The University of Manitoba

NEMATOPHAGOUS FUNGI OF MANITOBA

BY

© GILDETTA VALENTE-ESPOSITO

A thesis submitted to the Faculty of Graduate Studies

in partial fulfilment of the requirements for the degree

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Note to the reader: the letter u has been used to represent the symbol for micron (μ).

Abstract

An investigation into the occurrence of nematophagous fungi in Manitoba showed that they are abundant and widespread.

From 120 samples collected at 23 different sites, 106 isolations yielded 31 different species of nematode-destroying fungi. The various species were referrable to the subdivisions Zygomycotina, Basidiomycotina, and Deuteromycotina, and comprised twenty-three predators, five endoparasites, and three members of the Agaricales.

Sixteen species were new to Manitoba, and the lignicolous basidiomycetes Panus rudis Fr., Pleurotus elongatipes Pk., and Pluteus aurantiorugosus (Trog.) Sacc. were tested for nematophagous ability for the first time in Manitoba.

The 31 species belonged to the following genera: Arthrobotrys Cda., Dactylaria Sacc., Dactylella Grove, Duddingtonia R.C. Cooke, Geniculifera Rifai, Monacrosporium Oudem., Harposporium Lodhe, Verticillium Nees, Stylopage Drechs., Nematoctonus Drechs., Panus Fr., Pleurotus (Fr.) kumm. and Pluteus Fr.

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INTRODUCTION

Nematophagous fungi are a taxonomically diverse group of organisms that have ecologically similar habitats and share a pronounced predilection towards utilizing microscopic animals, especially nematodes, as food source. They can be assigned to many fungal subdivisions: the Mastigomycotina, Zygomycotina, Deuteromycotina and Basidiomycotina. The nematophagous fungi have not recently evolved, since "Fossil nematodes in pieces of Mexican amber, approximately 25 million years old appeared to have been parasitized by fungi showing a striking resemblance to present-day nematophagous species" (Jansson 1986). This long coevolution between host and parasite has resulted in a variety of modifications in certain fungal spores and hyphae for capturing nematodes (Mankau 1980).

The nematode-destroying fungi can be divided into two broad groups: (1) endoparasitic and; (2) predaceous fungi. Endoparasitic species do not exhibit extensive mycelium development outside the body of the host, nor can they usually be active without a host. For example in the Chytridiomycete Catenaria anguillulae Sorokin only the evacuation tubes of the zoosporangia protrude from the nematode body, thus allowing for the release of the zoospores. In other endoparasitic species, such as Harposporium helicoides Drechs. (Fig. 1), only the conidiophores and conidia project externally into the air or trail on the substrate. The endoparasitic species rapidly complete their life cycle and persist in the form of resting spores when conditions are unfavorable for active growth. The infective agents are usually spores which make contact with the prey in different ways. In C. anguillulae, the flagellate zoospores swim to the prey and encyst on the host cuticle prior to penetration. Haptoglossa heterospora Drechs. (Davidson and Barron 1973) has a peculiar way of infecting nematodes; a tertiary spore, representing an infection unit, is injected from a secondary glossoid spore into the cuticle of a passing nematode. Only those tertiary spores that pass directly

