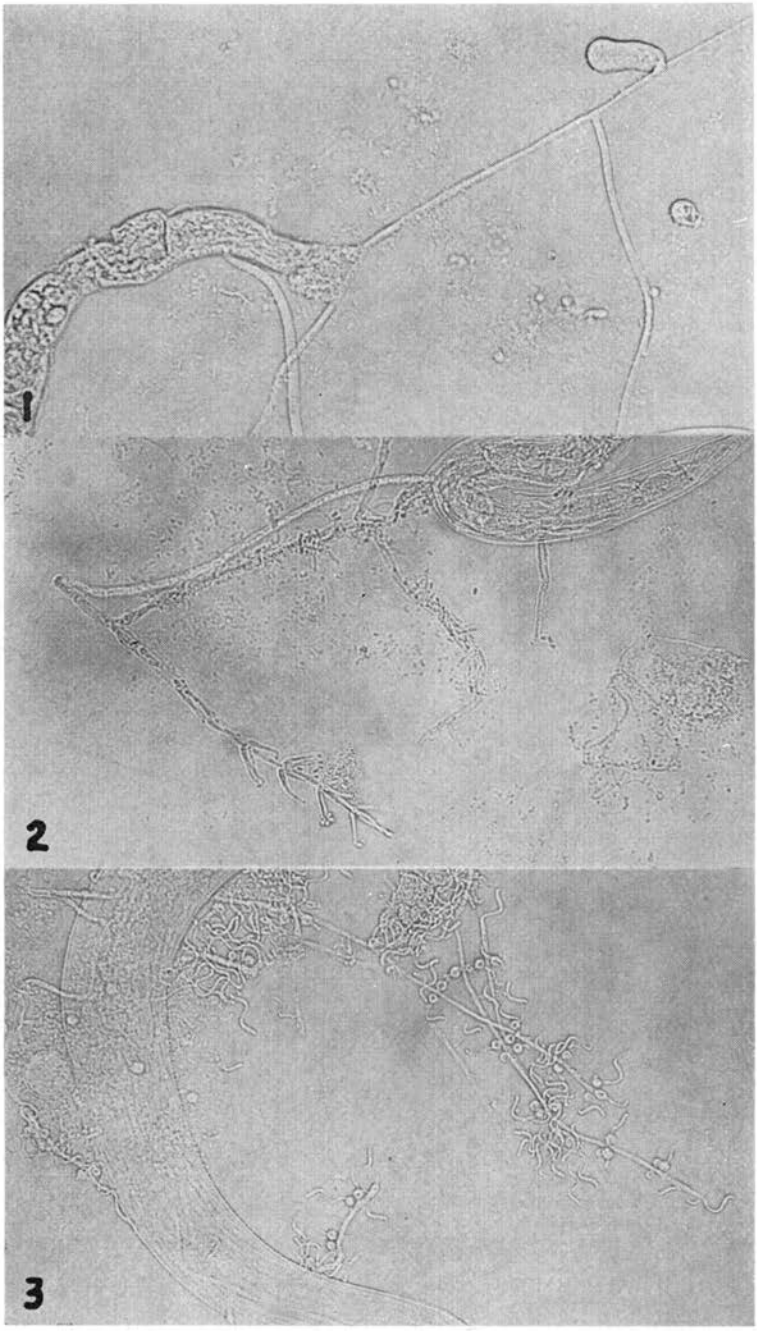

Figure 1. Hypha of *Cystopage lateralis* with chlamydospore and captured nematode. Figure 2. Nematode parasitized by *Acrostalagmus bactrosporus*. Figure 3. Nematode parasitized by *Harposporium helicoides*.

