VARIABILITY AND TRAPPING ACTIVITY OF NEMATOPHAGOUS FUNGI

LOBO

STUDIES ON THE VARIABILITY AND TRAPPING ACTIVITY OF SELECTED NEMATOPHAGOUS FUNGI

by

Kenneth John Lobo, B.Sc. (Botany)

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

Department of Plant Pathology,
McGill University,
Montreal.

February, 1966.

TABLE OF CONTENTS

page

SECTION THREE

OBSERVATIONS ON THE CONTROL OF PLANT PARASITIC NEMATODES BY NEMATOPHAGOUS FUNGI AND PREDACIOUS NEMATODES

STUDIES ON THE VARIABILITY AND TRAPPING ACTIVITY OF SELECTED NEMATOPHAGOUS FUNGI

GENERAL INTRODUCTION

Predacious fungi that capture and kill nematodes live, amongst other places, in the soil where it is assumed that they participate in the natural control of nematodes, including the plant parasitic species that cause tremendous losses to farmers. There are predacious fungi in the soil which prey on amoebae, rotifers, rhizopods and other small animals but they were not dealt with in this study.

Nematode-trapping fungi have been known for about eighty years but little work was done on them during the first half of this period. In the past three decades, a period inaugurated by Charles Drechsler in America, increasing attention has been paid to this interesting group of fungi and today there are about 200 described species.

Much work has been done on biological, physiological and ecological aspects of the nematode-trapping fungi but few attempts have been made to determine the limits of their morphological variability or to assess the effect of environment on their trapping activity. Genetic studies, too, have been largely neglected and the existence of host specificity has not been adequately investigated.

Chemical control of plant parasitic nematodes is

costly and potentially hazardous to man and animals. In recent years, interest has been focussed on control by the use of the nematode-trapping fungi. The fungi are present in agricultural soils and a slight shift in the balance of Nature which favours the predacious fungi may result in effective nematode control. The classic instance of the successful control of the cottony-cushion scale (Icerya purchasi), an insect that damages citrus trees, by the lady-bird beetle (Rodolia cardinalis) offers encouragement in this direction (Riley, 1893).

costly and potentially hazardous to man and animals. In recent years, interest has been focussed on control by the use of the nematode-trapping fungi. The fungi are present in agricultural soils and a slight shift in the balance of Nature which favours the predacious fungi may result in effective nematode control. The classic instance of the successful control of the cottony-cushion scale (Icerya purchasi), an insect that damages citrus trees, by the lady-bird beetle (Rodolia cardinalis) offers encouragement in this direction (Riley, 1893).

GENERAL REVIEW OF LITERATURE

I. INTRODUCTION

A review that encompassed the knowledge of nematode-trapping fungi to that date, was prepared by Drechsler in 1941. Other comprehensive accounts of these fungi have been provided by Duddington (1955, 1956, 1957a, 1957b, 1958, 1960, 1962a, 1962b) and Olthof (1962). Soprunov (1958) summarized the work done in the U.S.S.R. and Cooke and Godfrey (1964) published a simple key for the identification of the nematophagous fungi.

The book by Dollfus (1945), which gives a detailed treatment of animals and plants parasitic on nematodes, remains one of the major works of reference on the subject.

II. HISTORICAL OUTLINE

The earliest recorded recognition of the predacious mode of fungal life was by Lohde in 1874 when he described Harposporium anguilullae. Previous to this, Arthrobotrys superba and A. oligospora had been described by Corda (1839) and by Fresenius (1852) respectively, but they were unaware of the fact that, besides being able to live saprophytically, the fungi described by them could supplement their diet by capturing nematodes. Nearly forty years later, Zopf (1888) discovered that A. oligospora captured nematodes by forming traps composed of a complex system of anastomosing loops. In the following year, 1889, he described another nematode-trapping fungus, Myzoctium vermicolum. Maupas described Protascus subuliformis in 1915.

Nearly fifty years after Zopf's classical finding, Charles Drechsler (1933), in America, inaugurated what may be termed the modern era of the study of the fungi predacious on nematodes, amoebae, rhizopods and rotifers. His first papers dealt with Zoopagaceae, a family of obligate predators, subsisting mainly on amoebae. In 1937, he published a long paper on the predacious hyphomycetes, in which he described eleven species new to science. Since then, he has found several more species and today the predacious fungi are particularly associated with his name.

Linford and his associates (1937, 1938, 1939), in Hawaii, pioneered in the use of nematode-trapping fungi for the control of plant parasitic nematodes. In France, important work was done on the physiology of the fungi by Commandon and deFonbrune (1939) and by Lamy (1943). Studies on the control of nematode parasites of plants and animals were made by Deschiens et al. (1943) and by Roubaud and Deschiens (1941).

Duddington, in England, began his researches into the predacious fungi in 1940. He named several new species (Duddington, 1949, 1951a, 1951b, 1951c, 1955) and he and his associates investigated their biology and their potential as agents of biological control of plant parasitic nematodes (Duddington et al., 1956; Duddington and Duthoit, 1960; Duddington et al., 1961).

In this decade, the names of Duddington (1962b), Tarjan (1962), Feder (1963), Mankau (1963), Cooke (1964), and Olthof and Estey (1965) are prominent in studies of the nematophagous fungi.

III. TAXONOMY AND VARIABILITY OF NEMATOPHAGOUS FUNGI

Nematophagous fungi are usually classified under three groups, the Zoopagaceae, the nematophagous hyphomycetes, and other forms.

The Zoopagaceae or, according to Bessey (1950), the Zoopagales are set apart from the rest of the Zygomycetes by their exclusively predacious habit and the possession of true conidia which are not violently discharged. Most of them attack amoebae but a few capture nematodes. Duddington (1955) discussed their taxonomy in great detail.

The nematophagous hyphomycetes may be classified as endoparasites and ectoparasites. Duddington (1955) described the endoparasitic species as a rather mixed group having the common character of the main vegetative mycelium being within the body of the host, only the conidiophores being external. The majority of the ectoparasitic nematophagous hyphomycetes produce some type of trapping device. Olthof (1962) catalogued all the known ectoparasites according to the type of trapping organs they produce. Subramanian (1963), and Cooke and Dickinson (1965), discussed the taxonomic status of two genera of ectoparasitic nematophagous hyphomycetes, Dactylella Grove and Monacrosporium Oudemans, and transferred several species of Dactylella and Dactylaria to Monacrosporium.

Other forms of nematophagous fungi, not included in the Zoopagaceae and the nematophagous hyphomycetes, are

dealt with in the book by Dollfus (1945) and by Duddington (1955). The group contains some members of doubtful systematic position and some endozoic parasites which belong to the orders Lagenidales, Blastocladiales, Entomophthorales and Chytridiales.

Temporary variations in the morphology of nematophagous fungi have been observed as a result of environmental changes to which the fungi were subjected (Peach, 1954; Dixon, 1954; Juniper, 1956; Soprunov, 1958; Tarjan, 1961). Variations of a more permanent nature have also been reported: Duddington (1955) found that a number of isolates of Arthrobotrys robusta and Trichothecium cystosporium in his culture collection showed a strong tendency to produce traps spontaneously in the absence of nematodes. In 1962, he reported that Trichothecium flagrans lost the ability to produce traps, even in the presence of nematodes, within three months of its being isolated in pure culture.

IV. ECOLOGY AND PHYSIOLOGY OF NEMATOPHAGOUS FUNGI

Nematode-trapping fungi have been found with great regularity in decaying vegetable matter of various kinds, in soil, in dung, in moss cushions, in vinegar (Duddington, 1962a), and in fresh water (Peach 1952, 1954). Little, however, is known of their ecology. Systematic surveys, to determine the relative frequencies of the different species, have been conducted by Duddington in England (1951b), Shepherd in Denmark (1956), Soprunov in the U.S.S.R., (1958), Mankau and Clark in California (1959), and Estey and Olthof in Quebec (1965). One consistent result emerging from these surveys is that Arthrobotrys oligospora is the commonest of the nematode-trapping fungi.

Little work on the activity of predacious fungi in soil under natural conditions has been done. This is an important field of investigation in view of their possible utilization in the biological control of nematodes. Mankau (1962a) suggested that nematophagous fungi are poor competitors within the soil because the germlings that arise from their conidia are entirely dependent on captured nematodes for their development and subsequent survival. Drechsler (1937) and Linford and Yap (1939) had previously pointed out that nematode-trapping hyphomycetes do not compete well with other fungi for the use of organic substrates in the absence of nematodes. Cooke (1964), using his agar disc technique

Cooke, 1961), observed little fungistatic effect on spore germination and this only on the ring-forming fungi.

Cooke's (1963b) data indicate that nematode-trapping fungi vary considerably in their ability to capture nematodes and he suggests this factor as a possible explanation of the variable results obtained when these fungi have been used for nematode control. Cooke (1964), after observing the germination of conidia and the trap-forming ability of 16 nematode-trapping fungi in soil, classified all trap-forming nematophagous fungi into two broad groups: a predaciously efficient group comprising the branch, knob and ring formers with a well developed ability to form traps on the conidium or germling, especially in the presence of decomposing organic matter, and a predaciously inefficient group consisting of the network formers.

The majority of the fungi that produce traps do not do so when grown in pure culture and this is particularly true of those that produce 3-dimensional networks (Duddington, 1955). Arthrobotrys dactyloides and Dactylella doedycoides produce constricting rings and D. ellipsospora and D. cionopaga produce stalked and unstalked adhesive knobs respectively when grown in pure culture (Olthof and Estey, 1965; Drechsler, 1950). However, with the addition of nematodes, the species that produce traps spontaneously, now do so in greater profusion (Duddington, 1962a).

Some workers believe that a tactile stimulus is

involved in the formation of traps (Drechsler, 1941; Lawton, 1957). It was thought by Couch (1937) that a chemical stimulus was responsible for the initiation of traps. Evidence substantiating this was obtained by Commandon and deFonbrune (1938) and by Muller (1958) who demonstrated that water in which nematodes had been suspended induced trap formation. Pramer and Stoll (1959) extracted an active principle from a nematode-free culture filtrate in which the nematodes Neoaplectana glasseri had been reared; they called it "Nemin". Feder et al. (1960) used single air dried and then rehydrated female nematodes to stimulate ring formation in Dactylella doedycoides.

Much work has been done on the nature of the substance or substances that initiate trap formation. A number of vegetable extracts did not cause traps to be formed (Deschiens and Lamy, 1942), nor did plant hormones (Dixon, 1954). Various substances of animal origin gave positive results (Roubaud and Deschiens, 1939). Of these, human serum was particularly effective and Lamy (1943) demonstrated the existence of an optimal concentration for best results with horse serum, the undiluted serum being unable to induce trap formation. Pramer and Stoll (1959) obtained the same optimal concentration effect with their Nemin. Olthof (1962) found that several cutinaceous materials like hair, human nails and quills of hen's feathers could induce trap formation.