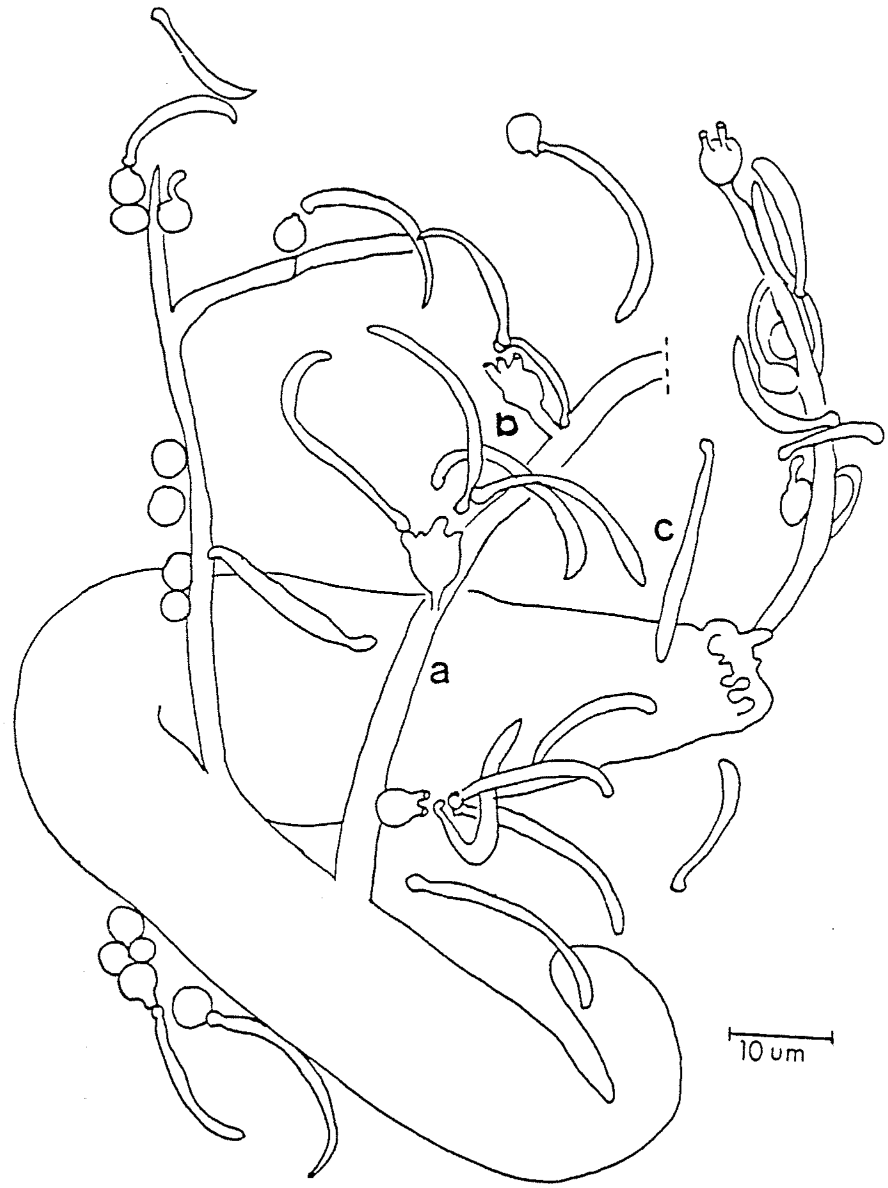

Figure 1. An endoparasitic development inside a nematode with conidiophores protruding from its cuticle (Harposporium helicoides Drechs.):
a. A conidiophore
b. A polyphialide
c. A conidium
Camera lucida drawing.

through the integument and the hypodermis of the nematode germinate into a single thallus. Spores may adhere to the cuticle of the nematode prior to germination and penetration in fungi such as Meria coniospora Drechs., or they may be ingested by the nematodes, as is the case in Harposporium species.

In contrast to the endoparasitic species, the predaceous species generally live as saprophytes. Once established, they colonize the substrate and, in the presence of nematodes, will produce the trapping device typical of the individual species. The term predaceous is applied to those fungi that can capture, kill and consume microscopic animals (Duddington 1955d), their specific trapping mechanisms have been described by Drechsler (1941b), Duddington (1962), Barron (1977b; 1981) and Gray (1987). These mechanisms are:

a) Adhesive hyphae: found in fungi such as Stylopage grandis Dudd. (Fig. 2A), in which an adhesive material produced on hyphal surfaces entraps the prey. A large area of the fungal hyphae can thus serve for nematode capture.

b) Adhesive branches: these consist of morphologically specialized branches which alone are covered with an adhesive material. The most common species in which such branches occur is Dactylella cionopaga Drechs. (Fig. 2B). Adhesive branches generally consist of one to several cells, but occasionally, a bridging hypha may join two adjacent branches and as a result of such anastomosis form a loop.

c) Adhesive net-works: these are the most common trapping mechanism. The net-works are covered with an adhesive matrix and develop from short lateral branches which curve and anastomose with the main hypha and with other branches. While Arthrobotrys musiformis Drechs. forms simple two dimensional net-works consisting of single loops, complex three dimensional net-works are more commonly formed by repeated development of single loops at one point.