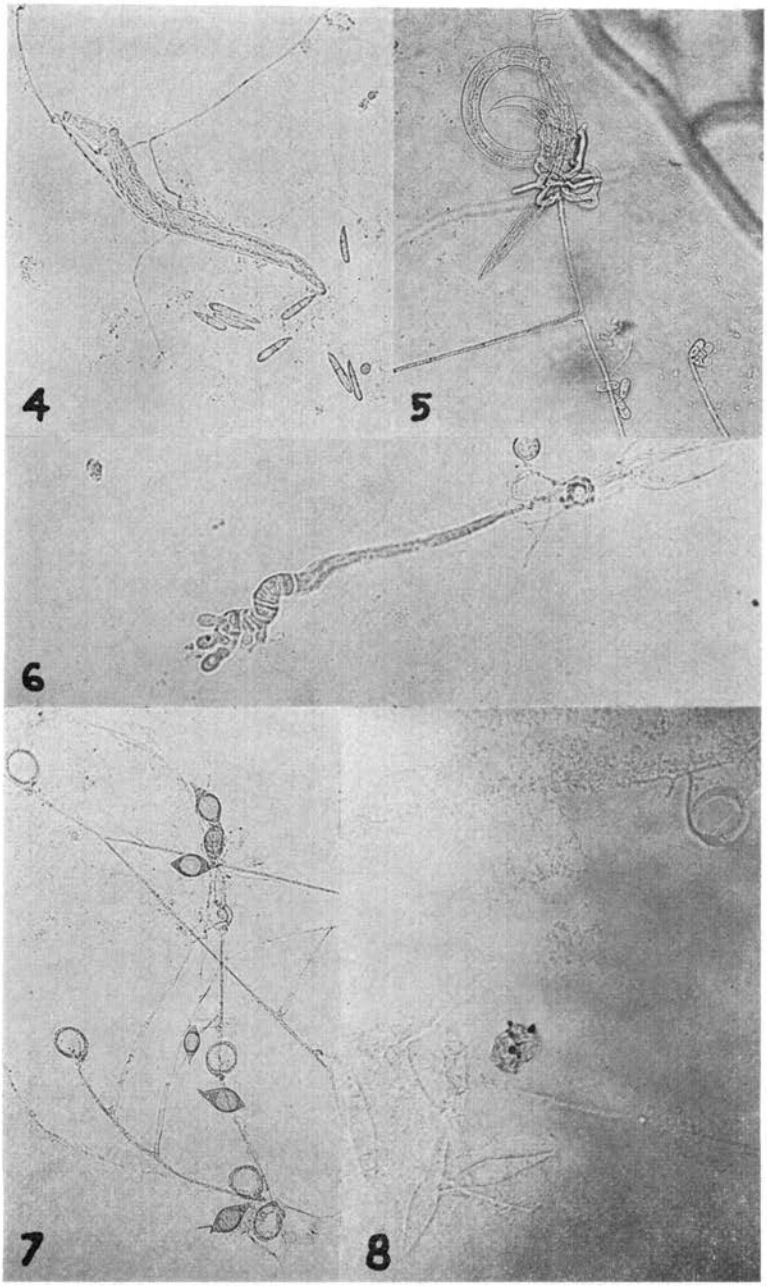

Figure 4. Conidia and constricting rings of *Arthrobotrys dactyloides*. Figure 5. Conidia, bails and loops of *Arthrobotrys cladodes* var. *macroides*. Figure 6. Conodiophore of *Meristacrum astropermum*. Figure 7. Conidia and constricting rings of *Dactylella bembicoides*. Figure 8. Conidia and non-constricting ring of *Dacctylaria candida*.

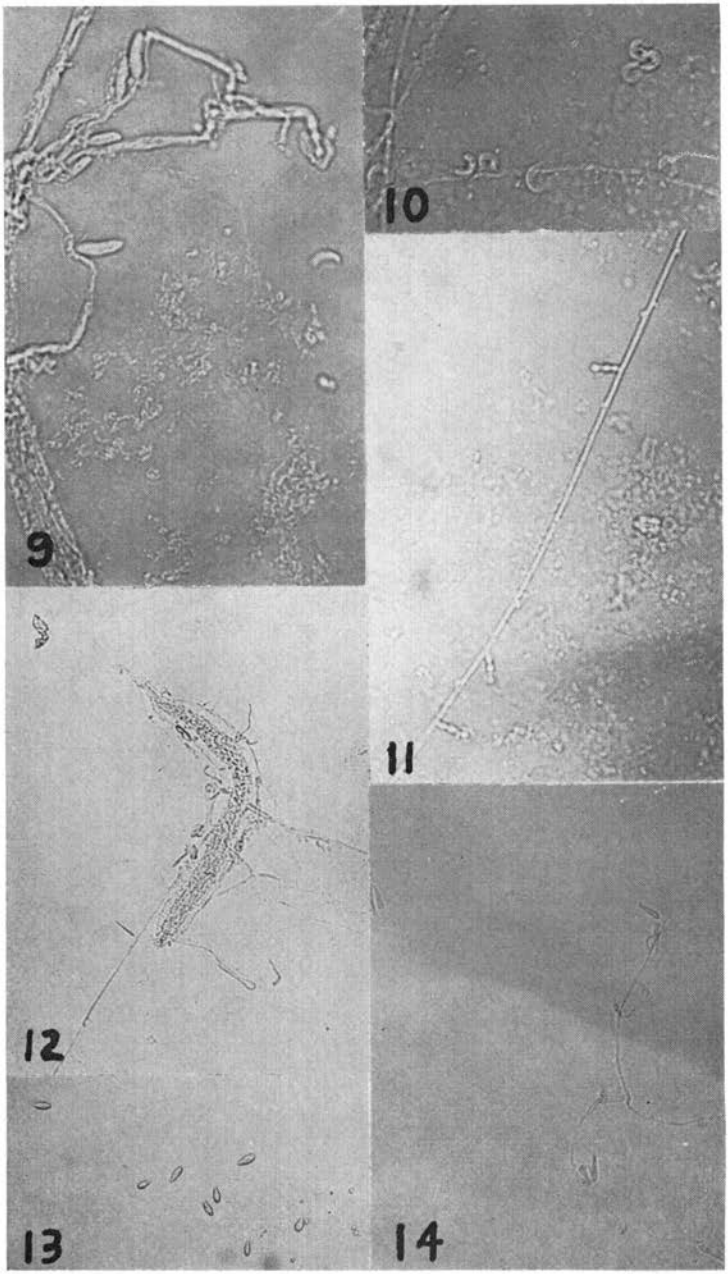

Figure 9. Conidia and terminal adhesive knob of *Nematoctonus raptocladus*. Figure 10. Conidia of *Nematoctonus robustus*. Figure 11. Adhesive knobs of *Nematoctonus robustus*.. Figure 12. Nematode parasitized by *Nematoctonus leisporus*. Figure 13. Conidia of *Stylopage hadra*. Figure 14. Conidia of *Nematoctonus sp.*

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