The active principle in water in which nematodes

were suspended was destroyed by boiling (Commandon and deFonbrune, 1938) whereas that in the nematode-free culture filtrate in which nematodes had been reared was not destroyed after 10 minutes at 100°C (Pramer and Stoll, 1959) and that in guinea pig serum was thermostable and not affected by alcohol (Lamy, 1943). The nature of the substance or substances causing trap formation has not been determined.

The spectacular closure of constricting rings has attracted the attention of several workers. Couch (1937) suspected that, because of the vigorous movement of nematodes, either a mechanical stimulus or heat was involved. He obtained closure by heat but on inserting a fine glass needle into a ring and rubbing it against the sides, thereby simulating the movement of a nematode, no closure resulted; however, on repetition of the same experiment, Commandon and deFonbrune (1939) found the stimulated cell to treble in volume in less than one-tenth of a second and a fraction of a second later the other two cells of the ring followed suit. Their cinematographic records stand testimony to this fact. Further corroboration was supplied by Muller (1958) who also observed that closure of rings could be induced by pressure and electric currents. Couch (1937) also tried weak acids and potassium hydroxide. Slight closure resulted from the application of lactic acid but in no instance was closure complete. Muller (1958) was able to slow down the process of closure by employing sucrose solutions.

While much of the nature of the stimulus involved in closure of a constricting ring has been elucidated, nothing is known of the mechanism by which swelling of the ring cells is achieved. Duddington (1955) suggested that molecular rearrangement of the cell colloids resulting in imbibition plus osmotic forces were involved; turgor pressure alone could not account for the speed of the reaction.

Besides the constricting rings, the sticky mechanisms of the nematode-trapping hyphomycetes were also studied by Commandon and deFonbrune (1939). Only nematodes, of all objects tested, stuck to knobbed outgrowths and 3-dimensional networks under normal moist conditions; but on detaching a trap from its mycelial hypha (dry conditions), a glass needle adhered to it.

Several factors are known to affect the formation of traps and the efficiency of their operation. Feder et al. (1960), from their work on Dactylella doedycoides, inferred that a fungus must be heterocaryotic to be able to produce its traps. Physiological age of a fungus culture affects its trapping efficiency, young networks being more sticky than older ones (Commandon and deFonbrune, 1938), and young constricting rings closing more rapidly than older ones (Couch, 1937). Other factors influencing trap formation are temperature (Feder, 1963) and type of medium a fungus is grown on (Tarjan, 1961).

According to Shepherd (1955), the captured nematode

is pierced by an infection peg of the fungus and the latter swells to form an infection bulb which may completely occlude the body cavity of the nematode. Trophic hyphae grow out from the infection bulb and ramify to extend throughout the body of the host. The contents of the nematode are absorbed by the trophic hyphae and eventually only the integument, filled with the empty trophic hyphae remains.

Although the physical course of events following capture is well known, the precise cause of death is still in doubt. Duddington (1955) thought it possible, though unlikely, that the nematode dies from physical exhaustion. Drechsler (1937) suggested that the nematode is killed mechanically by the infection bulb. Shepherd (1955) discounted this explanation as she observed trapped nematodes to become inactive before the infection bulb had become fully developed. Soprunov and Galiulina (1951) attributed the killing to a toxin produced by the fungus and Duddington (1955) appeared to favour this explanation. The work of Olthof and Estey (1963) adds corroboration to the toxin theory of killing.

The work of Drechsler (1937) and Duddington (1955) indicates that the predacious hyphomycetes show little host specificity, being able to trap almost all species of nematodes.

V. NEMATOPHAGOUS FUNGI AND NEMATODE CONTROL

The majority of the attempts at biological control of plant parasitic nematodes by predacious fungi have utilized the nematophagous hyphomycetes; this is because they possess trapping organs, cause spectacular havoc with nematodes in Petri dish cultures and lend themselves to artificial culture on a scale required for biological control (Duddington, 1955).

Linford and his colleagues, in Hawaii, conducted a series of experiments (1937, 1938, 1939) aimed at the control of root-knot nematodes that were causing considerable damage to pineapple plants. Of five nematode-trapping hyphomycetes added in pure culture to root-knot infested soil in which pineapple plants were growing, only Dactylella ellipsospora, a sticky-knob former, afforded a certain degree of protection; the others, Arthrobotrys oligospora, A. musiformis, Dactylaria candida and D. thaumasia were ineffective (Linford and Yap, 1939).

Linford et al. (1938) had earlier shown that nematophagous fungi already present in the soil were stimulated to increased activity following the addition of organic matter. By adding chopped pineapple tops to root-knot infested soil, they obtained a degree of protection that was statistically significant at the 0.1 per cent level. This treatment produced a rapid rise in the population of free-living nematodes and it was followed by an equally

rapid fall. They postulated that the addition of organic matter stimulated an increase in the population of saprophagous nematodes which, in turn, enhanced the trapping-activity of the nematode-trapping fungi which did not discriminate between saprophagous and root-knot nematodes, reducing the numbers of both. Cooke's (1962b) results refute Linford's hypothesis that the increase in saprophagous nematodes acted as a stimulus to the nematode-trapping fungi. However, fungal stimulation by the addition of organic matter has been confirmed by several workers (Cooke, 1962b; Duddington and Duthoit, 1960; Duddington et al. 1961; Mankau 1962b; Paasuke, 1962).

Deschiens et al. (1943) used Dactylella bembicoides and Arthrobotrys oligospora to protect Begonia cuttings from root-knot nematodes. The authors stated that their results showed that the fungi conferred an important level of protection, but results for individual plants were not given and Duddington (1957a) indicated that their results were not statistically significant.

Duddington and his co-workers attempted the control of the sugar beet nematode, Heterodera schachtii (1956), the potato root nematode, H. rostochiensis (1956), and the cereal root nematode, H. avenae (1961), by the addition of organic matter. The results in the first two cases were disappointing but a statistically significant reduction in numbers of nematode larvae in roots of treated oat seedlings as compared

with the controls was obtained. Duthoit and Godfrey (1963) observed a significant reduction, in H. avenae larval invasion of oats, by Arthrobotrys oligospora and A. robusta.

Hams and Wilkin (1961) carried out an extensive series of pot and field experiments on nematode control by predacious fungi. Assessing their results by crop yields, they observed that Arthrobotrys robusta alone gave a significant increase in yield of peas and potatoes grown in soil infested with Heterodera gottingiana and H. rostochiensis, respectively. Further trials carried out on the cereal root nematode in oats and the potato root nematode in potatoes using Dactylaria candida, Trichothecium cystosporium, Phialospora heteroderae and Cylindrocarpon radicicola gave entirely negative results. The last two fungi attack cysts only and not larvae.

Mankau (1961b) obtained a distinct reduction, in the severity of root-knot of tomato plants, by Arthrobotrys arthrobotryoides. Dactylella ellipsospora and A. conoides extended survival of tomato plants grown in root-knot infested soil; however, none of the fungi tested were able to prevent serious damage to tomato and okra (Hibiscus esculentis) plants. In some instances, the fungi added to the soil could not be recovered (Mankau, 1961b).

Attempts by Mankau (1961a) to establish nematode-trapping fungi in soil gave variable results and were often unsuccessful. He suggested that they were in competition

with antagonistic elements of the soil and concluded that our present knowledge was insufficient to enable use of them to be made in the control of plant parasitic nematodes. Van der Laan (1956, 1964) came to a similar conclusion from his studies on the biological control of Heterodera rostochiensis.

In recent years, Russian workers have achieved remarkable successes in controlling root-knot nematodes. Gorlenko (1956) reported control of root-knot of cucumber by Trichothecium pravicovi and Arthrobotrys kirghizica. Increasing the dose of the fungus increased the effectiveness of control and the addition of several small doses was more effective than a single large dose. Soprunov and Tendetnik (1960) concluded, after five years of testing, that Arthrobotrys oligospora and A. dolioformis were the most promising fungi for nematode control. Soprunov (1958) reviewed work done in the U.S.S.R.

French workers attempted the control of nematodes pathogenic to animals. Roubaud and Deschiens (1941) showed that sheep, fed on pasture artificially infested with Strongyloides papillosum and a Bunostomum species and then treated with spores of predacious fungi, remained healthy while those fed on untreated infested pasture were infected with the nematodes. Only two sheep were used in each treatment and this result was considered inconclusive by Duddington (1957a). These workers did, however, establish the fact that nematode-trapping fungi are without parasitic

action on plants and are non-toxic to animals.

The Russian workers Ei and Alakhverdyants (1962) reported the production of dried preparations of nematode-trapping fungi in readiness for the control of strongyles in horses and sheep. Ei et al. (1961) previously showed that dried fungal preparations were still viable after passage through the intestines of test animals.

SECTION I. STUDIES ON THE VARIABILITY OF NEMATOPHAGOUS FUNGI

I. MORPHOLOGY OF SELECTED NEMATOPHAGOUS FUNGI

A. INTRODUCTION

Variation in morphology and trapping efficiency of the nematode-trapping hyphomycetes appears to be the rule rather than the exception. Not only does variation exist between different isolates of the same species but also between cultures of the same isolate (Duddington, 1955; Drechsler, 1937). Duddington (1962a) reported the loss of ability to produce networks, even in the presence of nematodes, by Trichothecium flagrans within three months of being isolated in pure culture, although this fungus had been named because of the high efficiency of its networks and its effectiveness in capturing nematodes in enormous numbers. He further reported that he was later given an isolate of the same species that formed networks spontaneously and copiously in pure culture. He stated, "I am unable to give any reason for this, any more than I can account for the fact that certain strains of reticulate hyphomycetes will sometimes form networks spontaneously in pure culture for no apparent reason." He had earlier (1955) reported that a number of isolates of Arthrobotrys robusta and Trichothecium cystosporium in his culture collection showed a strong tendency

to produce traps spontaneously.

According to Duddington (1962a), the adhesive knobs of Dactylella ellipsospora show proliferation, and different strains of this species appear to vary in this tendency to proliferate.

Drechsler (1937), in his description of Arthrobotrys dactyloides, stated that in most cultures the conidia were uniformly uniseptate, slightly curved and elongate ellipsoidal in shape. Occasionally, however, he observed material with an admixture of conidia, shortened and widened in varying degree and containing two septa. This variation in conidial morphology appeared so much in excess of the variation usual in members of the predacious species that he at first thought it might be a species of a different genus.