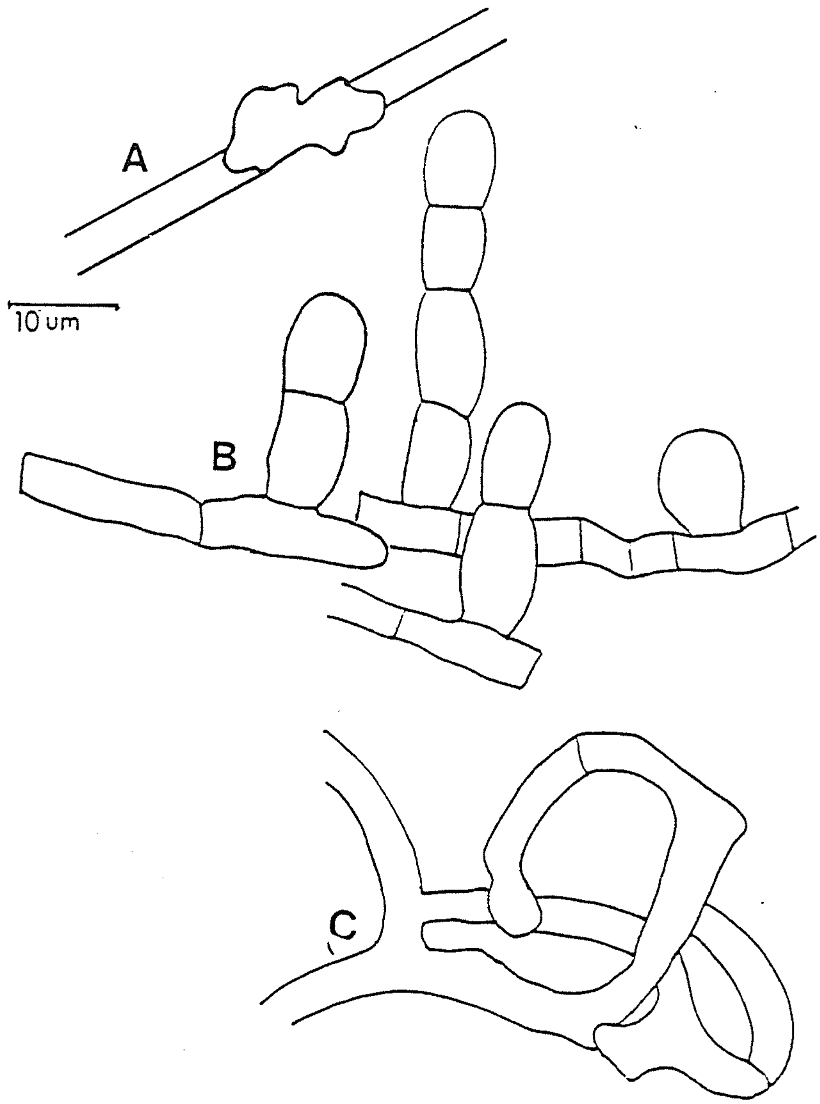

Fig. 2. Examples of trapping mechanisms of predaceous fungi:

A. An adhesive hypha (Stylopage grandis Dudd.).
B. Adhesive branches (Dactylella cionopaga Drechs.).
C. An initial stage in the development of adhesive net-works (Arthrobotrys oligospora Fres.).
Camera lucida drawings.

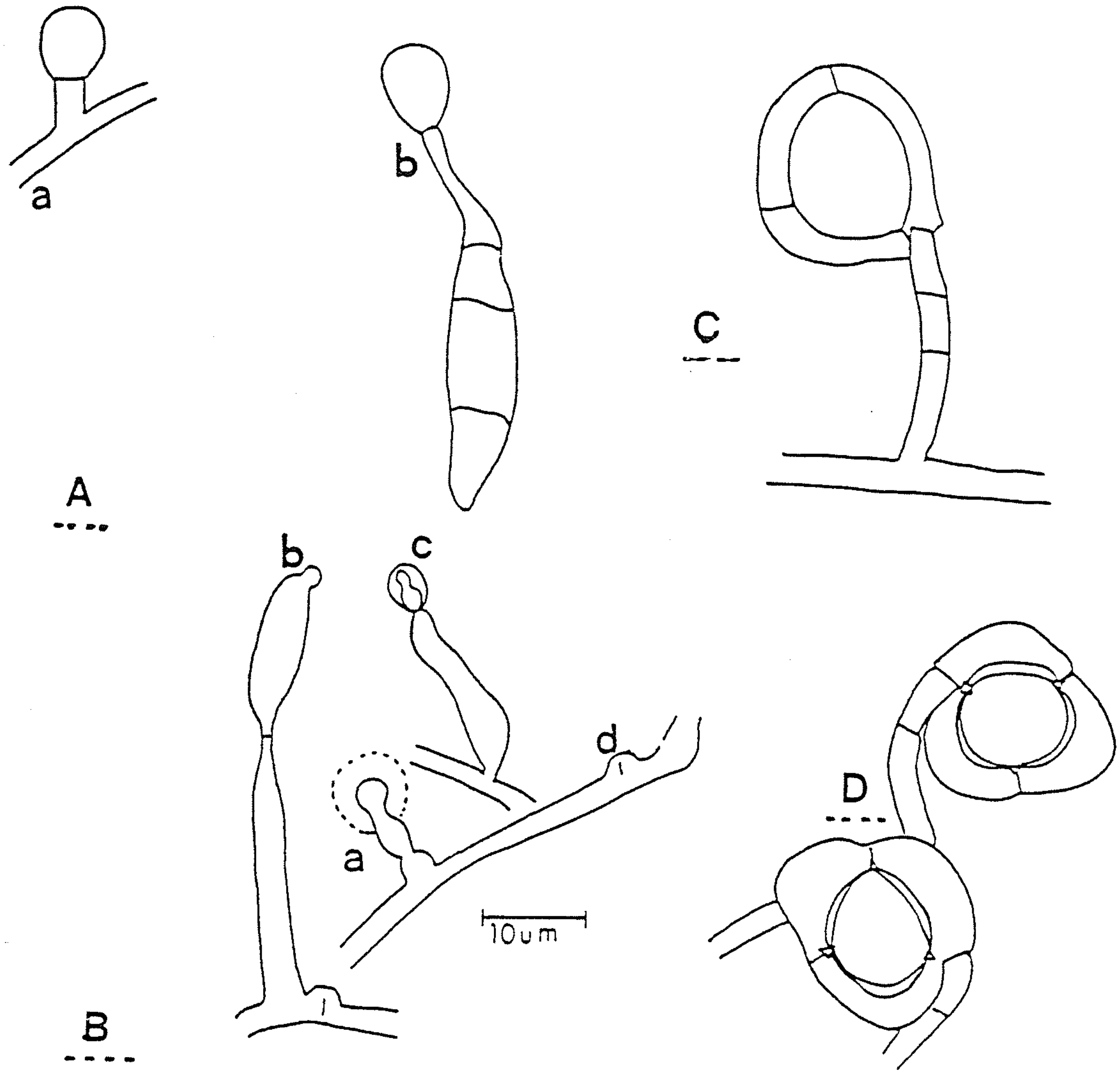

Figure 3. Examples of trapping mechanisms of predaceous fungi: -

A. Adhesive knobs (Dactylella drechsleri Tarjan),
   a. An adhesive knob on hypha
   b. An adhesive knob on a conidium.

B. Adhesive hour-glass knobs (Nematoctonus amatus Thorn and Barron),
   a. An adhesive hour-glass knob on hypha
   b. An initial stage of hour-glass knob on a conidium
   c. An hour-glass knob on a conidium
   d. A clamp connection.

C. A non-constricting ring (Dactylaria candida (Nees) Sacc.),

D. Constricting rings (Arthrobotrys dactyloides Drechs.). Camera lucida drawings.

These are seen in A. oligospora Fres. (Fig. 2C), and nematodes are caught by entanglement and adhesion to the net-works.

d) Adhesive knobs: typical of predaceous species of the Deuteromycotina and Basidiomycotina, either sessile or stalked and covered by adhesive material. Nematodes are trapped when they come in contact with one or more knobs. It is not uncommom to see a nematode struggling to free itself, but even when the knobs are detached from the hyphae, they remain attached to the nematode's cuticle and initiate subsequent infection. Dactylella drechsleri Tarjan is an example of a fungus which produces such trapping devices (Fig. 3 A). The conidia of such fungi not only produce typical hyphae on germinating, but can also give rise to an adhesive knob in the presence of nematodes (Fig. 3Ab). Species of the genus Nematoctonus Drechs. which possess typical hour-glass shaped knobs covered by a large drop of adhesive material are the only fungi currently said to have non-detachable knobs (Fig. 3B).