Because of the variation and because environmental conditions affect the morphology of organisms, it was decided to describe the morphology of the nematode-trapping species dealt with in this thesis. Further, the descriptions will serve as a basis of comparison with new strains derived from these species.

Five of eleven species of nematode-trapping hyphomycetes, isolated from Southern Quebec agricultural soils by Olthof in 1959 (Olthof 1962), were used in these studies. They were:

Arthrobotrys oligospora Fresenius (1852)

" superba Corda (1839)

Arthrobotrys robusta Duddington (1951a)

" dactyloides Drechsler (1937)

Dactylella ellipsospora (Preuss) Grove (1886)

B. MATERIALS AND METHODS

Corn meal agar was prepared according to the formula of Duddington (1955). Twenty grams of Corn meal were added to 1.2 litres of tap water and heated at 70°C for 1 hour. The mixture was filtered through a Buchner funnel, the volume made up to 1 litre, 1.5 per cent agar powder added and the medium steamed to dissolve the agar. The medium was then autoclaved at 121°C for 15 minutes. Twenty millilitres of this medium were poured into each of several Petri plates and for each species of nematode-trapping fungus to be studied four plates were inoculated with a small block of a culture of the fungus. The cultures were incubated at room temperature, the plates being wrapped in polythene bags to prevent drying. After 24 hours, a drop of a suspension of Rhabditis oxycerca nematodes, which had been washed ten times with sterile water, was added to two of the four plates of each fungus.

Camera lucida drawings and measurements were made of the living material directly from the agar plates. Photomicrographs were taken of conidia stained with 0.1 per cent lactophenol cotton blue.

C. DESCRIPTION OF SPECIES

The characters described are those found in 90mm Petri plate cultures infested with nematodes. In pure culture, traps are usually absent, conidial dimensions are often smaller and the morphology of the conidiophore may also be different.

1. ARTHROBOTRYS OLIGOSPORA Fresenius (1852)

The vegetative mycelium showed a radial arrangement and within 24 hours of adding nematodes the predacious apparatus, which consisted of 3-dimensional adhesive hyphal networks, was well developed. The networks started as discrete loops which usually gave rise to other loops and anastomosed with neighbouring ones to become compounded. Nematode-trapping activity reached a peak in 48 hours and then began to decline. After about 2 weeks trapping activity came completely to a halt.

Sporulation began shortly after the onset of nematophagous activity and the bicellular, obovoid, hyaline conidia were produced in whorls along conidiophores which were usually unbranched and attained a height of 250 to 500u. The conidiophores were broader at the base than at the tip and the conidia were 20 - 28u in length and 10 - 14u in width. The conidial dimensions were slightly smaller than

those given by Drechsler (1937) for this species.

In old cultures, whether pure or infested with nematodes, brown chlamydospores occured regularly. They varied in shape from cylindrical to spherical and their walls usually appeared to be two layered.

2. ARTHROBOTRYS SUPERBA Corda (1839)

The main difference between this fungus and Arthrobotrys oligospora was that the conidial septum was in the middle, partitioning off two approximately equal cells. The cylindrical conidia were also smaller and narrower than those of A. oligospora, being 14 - 19u in length and 6 - 10u in width. In pure culture, the conidiophore was not straight as in A. oligospora and A. robusta but was irregularly geniculate bearing a whorl of conidia at each nodose bend. Chlamydospore formation was not observed to occur.

The description of this species agrees closely with that of Drechsler (1937).

3. ARTHROBOTRYS ROBUSTA Duddington (1951a)

The vegetative phase of this fungus was identical with that of A. oligospora and A. superba and the trapping mechanism was also very similar. The conidia were again bicellular, with the septum towards the middle, but they

were produced in terminal heads and not in whorls along the conidiophore. The conidiophore was usually branched and about 200 - 400u tall. The conidia appeared slightly plumper than those of A. oligospora and were bluntly rounded at the proximal end while those of A. oligospora were acutely pointed. The conidia were 18 - 27u in length and 8 - 12u in width. Chlamydospores and enlarged storage hyphae were not found.

This description agrees well with that given by Duddington (1951a) for the species.

4. ARTHROBOTRYS DACTYLOIDES Drechsler (1937)

The predacious apparatus of A. dactyloides was a constricting ring composed of 3 arcuate cells borne on a 2-celled stalk. The constricting rings were produced spontaneously in pure culture, but very sparsely. On adding Rhabditis nematodes, however, they were produced in great profusion but took longer to make their appearance than did the hyphal networks of the retiary species. The conidia were uniformly uniseptate, elongated and slightly curved, 32 - 50u in length and 7 - 10u in width and produced in loose clusters on erect conidiophores 200 - 400u tall.

Swollen 3-celled conidia, such as those observed by Drechsler (1937) were not seen, nor were they reported by Olthof (1962) or Thomas (1963) who had worked with the same isolate for three years and two years respectively.

Chlamydospores were not observed.

5. DACTYLELLA ELLIPSOSPORA (Preuss) Grove (1886).

This fungus produced stalked adhesive knobs in pure culture, but produced them more profusely when nematodes were present. Each erect conidiophore, 150 - 250u in length, bore a single conidium but occasionally the conidiophore elongated pushing the conidium to one side and a new conidium arose at the tip. As many as four conidia arose from one conidiophore in this manner. The conidia were broadly spindle shaped and typically divided by four septa into five cells of which the middle one was the largest. Occasionally, one and two septate conidia were observed but these were thought by Drechsler (1937) to be immature ones. The conidia were 28 - 65u in length and 8 - 18u in width, corresponding closely to the measurements given by Drechsler (1937) for this species.

D. DISCUSSION

The descriptions of these fungi are almost identical with those of Olthof (1962) who originally isolated them. There is no evidence of any morphological change in spite of repeated subculturings by two workers who handled them for a total of 6 years. Attention is drawn to the fact that none of the retiary species described produced traps spontaneously in pure culture.

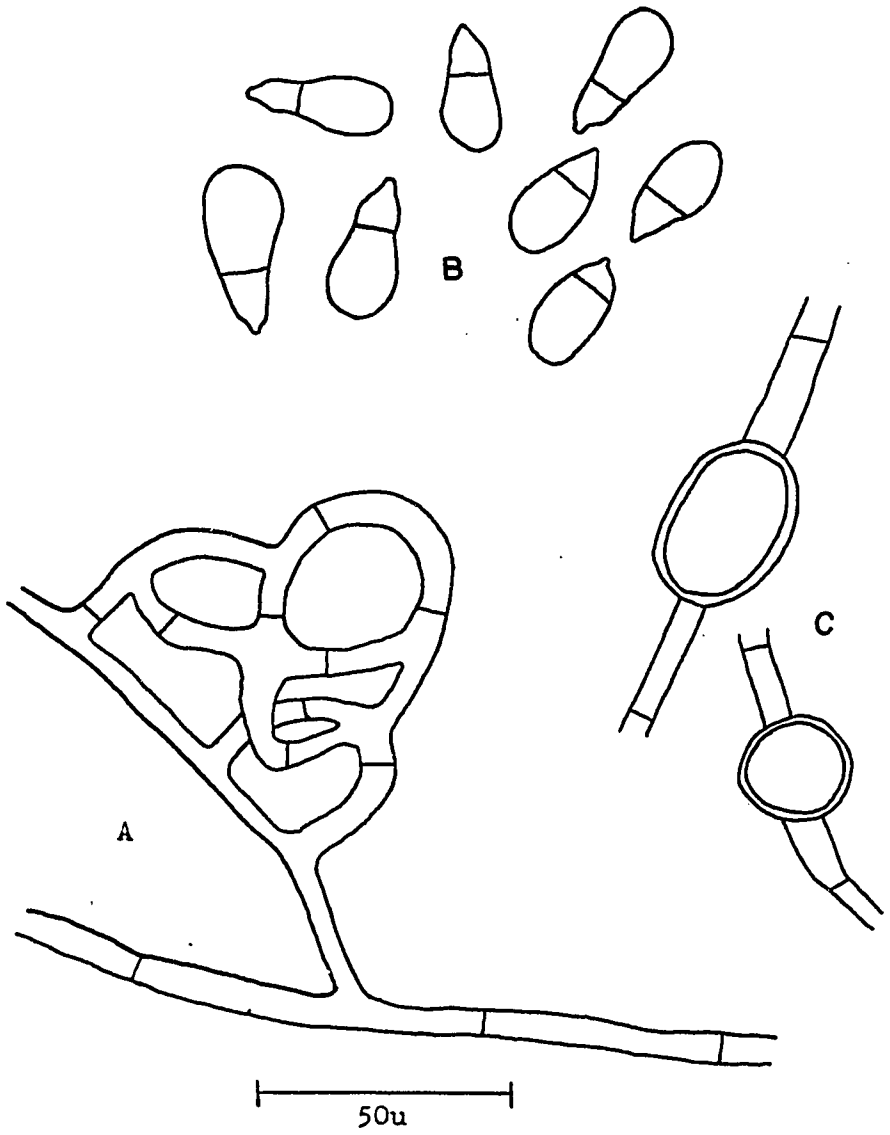


Figure 1. Arthrobotrys oligospora Fresenius
A. Hyphal network.
B. Conidia.
C. Chlamydospores.

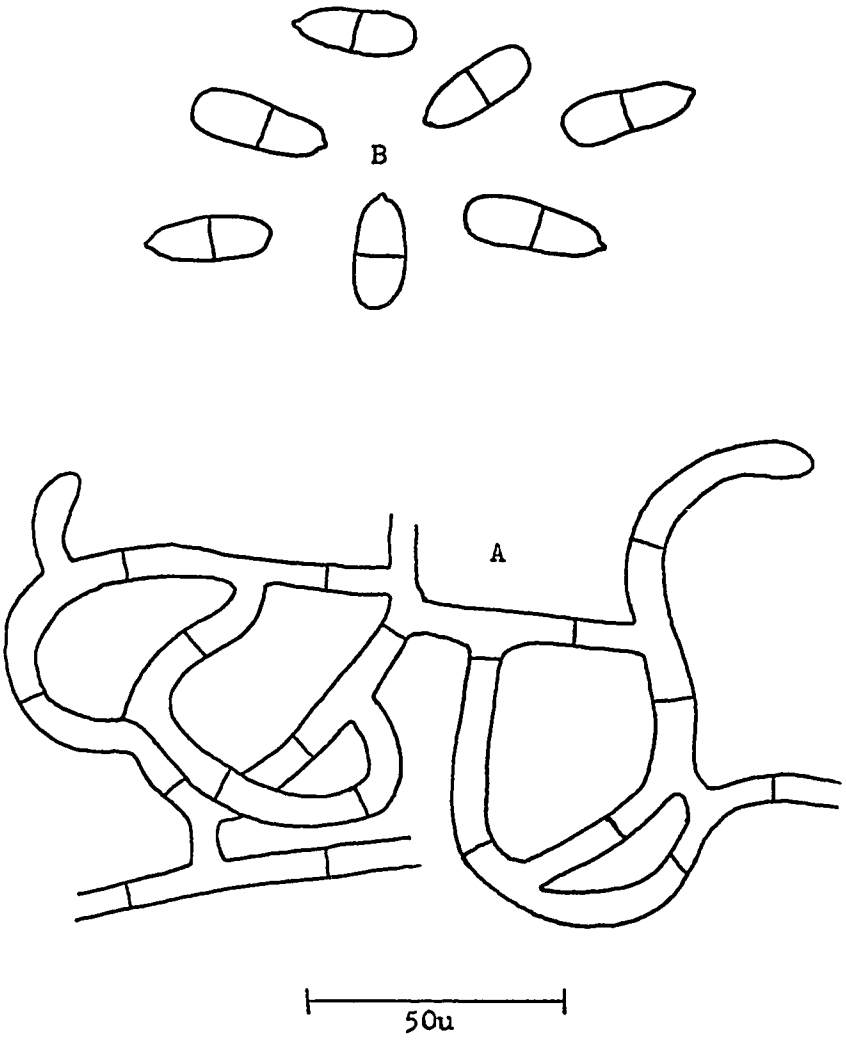


Figure 2. Arthrobotrys superba Corda
A. Hyphal network.
B. Conidia.