e) Non-constricting rings: here a nematode is caught when it enters the lumen of a three-celled ring, and becomes tightly wedged therein. Such structures are produced in species assignable to the Deuteromycotina. However, it is not unusual for fungi which produce non-constricting rings, to also produce sticky knobs e.g. Dactylaria candida (Nees) Sacc. (Fig. 3C). Detached rings with are viable and can initiate infection of entrapped nematodes.

f) Constricting rings: produced by such fungi as Arthrobotrys dactyloides Drechs. (Fig. 3D) are the most sophisticated trapping mechanism to be found amongst the predaceous members of the Deuteromycetes. When a nematode enters the lumen of the three-celled ring, there is a rapid, irreversible inward expansion of the three cells comprising the ring with sufficient force to constrict the body of the nematode. Drechsler (1950a) described how the constricting rings developed from the main hyphae in Dactylella aphrobrocha Drechs. He reported

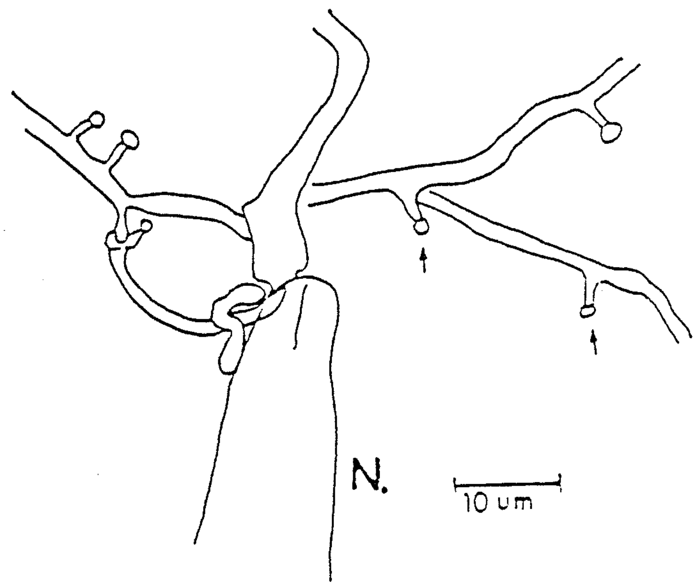

Figure 4. Stalked secretory cells for the release of nematoxin (arrows) (*Pleurotus* species).
N. A nematode immobilized by nematoxin and penetrated by the fungal hyphae.
Camera lucida drawing

that the ring arose as a curved branch from a prostrate vegetative hyphal element. Septa were formed in the part of this branch that became the stalk of the ring. Then, as the curving branch approached the stalk, a small bud grew out from the stalk and anastomosed with the tip of the curved branch. The branch tip continued to grow and then anastomosed with the base of the first ring cell. A septum then formed across this anastomosis and another septum formed to separate the third cell from the stalk.

Recently, Barron and Thorn (1987) have described another mechanism whereby some fungi can trap nematodes. Some species of the genus Pleurotus (Fr.) Kumm. produce stalked secretory cells which, on contact with nematodes, release a powerful toxin capable of quickly immobilizing them (Fig. 4).

Distinguishing between endoparasitic and predaceous fungi is a useful practice and generally one can assign fungi to one or the other group quite easily. However, there are species of Nematoctonus, eg. N. amatus Thorn and Barron which produce adhesive knobs on both the hyphae and the conidia. The adhesive knobs of the hyphae enable the fungus to catch, kill and consume their prey, while those produced by the conidia enable the fungus to start a new infection on a passing nematode, this pattern seems to form a bridge between predation and endoparasitism.

The purpose of this thesis is to describe and illustrate some of the nematophagous fungi which occur in Manitoba. Particular attention was given to the habitat and to the nature of the samples collected in an attempt to isolate the largest variety of organisms consuming nematodes. Nematophagous fungi are generally present in undisturbed habitats where nematodes are abundant, but individual species tend to have a favorite habitat. Samples were collected with a view to explore associations with particular soil types, other substrates, vegetation and specific environments.

LITERATURE REVIEW

Occurrence of nematophagous fungi

The endoparasitic fungus Harposporium anguillulae was the first fungus recorded as nematophagous (Lohde 1874). Fresenius (1852) had already named a fungus producing tall conidiophores with clusters of two-celled conidia Arthrobotrys oligospora, but he was not aware of its predatory ability. Nearly twenty years later, improved plating techniques employed by Woronin (1870) allowed for the observation of net-works produced on the hyphae of A. oligospora, but even then the function of these structures was not understood. Finally in 1888, Zopf observed nematodes caught in these net-works and saw the fungal hyphae penetrating the nematode cuticle and initiating an infection. The discovery did not attract great interest because it was thought that fungi would trap and consume nematodes only in times of starvation.

Fifty years later, Charles Drechsler (1933a; 1933b; 1933c and 1933d) using clear media observed that not only were nematodes caught by entanglement in the loops, but they were held there by a powerful adhesive produced by the fungus over the surface of such net-works. This began a long series of outstanding publications by Drechsler in which he described new genera and species and also pointed out the differences between the predaceous and endoparasitic fungi. As a result of his life-long interest in this group of organisms, Drechsler described approximately 100 new species distributed in many genera. Several species belonging to genera assignable to the subdivisions Zygomycotina or Mastigomycotina appeared to subsist on nematodes or on species of rhizopods. A few revealed a sexual life cycle, for e.g. Acaulopage rhicnospora Drechs. where sexual reproduction occurs by fusion of gametangia and results in the production of thick walled zygospores.

Predaceous Hyphomycetes were described by Drechsler in the following publications: 1936; 1937a, 1937b; 1940a; 1940b; 1943a; 1944a; 1944b; 1947; 1950a; 1950b; 1952; 1954b; 1962; and 1975. Most of these species belonging to the genera Arthrobotrys Cda., Dactylaria Sacc. and Dactylella Grove preyed on nematodes. Drechsler (1941a; 1942; 1946b; 1946c; 1950c and 1959a) also described endoparasitic hyphomycetes of the genera Acrostalagmus Cda., Harposporium Lohde, Cephalosporium Cda. and Spicaria Hasting that form conidia from phialides. All these genera of predaceous and endoparasitic Hyphomycetes are assignable to the subdivision Deuteromycotina.

Drechsler (1941a) also encountered predaceous fungi with clamp connections on their hyphae. He erected a new genus: Nematoctonus Drechs., for the first two endoparasitic species N. tylosporus Drechs. and N. leiosporus Drechs. which produced the typical hour-glass shaped adhesive knobs on conidia. Later, he reported two other endoparasitic species with adhesive knobs on conidia (Drechsler 1943b) and three predaceous Nematoctonus spp. with adhesive knobs on the hyphae (Drechsler 1946a; 1949; 1954a). This genus is assignable to the subdivision Basidiomycotina. The fact that nematophagous fungi are found in the four different subdivisions Zygomycotina, Mastigomycotina, Deuteromycotina and Basidiomycotina, confirms that the predaceous habit has arisen many times in more than one evolutionary line of fungi.

Another major contribution to the study of nematode trapping fungi was made by the British mycologist C. L. Duddington. Duddington (1940; 1946; 1949; 1950; 1951a; 1951b; 1951c; 1951d; 1953; 1954 and 1955c) recorded nematophagous fungi and described new species occurring in Britain. Duddington (1955a) wrote about techniques for handling predaceous Hyphomycetes and Duddington (1955b, 1955d, 1956, 1962 and 1963) addressed such topics as the physiology and taxonomy of this group of fungi and the inter-relationship between the nematophagous fungi and the nematodes.

Other British mycologists have also contributed to the literature of the nematophagous fungi by describing new species of endoparasitic or predaceous Hyphomycetes: Goodey (1951), R.C. Cooke (1964; 1969a; 1969b), R.C. Cooke and Dickinson (1965), R.C. Cooke and Satchuthananthavale (1965) and Rifai and R.C.Cooke (1966). Jones (1964) added Nematoctonus robustus Jones, isolated from leaf litter in Ghana, to the seven species described by Drechsler. In 1972, Giuma and R.C.Cooke described and illustrated Nematoctonus tripolitanus Giuma and R.C. Cooke, a new species collected in Libya.