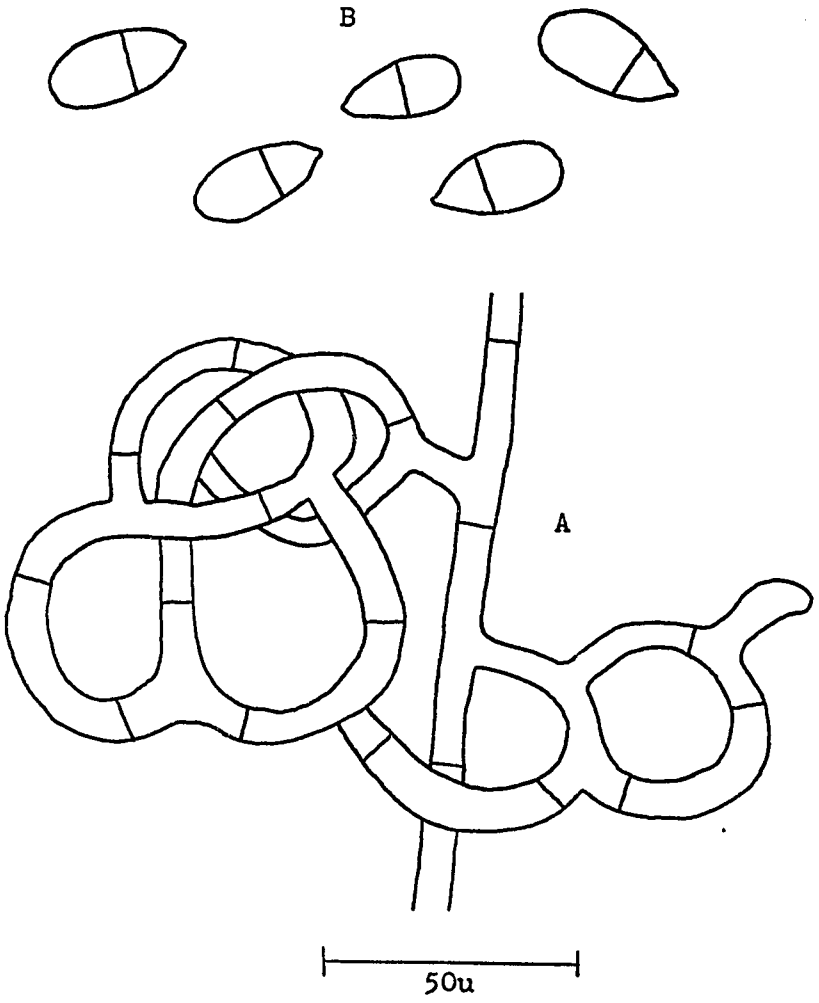
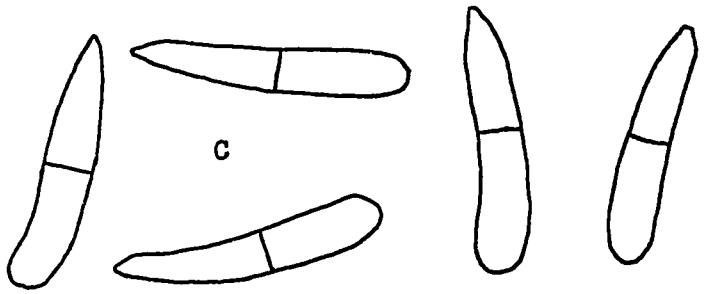
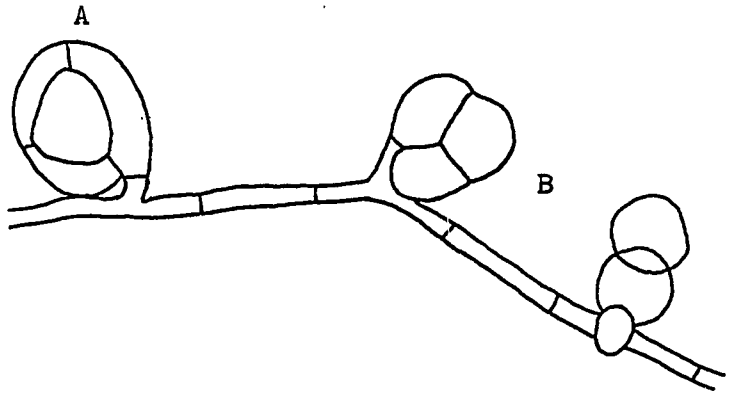


Figure 3. Arthrobotrys robusta Duddington

A. Hyphal network.
B. Conidia.





50u

Figure 4. Arthrobotrys dactyloides Drechsler

A. Constricting ring - uninflated.
B. Constricting rings - inflated.
C. Conidia.

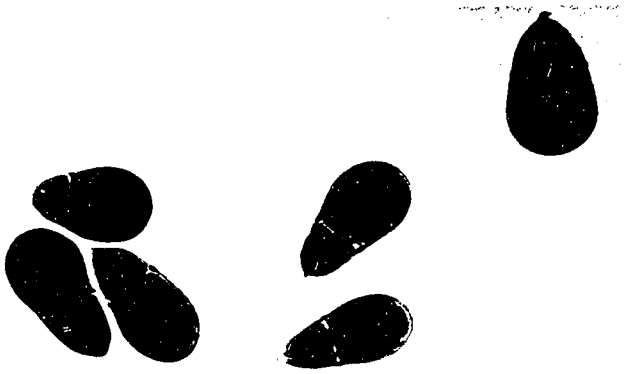


Figure 5. Photomicrograph of Arthrobotrys oligospora conidia

Figure 6. Photomicrograph of Arthrobotrys superba conidia

Figure 7. Photomicrograph of Arthrobotrys robusta conidia

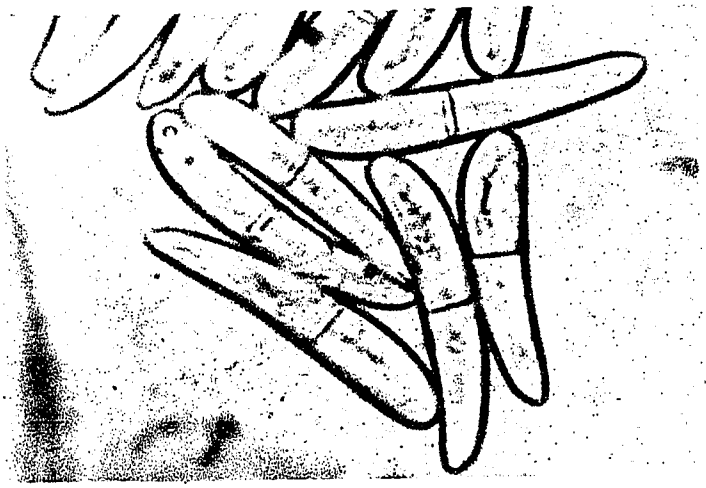


Figure 8. Photomicrograph of Arthrobotrys dactyloides conidia

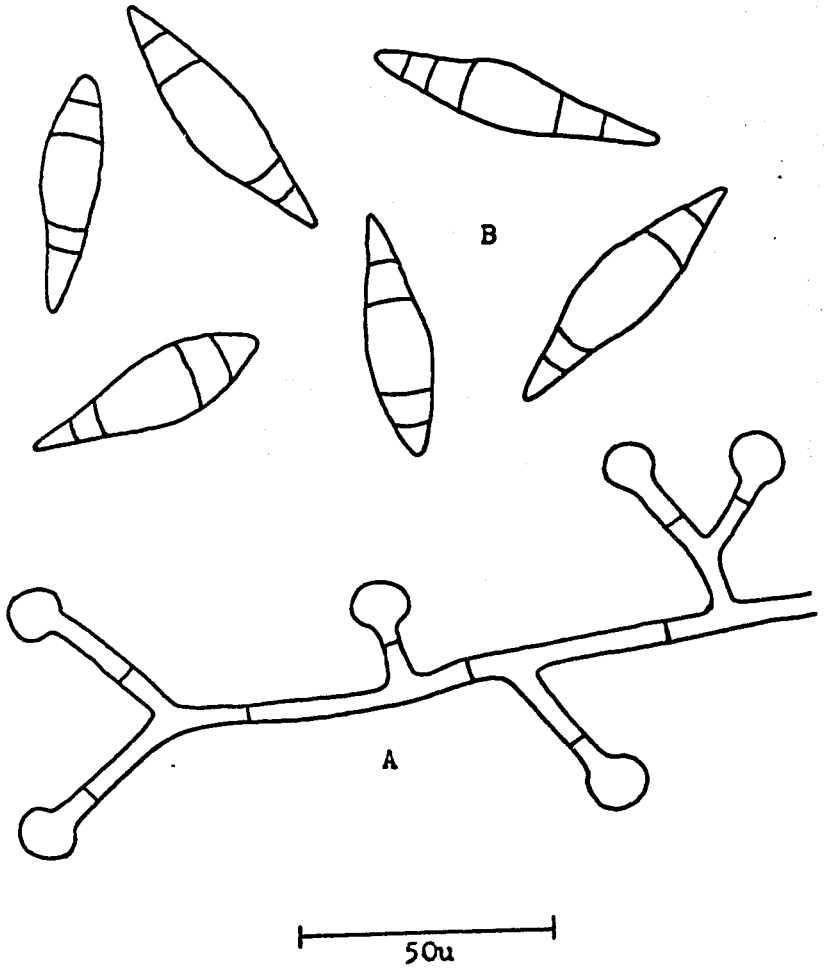


Figure 9. Dactylella ellipsospora (Preuss) Grove
A. Hypha showing knobbed outgrowths.
B. Conidia.