Since early times, French researchers have been interested in the study of nematophagous fungi as biological control agents of nematodes parasitic on plant and animals (Comandon and De Fonbrune 1938, 1939; Descazeaux and Capelle 1939; Deschiens 1939a, 1939b). In 1946 Dollfus described all the species known at that time to attack nematodes, while Virat (1977) and Peloille and Cayrol (1979) added Candelabrella javanica Rifai and R.C.Cooke, Duddingtonia flagrans (Dudd.) R.C. Cooke. and A. oviformis Soprunov to the French records

Another outstanding contributor to the knowledge of the predaceous fungi was the Russian mycologist Soprunov, who in 1958 published descriptions of all the then-known and of several new predaceous Hyphomycetes isolated in Turkmenistan. The new species included A. oviformis Soprunov and A. dolioformis Soprunov, and Trichothecium pravicovi Soprunov.

Jarowaja (1968; 1971), a Polish mycologist, described two constricting-ring trappers, Dactylaria effusa Jarowaja and Dactylella inquisitor Jarowaja.

Unquestionably, the major Canadian contributor to our knowledge of the nematophagous fungi is G.L. Barron, who, alone and in collaboration with a series of co-workers, has investigated many aspects of the biology and taxonomy of the nematode-destroying fungi. In (1970), Barron noted that conidia of Harposporium helicoides Drechs. were ingested intact, and germinated in the nematode gut causing infection. He also pointed out (Barron 1973a) that Rhophalomyces elegans Cda. parasitizes both larval and adult stages of a species of Rhabditis, as well as nematode eggs and recorded important observations on special structures such as constricting rings and chlamydospores of the predaceous Hyphomycetes (Barron 1975b, 1979a). Barron (1977b) also published a book on the nematophagous fungi and presented an up to date overview (Barron 1981) on the most important features of the nematode-destroyng fungi.

Barron (1973b; 1975a; 1976a; 1976b; 1985) and Barron and Percy (1975) has described many new species of endoparasitic nematophagous fungi of the subdivisions Mastigomycotina and Zygomycotina. Other new species described by him were endoparasitic Hyphomycetes (Barron 1977a; 1979b; 1980) and the predaceous Hyphomycete Arthrobotrys botryospora Barron (1979c) with aseptate conidia. Barron and Davidson (1972) described A. anomala Barron and Davidson, an adhesive net-work trapper with narrow cylindrical conidia.

Barron and Dierkes (1977) showed that a Hohenbuehelia sp. was the perfect state of an Ontario isolate of Nematoctonus.

Thorn and Barron (1984) studied the ability of lignicolous Basidiomycetes to attack and consume nematodes. Testing 27 species they found five of Hohenbuehelia, five of Pleurotus and one of Resupinatus capable of destroying nematodes. Thorn and Barron (1986), in an Ontario based study, isolated five species of Nematoctonus and described a few more new species obtained in culture derived from basidiospores of several Hohenbuehelia spp.

Schenk et al. (1977) described Arthrobotrys amerospora Schenk, Kendrick and Pramer, a new species with aseptate conidia trapping nematodes by adhesive net-works.

The isolation of new species continued, Stirling and Mankau (1978) described Dactylella oviparasitica Sterling and Mankau, parasitic on eggs of Meloidogyne incognita Chitwood. McCulloch (1977b), during a survey of nematophagous fungi of Australia, isolated A. pauca McCulloch, a species similar to A. entomopaga, but with conidia produced on peg like sterigmata, Monacrosporium robustus McCulloch, a species capturing nematodes by means of sessile adhesive knobs, and two parasitic forms, Entomophthora vermicola McCulloch and Meristacrum pendulatum McCulloch.

In 1984, Tubaki and Yamanaka described a new species isolated from pine sap in Japan: Arthrobotrys ellipsospora Tubaki and Yamanaka, with small conidia and trapping by adhesive branches, and in 1985, Kuthubutheen et al. isolated in Malaysia a new nematode trapping synnematous species Arthrobotrys dendroides Kuthubuteen, Muid and Webster.