II. NUCLEAR CONDITION OF NEMATOPHAGOUS FUNGI

A. INTRODUCTION

An appreciation of the importance of variability for the perpetuation of a fungus has resulted largely from the failure of plant hosts bred for resistance to a given pathogenic fungus to remain resistant when grown on a large scale. Mutations and heterocaryotic phenomena are necessary if the fungi are to increase their variability. Evidence strongly suggests that isolates of fungi from nature are often heterocaryotic or are aggregates of different clones of any given species (Buxton, 1960).

The cells of most hyphomycetes are multinucleate (Duddington, 1962a) and it was shown by Hansen (1938) that, in a number of imperfect fungi, strains of species could be obtained by making subcultures of a large number of single-spore isolations. The simplest way of accounting for the known variability in the nematode-trapping fungi is by assuming the existence of heterocaryosis. This assumption is strengthened by the work of Feder et al. (1960) on constricting ring formation in Dactylella doedycoides which suggests that this fungus is heterocaryotic with regard to the capacity to form rings.

Because knowledge of heterocaryosis is an important consideration in the understanding and the interpretation of

results of studies on the biology and the use of nematode-trapping fungi in the control of plant parasitic nematodes, an investigation of the nuclear condition in these fungi was undertaken.

It is recognized that a fungus does not need to have multinucleated cells to be heterocaryotic. Conversely, a fungus with multinucleated cells need not be heterocaryotic as all the nuclei may be of the same type. Bearing this in mind, the nuclear condition of the mycelium, conidia and conidiophore was studied.

B. MATERIALS AND METHODS

1. FUNGI

The observations reported in this study were made on the nuclei in the conidia and vegetative mycelia of Arthrobotrys oligospora, A. superba, A. robusta and A. dactyloides. These fungi were grown on weak Corn meal agar (Duddington, 1955) and on Water agar for cytological observations. The original cultures of each species were maintained on Difco Bacto Lima bean agar at 4 - 6°C.

2. CYTOLOGICAL PREPARATIONS

a) Ungerminated conidia were affixed to microscope slides

after applying Haupt's (1930) adhesive.

b) Thin slide-cultures were prepared by placing a drop of the medium on a microscope slide and spreading it into a thin layer with a glass rod. The inoculated slides were incubated, at room temperature, in Petri plates which had moistened filter papers in the bottoms and the lids.

c) Pieces of cellophane small enough to go with ease into a Petri plate were placed in boiling water for about 30 minutes, peeled of their impervious coverings, placed between well moistened filter papers in a Petri plate and autoclaved. The cellophane pieces were removed as needed, soaked in sterile water and placed on the surface of the medium in Petri plates. They were inoculated with conidia and incubated at room temperature. Some of the resultant cellophane cultures were examined while still on the surface of the medium by phase contrast microscopy to determine the nuclear condition of the living mycelium, while others were stained. Still others were left till sporulation had taken place and then stained to determine the nuclear condition of the conidia, as the vast majority of conidia affixed to microscope slides were washed off during the staining process.

3. MICROSCOPY

For the study of stained preparations, a Zeiss automatic photomicroscope was used. It was fitted with plan-

achromat objectives 40/0.65 and 100/1.25 in conjunction with a Zeiss achromatic aplanatic condenser (N.A. 1.4Z).

The nuclear condition in living cells was studied with a Reichert "Neozet" binocular research microscope fitted with a 1mm universal "Polyphos" condenser and matched phase objectives x 40 (N.A. 0.65) and x 100 (N.A. 1.30). Photomicrographs were taken with Kodak High Contrast Copy film.

4. FIXATION

Throughout this work, either acetic acid alcohol (1:3) was used or the staining procedure was carried out without previous fixation.

5. STAINING

a) Azure A-SO_2. (Huebschman, 1952). The cytological preparations were hydrolyzed in 1N HCl at 60°C for 6 minutes to remove basophilly of the cytoplasm. They were then stained in a mixture of 50ml of 2 per cent aqueous Azure A (Flax and Pollister, 1949), 3ml of 10 per cent $NaHSO_4$ and 3ml of 1N. HCl for 30 minutes. The stained preparations were washed in two changes of distilled water for 5 minutes, a drop of 10 per cent alcohol added, dried and mounted in Euparal.

b) HCl-Giemsa. (Robinow, 1957). Hydrolyzed preparations

were stained overnight in a solution prepared by adding 2 drops of Gurr's Improved Giemsa stain (R66) per ml of buffer solution at pH 6.8. Stained preparations were mounted in buffer, covered with a No. 1 cover glass and sealed.

c) Other stains. Acetic orcein (La Cour, 1941), Lacto-proprionic orcein (Dyer, 1963), Aceto-carmine (Geitler, 1949), and Basic fuchsin (de Lamater, 1948) were also used.

C. RESULTS AND DISCUSSION

1. LIVING PREPARATIONS

Phase contrast microscopy of slide and cellophane cultures did not reveal the nuclei in the living spores or the vegetative mycelium because of their high natural refractility. Mineral oil was spread on the preparations to depress the refractility to no avail. However, vegetative hyphae that had grown off the cellophane into the surrounding medium showed 2 - 7 nuclei per cell with the average around 3.5. The range in numbers of nuclei per cell and the average number of nuclei per cell were the same in all the 4 species studied.

2. STAINED PREPARATIONS

The stained cellophane strips yielded the best

preparations. Of the staining methods used, the Azure A-SO_2 method gave transparent preparations of spores and vegetative mycelium with well stained nuclei. The HCl-Giemsa method stained nuclei faintly, while acetic orcein, lacto-proprionic orcein and aceto carmine did not stain the nuclei.

Stained preparations of vegetative mycelium showed the same range in numbers of nuclei as did phase contrast microscopy of living preparations. The usual number of nuclei per cell was 3 or 4 while as many as 7 per cell was not uncommon (Figs. 10, 12, 14, 16). Occasionally, a short lateral branch had one nucleus per cell but it soon anastomosed with a hypha that had more than one nucleus per cell.

Conidia of all the four species showed between 3 and 15 nuclei per cell with an average of 10 to 12 (Figs. 11, 13, 15, 17). Photographs of conidia showed only about half the number of nuclei actually present. This was because the nuclei lay at different depths in the conidia.

The nuclear count was slightly higher when the fungi were grown on Corn meal agar. This finding appears to agree with the finding of Huebschman (1952) that the number of nuclei in the conidia of Neurospora crassa could be increased by growing the fungus on a nutritionally rich medium.

It is desirable to ascertain the number of nuclei that go from the terminal cell of a conidiophore into the formation of a conidium. The use of phase contrast microscopy

Figure 10. Multinucleate hyphal cells of Arthrobotrys oligospora

Figure 11. Multinucleate cells in a conidium of Arthrobotrys oligospora

Figure 12. Multinucleate hyphal cells of Arthrobotrys superba

Figure 13. Multinucleate cells in conidia of Arthrobotrys superba

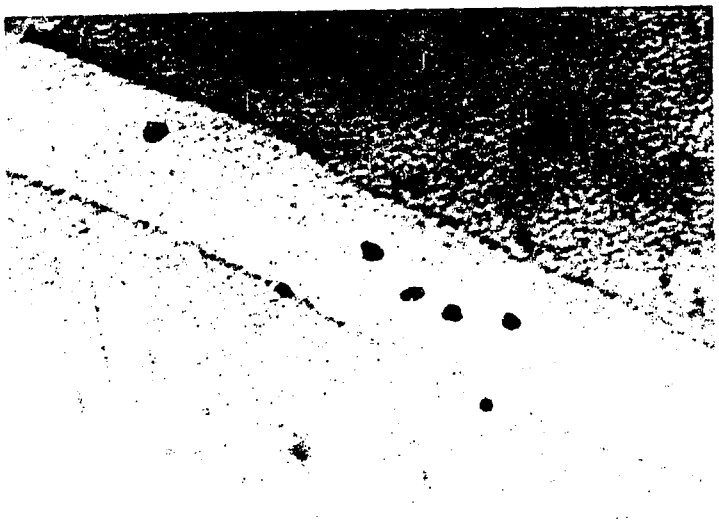


Figure 14. A multinucleate hyphal cell of Arthrobotrys robusta

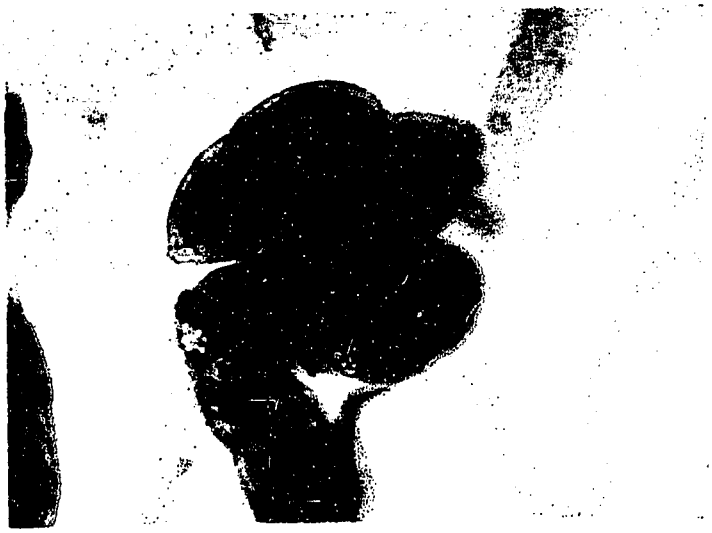


Figure 15. Multinucleate cells in conidia of Arthrobotrys robusta

Figure 16. Multinucleate hyphal cells of Arthrobotrys dactyloides

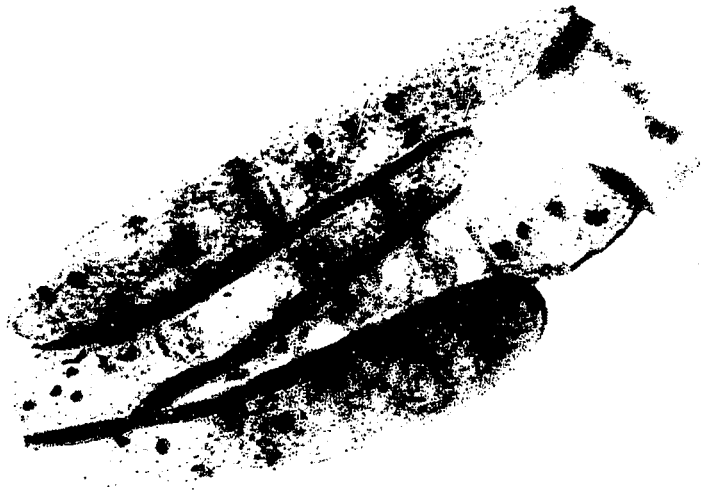


Figure 17. Multinucleate cells in conidia of Arthrobotrys dactyloides

might have been of help in this connection if the refractility of the specimens could have been eliminated. Work on chromosome numbers might be of advantage in determining relationships of the different species.

III. THE EFFECT OF ENVIRONMENT ON VARIABILITY OF NEMATOPHAGOUS FUNGI

A. INTRODUCTION

Much work has been done on the effect of various environmental factors, such as light conditions, type of medium, temperature and pH of the medium on growth and cultural characteristics of various nematode-trapping fungi (Olthof, 1962). There are reports (Juniper, 1956; Tarjan, 1961) of variation in growth rate, amount of sporulation and size of conidia but these apparently were not permanent features. The aims of the workers in this field were either to distinguish between closely related species or to find the optimal conditions for their culture in bulk as a prelude to their employment as agents of biological control of plant parasitic nematodes. Olthof and Estey (1965) reviewed the work done and made a detailed study of the growth and cultural characteristics of ten nematophagous hyphomycetes isolated from Quebec agricultural soils.

The separation of related species on the basis of morphological character differences has been justified by the need for a practical system of classification but the drawback of this method is that phenotypic variation in a single strain in response to changes in environment could result in the variants being described as two or more

distinct species. The work of Hansen (1938) suggests that isolates of fungi from nature, especially the Fungi Imperfecti, are often heterocaryotic. If this is the case with the nematode-trapping hyphomycetes, then the use of various culture media, light, temperature and hydrogen ion concentrations may act as selective "baits" for their different components.

The purpose of this study was to determine if sectoring or other visual evidences of nuclear dissociation could be induced by varying the ecological conditions under which Arthrobotrys oligospora was grown. It was hoped that this would lead to the selection of new stable strains.

B. MATERIALS AND METHODS

Four Petri plates, each containing 15ml of the appropriate medium and inoculated with a single conidium of Arthrobotrys oligospora, were employed for each of the different temperatures, light conditions and hydrogen ion concentrations.

a) Media. Fourteen media were used to study the effects of culture media and light conditions on variability of the fungus. They were Potato dextrose agar, Corn meal agar, Malt extract agar, Chlamydospore agar, Lima bean agar, Prune agar, Rice extract agar, Littman oxgall agar, Czapek Dox agar, Wort agar, Mycophil agar, Mycologogical agar, Oat meal agar and

Water agar. The media, with the exception of Water agar, were prepared from Difco Bacto dehydrated media according to the directions of the manufacturer.

The media used in studies relative to temperature and hydrogen ion concentration were weak Corn meal agar and Czapek Dox agar amended with 0.1 per cent yeast extract.

b) Light conditions. Continuous light supplied by normal fluorescent lamps of a laboratory, twelve hours of dark followed by twelve hours of light of the same intensity as that used for continuous light, and continuous darkness were the conditions used.

c) Hydrogen ion concentrations. In the study of the effect of hydrogen ion concentration on variability of the fungus, pH values 4, 5, 6, 7 and 8 were used. Buffering of the medium to obtain the desired pH values was accomplished by the use of McIlvaine's buffer system (McLean and Cook, 1958) in the following manner:

Five hundred millilitres of double strength yeast-amended Czapek Dox broth were divided into 5 lots of 100ml and each lot received the following amounts of 0.1M citric acid solution and 0.2M disodium hydrogen phosphate solution:

pH	Citric acid	Na_2HPO_4
4	61.45ml	38.55ml
5	48.50	51.50
6	36.85	63.15
7	17.65	82.35
8	2.75	97.25

After the addition of the buffer, each medium received 15g per litre of agar with the exception of the medium at pH 4 to which 30g per litre were added. The media were steamed to dissolve the agar and autoclaved at 121°C for 15 minutes. After cooling to 40°C, the pH of each medium was determined by means of a Beckman electric pH meter and the pH adjusted with either concentrated HCl or NaOH.

d) Temperature levels. Six temperature levels were employed in studying the effect of temperature on variability of the fungus. These were 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. All other incubations were carried out at room temperature, which was about 23°C.

C. RESULTS AND DISCUSSION

The plate-cultures were examined every 24 hours but no sectoring or island formation was observed. These findings did not rule out the existence of heterocaryosis

in Arthrobotrys oligospora as each cell of its conidium was shown to have 3 - 15 nuclei and the chances of selecting one type of nucleus, if more than one exist, were infinitesimally small considering the number of replications employed in this study. The indications were that the fungus, if heterocaryotic, was stable and its nuclear ratio was not easily altered.

D. OTHER OBSERVATIONS

(i) Three cultures of Arthrobotrys superba on weak Corn meal agar, wrapped in polythene and left on the laboratory table for 2 weeks, showed budding of a large number of conidia while they were still attached to the conidiophores. Some of the resulting conidial chains were branched (Fig. 18). The conidial buds were subcultured but this variant was not seen again. Apparently, some environmental factor, which could not be detected, was responsible for this unusual phenomenon.

(ii) Four plates of weak Corn meal agar, buffered with McIlvaine's Buffer System (McLean and Cook, 1958) at pH 6 and inoculated with Arthrobotrys oligospora and nematodes of the species Rhabditis oxycerca were incubated for a week at 30°C. No traps were formed and on removing them from the incubator they were left on the laboratory table for two days. Prior to discarding the cultures, they were scanned with a stereoscopic microscope and every one of them showed a

prolific production of aseptate microconidia (Fig.19). After four days, they started to produce the normal bicellular conidia. Single microconidial cultures yielded the wild type but one culture showed the production of a new strain. It was distinguished from the wild type by the fact that in pure culture the mycelium lysed completely within three weeks of planting, leaving erect conidiophores with normal conidia,and a large number of chlamydospores which were produced earlier than is usual for A. oligospora. The character of early lysis of the mycelium has not been lost after five successive subculturings.

(iii) In 1962, Thomas (1963) placed under oil several cultures of the nematode-trapping hyphomycetes with which he was working. In 1965, Arthrobotrys oligospora was subcultured from four of these slants under oil and every one of them produced traps spontaneously in pure culture. The character of spontaneous trap production has remained stable and has not been previously reported for A. oligospora. In every one of the slant cultures the fungus had overgrown the medium and penetrated it extensively. Although being kept under oil lowers the metabolic activity of the fungus, it could be that starvation conditions arose giving rise to a selection of a certain type of nucleus or to an alteration of the nuclear ratio.

(iv) At times, Arthrobotrys oligospora on weak Corn meal agar produced conidia in close compact whorls, one

Figure 18. A chain of conidia produced from a single conidium of Arthrobotrys superba while still on the conidiophore above the substrate.

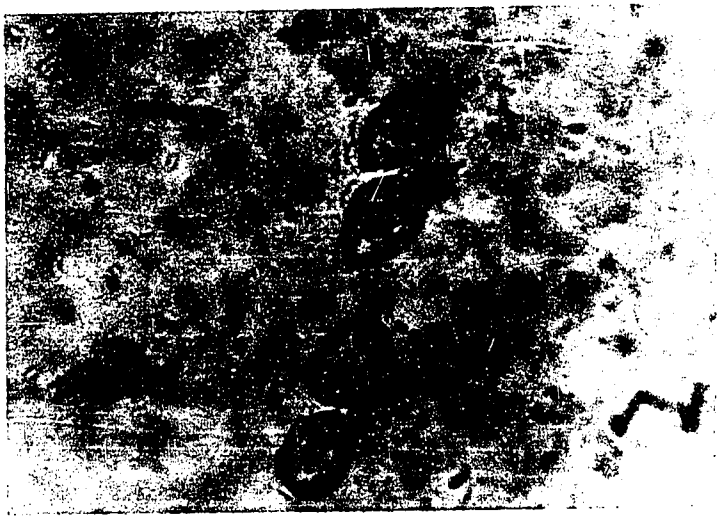


Figure 19. Aseptate microconidia of Arthrobotrys oligospora produced by the fungus at 30°C on buffered weak corn meal agar at pH 5, after the addition of nematodes.

immediately above the other, to resemble a feather (Fig.20). At other times, on the same medium, the whorls of conidia were well spaced and the conidiophores were branched to give the appearance of a loose aerial tangle of conidiophores and conidia (Fig.21).

(v) Arthrobotrys superba produced traps spontaneously in pure culture on Water agar. This finding is consistent with the starvation suggestion mentioned in a previous paragraph.

Figure 20. Arthrobotrys oligospora - compact whorls of conidiophores, the conidia absent.

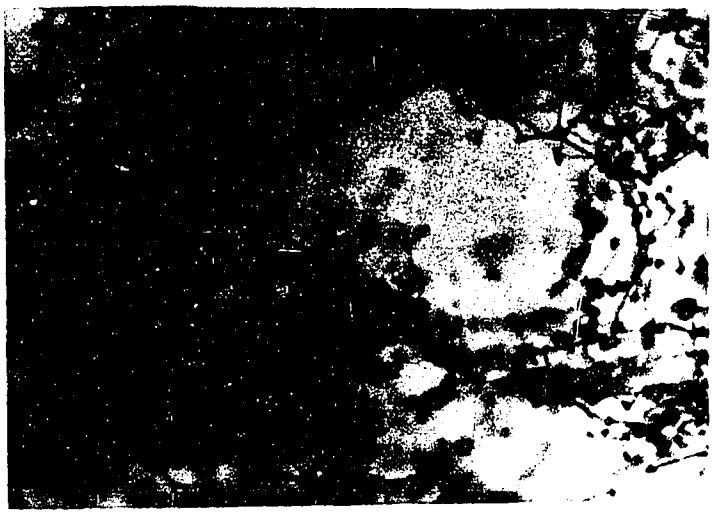


Figure 21. Arthrobotrys oligospora - [illegible] conidia [illegible].

IV. THE SELECTION OF NEW STRAINS

A. INTRODUCTION

The conidia of Arthrobotrys oligospora, A. superba, A. robusta and A. dactyloides have been shown to be multinucleate (page 38). Multinucleate spores cannot be regarded as individuals genetically but must be treated as colonies that may have genetically different nuclei. Hansen and Smith's (1932) comprehensive study of the dual phenomenon in Botrytis cinerea provides proof of this. Their forty seven cultures of B. cinerea collected in California yielded several distinct morphological types when sub-cultured as conidia. The morphologically different strains frequently anastomosed but the original components could be isolated from the resulting heterocaryons.

Hansen (1938), from an analysis of 916 wild type isolates belonging to 30 different genera of imperfect fungi, concluded that a mixed condition, which he called the "dual phenomenon", was a characteristic of the wild type in nearly all the imperfect fungi he examined. He further stated, "The frequency with which the dual phenomenon is observed to occur in imperfect fungi suggests that duality may be the natural condition for many of them".

Since then, other workers have selected new strains, some of them homocaryons, of several imperfect fungi by

successive single spore or hyphal tip isolations.

In this study, especial attention was paid to the production of new strains of Arthrobotrys oligospora, A. superba and A. robusta. These fungi were chosen because, apart from differences in spore size and shape and in the type and degree of whorling, they have almost identical morphologies and the traps they produce are indistinguishable from one another. It was considered possible that they might be strains of one species rather than different species.

B. MATERIALS

The fungi used in these studies were Arthrobotrys oligospora, A. superba, A. robusta and A. dactyloides.

The medium used for the selection of new strains was Water agar. This medium was chosen because the work of Jinks (1952) on Penicillium, Buxton (1954) on Fusarium, and Huebschman (1952) on Neurospora crassa had shown that the average number of nuclei in the vegetative mycelial cells and conidial cells could be altered by changing the nutritional status of the medium on which they were produced, a poor medium giving a low average number of nuclei. This was found to hold true for the fungi studied (page 38) and it was thought that the chances of being able to produce new strains, should these fungi be heterocaryotic, would be improved by culturing them on Water agar.

Stock cultures and new strains were maintained on slants of Difco Bacto Lima bean agar.

C. METHODS

Four methods were employed for the selection of new strains from their wild types:

1. SUCCESSIVE SINGLE SPORE ISOLATIONS

A dilute suspension of conidia, produced on Water agar, was pipetted onto a Petri dish of Water agar and spread around by rotating the plate. Using a stereoscopic microscope, at x50 magnification, single conidia which were well separated from others were selected and their positions marked by pricking the agar in the immediate neighbourhood with a transfer needle. After checking under a binocular microscope at x200 magnification to ascertain that they were indeed single conidia, they were transferred to slants of Water agar. When the cultures on the slants sporulated, the whole process was repeated so that a succession of single spore isolates was obtained.

2. SUCCESSIVE ISOLATIONS OF GERM TUBES OF SINGLE SPORES

It was felt that better success in selecting new

strains might be achieved through utilizing the germ tubes of conidia rather than whole conidia, because the conidia are bicellular and each cell of the conidium germinates. The procedure adopted was essentially the same as that for successive single spore isolations with the exception that, after placing the conidia on the medium, the plates were kept for a day at room temperature to allow the conidia to germinate. Single germ tubes were located and after checking under higher magnification to verify their being single, they were pinched off with a sterile dental canal pulp file and transferred to slants of Water agar.

3. SUCCESSIVE HYPHAL TIP ISOLATIONS

Hyphae from single conidia produced on Water agar, were allowed to grow for three days on Water agar and discrete hyphal tips pinched off with a dental canal pulp file were transferred together with the agar on which they were lying to slants of the medium.

4. SUCCESSIVE SINGLE INTERCALARY-CELL ISOLATIONS

Essentially, the method of Whitney and Parmeter (1963), was used.

Single conidia were cultured on cellophane pieces lying on the surface of 3 per cent Water agar. With a medium

of softer consistency, difficulty was experienced in cutting single cells as the cellophane either shifted or was pushed down into the medium during the cutting process.

For the cutting of particular cells, micromanipulators both manual and pneumatic were at first employed but little success attended these efforts and they were abandoned in favour of the razor-blade chip knife method of Whitney and Parmeter (1963). The knife was made by inserting a razor-blade chip into a needle holder after removing the needle. It was sterilized by immersing in 90 per cent alcohol followed by flaming.

The living hyphae on the cellophane were of a golden hue and highly refractile. On touching a cell, it burst to release its contents, becoming transparent in the process. A single cell was obtained by puncturing the cells on either side of it (Fig.22). Its position was marked by piercing the cellophane near it and then it was examined at x200 magnification to make certain that it was a single cell. The cellophane supporting the single cell was cut out and transferred to a plate of Water agar. When the resulting cultures sporulated, the process was repeated to give a succession of cultures arising from single intercalary cells,

D. RESULTS

Repeated single spore, germ tube and hyphal tip

isolates failed to produce colonies that were visably different from the wild types of the four fungi studied. Successive isolations of single intercalary cells was a successful method and the following new strains or variants were obtained; in each instance they were similar to the parent colony, except for the characters mentioned:

1. ARTHROBOTRYS OLIGOSPORA

Three new strains of A. oligospora were selected.

a) Strain 121A. It produced traps spontaneously in pure culture (Fig.23). This strain appeared to be identical with the one recovered from a culture that had been stored under oil (page 49).

b) Strain 223B. In this strain the conidia germinated while still on the conidiophore above the medium to give satellite conidia, each on a short conidiophore (Fig.24). When some trap inducing substance, such as beef, was placed on the medium, traps were produced by conidia while still attached to the conidiophore (Fig.25).

c) Strain 23A. The conidiophores of this strain were fairly well branched and the conidiophore axis was geniculate bearing the conidia at the "knees" as in A. superba.

Of these three strains, only 121A was stable after repeated subculturing while the other two soon reverted to the wild type from which they were obtained.

Figure 22. A single isolated intercalary cell of Arthrobotrys oligospora on cellophane.

Figure 23. Arthrobotrys oligospora strain 121A. Three dimensional adhesive networks produced spontaneously on weak Corn meal agar in the absence of nematodes.

Figure 24. Arthrobotrys oligospora strain 223B. Conidia germinated while still on the conidiophore to give satellite conidia, each on a short conidiophore.

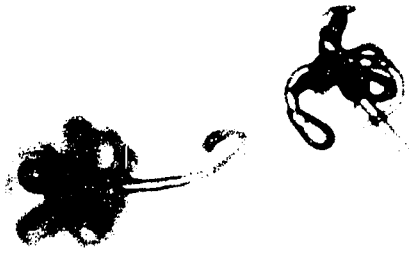


Figure 25. Arthrobotrys oligospora strain 223B. Conidia produced traps while still on the conidiophore above the substrate.

2. ARTHROBOTRYS SUPERBA

Two new strains of A. superba were selected.

a) Strain 146A. It produced chlamydospores in pure culture and when nematodes were added (Fig.26). The chlamydospores were brown, intercalary structures and produced in chains which were usually branched. Most of the individual elements had double walls. There are no published reports of A. superba producing chlamydospores.

b) Strain 145B. It grew on solid media, about one and a half times as fast as the wild type and in Czapek Dox broth amended with yeast extract it made about twice as much growth as the wild type. No difference in conidial shape, septation or dimensions was observed. The conidiophores collapsed and the individual conidia, in the "head" of conidia lying on the medium, germinated to produce what resembled a bunch of spikes.

Both these new strains were stable.

3. ARTHROBOTRYS ROBUSTA

Four new strains of A. robusta were obtained.

a) Strain 116D. It produced chlamydospores in pure culture and when nematodes were added (Fig.27). Chlamydospores usually occured singly at the ends of short lateral hyphal branches, but intercalary chlamydospores were also present.

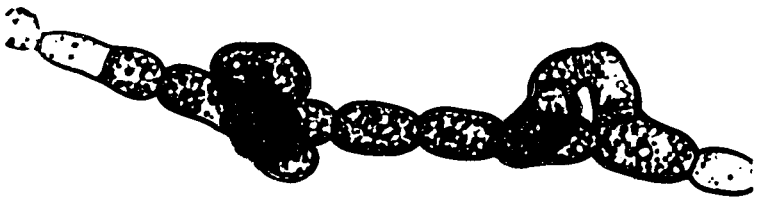


Figure 26. A branched chain of chlamydospores produced by Arthrobotrys superba strain 146A.

They were globular, brown in colour and larger than those of A. oligospora. Conidia were produced in whorls along the conidiophore.

b) Strain 122C. It had a slightly slower rate of growth on solid Corn meal agar than the wild type and made only about one-eighth the growth of the wild type in Czapek Dox broth amended with yeast extract. Whorling of conidia along the conidiophore also occured (Fig.28).

c) Strain 146D. The only difference between this strain and the wild type was that the conidia were borne in whorls; otherwise the two were indistinguishable.

d) Strain 152B. It was identical with strain 146D but the conidia were elongated and comparatively narrow, almost cylindrical in outline with the septum often towards the middle of the conidium (Fig.29). The conidia were similar to, but larger than, those of A. superba.

All four new strains were stable after repeated subculturing on weak Corn meal agar and on Lima bean agar.

4. ARTHROBOTRYS DACTYLOIDES

Two new strains of A. dactyloides were selected.

a) Strain 124D. It produced masses of 2-celled microconidia (Fig.30) together with normal conidia. The microconidia were about one-third the size of the normal conidia and resembled the latter in appearance. When single spores were subcultured

Figure 27. Terminal chlamydospores produced by Arthrobotrys robusta strain 116D.

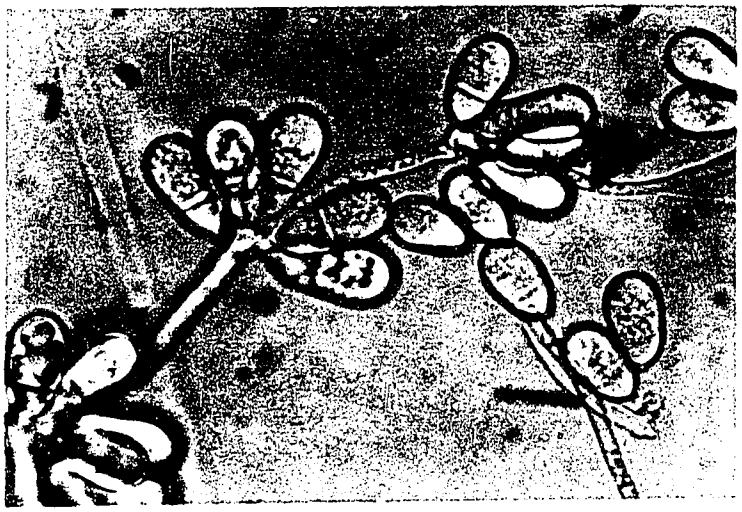


Figure 28. Arthrobotrys robusta strain 122C showing whorling of conidia along the conidiophore.

Figure 29. Elongate and narrow, almost cylindrical, conidia of Arthrobotrys robusta strain 152B, with the septum towards the middle of the conidium.

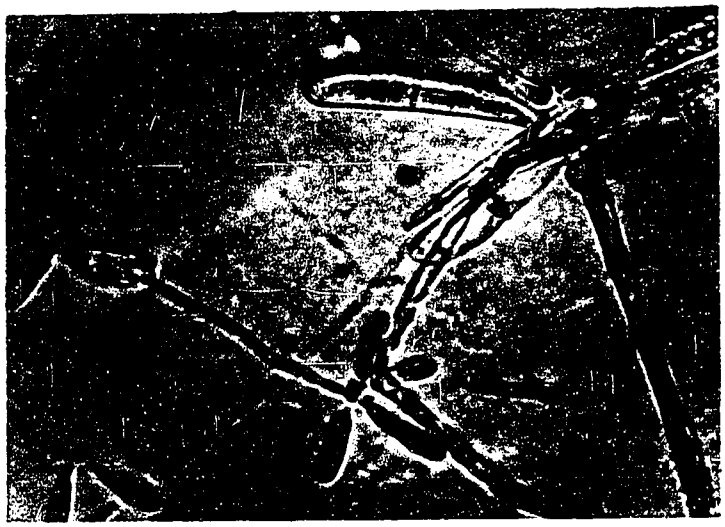


Figure 30. Arthrobotrys dactyloides strain 124D showing 2-celled microconidia and normal A. dactyloides conidia.

or when their germ tubes were cut off and cultured, they gave rise to both types of conidia.

b) Strain 148B. It produced swollen 3-celled conidia together with the normal conidia (Fig.31). When single germ tubes originating from these swollen spores were cultured, about 90 per cent gave rise to normal conidia only. In 10 per cent of cases, both types of conidia were produced.

E. DISCUSSION

For every new strain that was recognized as being morphologically different from its wild type probably several others, which were physiologically different, went undetected; still, several new strains were produced in a relatively short period of time. This is believed to be circumstantial evidence for the existence of heterocaryosis in these fungi.

It is postulated that the wild types are heterocaryons whose nuclear ratios, when altered, result in the development of morphologically different strains. This theory is consistent with the findings that A. oligospora strains 223B and 235A were not stable but reverted to the wild type, suggesting that the altered nuclear ratio could not be maintained. Further substantiation could be found in the fact that about 90 per cent of the germ tubes of the swollen 3-celled conidia of Arthrobotrys dactyloides strain 148B gave rise to the wild type conidia, and about 10 per cent of them

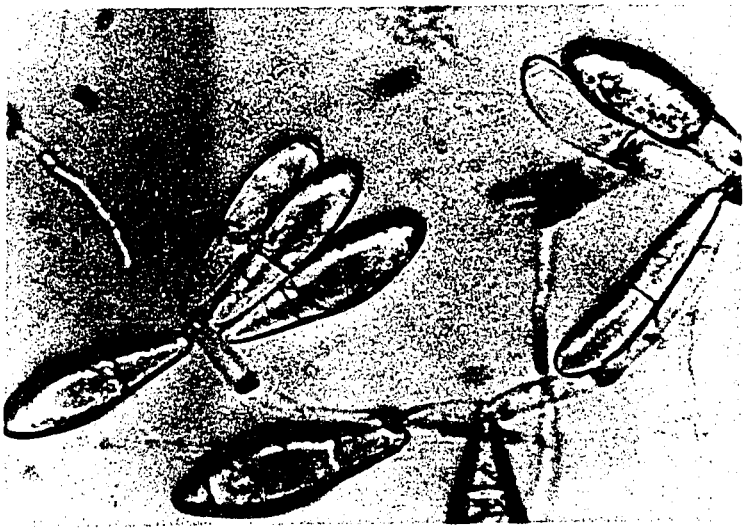


Figure 31. Arthrobotrys dactyloides strain 144b showing swollen 3-celled conidia and normal A. dactyloides 2-celled conidia.

produced both the swollen and the wild type conidia. The results of Feder et al. (1960) may also be explained on the basis of altered nuclear ratios. They distinguished type B spores of Dactylella doedycoides from type A spores because of their inability to form traps spontaneously and in the presence of nematodes. However, only a percentage of the spores which arose from cultures of the type B spores retained the type B condition; the others reverted to the type A which forms traps spontaneously.

Drechsler (1937), commenting on the identities of A. oligospora and A. superba stated that the latter did not produce chlamydospores. However, these studies demonstrate that some strains of A. superba have the genetic potentialities for chlamydospore production because strain 146A produced them in abundance.

Of the four new strains of A. robusta selected, strain 152B had conidia which resembled those of A. superba both in shape and position of septa. This might be an indication of a closer relationship between these two species than is at present considered.

Duddington (1951a) used as important criteria for distinguishing A. robusta a new species, his findings that it did not produce chlamydospores or whorls of conidia, but had branched conidiophores. All four new strains of A. robusta selected during these studies showed whorling of conidia, less branching of the conidiophore and three did not produce

chlamydospores. These findings cast doubts on the usefulness of such characters in delineating new species. However, as the nematode-trapping hyphomycetes lack a perfect stage, it may be necessary to make use of them until such time as more valid criteria are developed.

The type species for the form genus Arthrobotrys is A. superba and two of the main characteristics of this species are stated (Drechsler, 1937) to be the production of bicellular conidia and the nodal development of conidia in whorls, but A. dactyloides produces bicellular conidia which are not in whorls. Drechsler (1937) suggested that this should not be regarded as sufficient grounds for excluding a species from the genus Arthrobotrys if the arrangement of the conidia is truly capitate as it is in A. dactyloides. Duddington (1951a) agreed with this observation when he named A. robusta, a species which does not produce its conidia in whorls along the conidiophore. Drechsler (1937), when assigning A. dactyloides to the genus Arthrobotrys, stated that he did so with some misgivings because of the occasional production of swollen tricellular conidia in the manner of species in the genera Dactylella and Dactylaria.

In view of the finding that A. dactyloides strain 148B consistently produced tricellular conidia, it is suggested that it may be more appropriate if A. dactyloides were assigned to the genus Dactylella.

V. THE SYNTHESIS OF NEW STRAINS BY ANASTOMOSIS

A. INTRODUCTION

The problems encountered in species delineation arise primarily from the criteria used to describe them and from the difficulties in establishing their limits. Characters used to define and separate closely related species of nematode-trapping hyphomycetes frequently are of degree only and although they are justified by the need for a practical system of classification, the use of these quantitative criteria becomes an important consideration in determining whether anastomosis between two species constitutes a valid reason for considering the two as strains of a single species. For the purposes of this thesis, anastomosis is used as if it were a synonym of plasmogamy or the compatible fusion of two hyphae and the mingling of their cytoplasm.

Most attempts at making heterocaryons between different species of fungi have ended in failure (Gossop et al., 1940). In contrast to this, however, Taschdjian and Muskatblit (1955) observed anastomosis between three species of Trichophyton (the cause of Black Dot Ringworm in man) and this led them to amalgamate the three under one species. They state, "It is recognized that anastomosis will occur between mycelia of the same species (Langeron and Vanbreuseghem, 1952). While absence of fusion between two mycelia is not

sufficient per se to warrant placing two organisms into different species, presence of fusion indicates that two strains do belong to one and the same species".

Arthrobotrys oligospora, A. superba and A. robusta resemble one another morphologically and variant strains of A. superba and A. robusta (strains 146A and 116D respectively), that produce chlamydospores like those of A. oligospora, were selected from their wild types. A. robusta strains 116D, 122C, 146D and 152B, which produce their conidia in whorls along the conidiophore like those of A. oligospora, were selected from the wild type. Because of these facts and because of the selection of A. robusta strain 152B that has spores that resemble those of A. superba, it was thought that A. oligospora, A. superba and A. robusta might anastomose with one another.

B. MATERIALS AND METHODS

The fungi used in these studies were the normal or wild type strains of Arthrobotrys oligospora, A. superba and A. robusta.

The medium used for the study of anastomosis was weak Corn meal agar (Duddington, 1955). Stock cultures were maintained on Difco Bacto Lima bean agar.

a) Single conidia of two species were placed some distance apart on the medium in Petri plates. When the colonies grew together, a small square of the cultures at the line of

contact was removed and transferred to another plate of the culture medium. On sporulating, the conidia were washed off with sterile water and a single conidium inoculated on each of several slants of weak Corn meal agar. All cultures were incubated at room temperature. The resulting colonies were compared with the species that had been mated.

b) A modification of the Curry (1949) drop method was also used. About 2ml of the culture medium was pipetted on the inside of the lid of a sterile Petri plate. The medium was spread into a very thin layer and the lid fitted onto the plate which contained about 10ml of sterile water. In effect, a moist chamber was produced. The agar was inoculated with conidia of two species opposite one another and when the colonies met, microscopic examination for the occurence of anastomoses between the two species was carried out at x200 magnification without removing the lid.

c) In another approach to the study of anastomosis, vital stains of different colours were incorporated into the culture medium. Squares of cultures of the two species, one grown on a medium stained red and the other on one stained blue, were planted on opposite sides of a plate of Water agar. It was hoped that the fungi would use the stained Corn meal agar as a food base and the anastomosing hyphae would be coloured differently, thus making it easy to recognize the occurence of anastomosis between hyphae of different species.

d) Sterile, peeled cellophane squares were aseptically

placed on weak Corn meal agar. Conidia of one species were steeped in a vital stain and pipetted in a narrow streak on the cellophane. A day later, conidia of the other species, steeped in a vital stain of a different colour, were streaked in a line opposite the first one. When the hyphae from the conidia of the two species met, they were examined microscopically for the occurence of anastomoses.

With a razor-blade chip knife, the hyphal cells around the anastomosed cells in the anastomosis figure were punctured and the cellophane bearing the figure cut out and planted on weak Corn meal agar. Four such transfers were made from matings of two species.

By these methods it was hoped to eliminate all possibility of error in determining that anastomosis between hyphae from two different conidial sources had occured:

(i) the positioning of conidia on opposite sides would make it fairly easy, on tracing a hypha back to its conidium, to recognize which species it came from.

(ii) a long hypha would probably be from the species planted first and a short hypha from the one planted a day later.

(iii) the coloured hyphae and/or conidia would aid in distinguishing hyphae arising from different species.

Anastomosis was recognized to have taken place between the species when two fused hyphae could be uninterruptedly traced to their two opposite conidial sources.

C. RESULTS

Good observations were obtained with the modification of the Curry drop and with the cellophane culture technique. The hyphae, of the colonies arising from media in which stains were incorporated, did not take up the vital stains; however, the conidia did take up the stains and most of them retained it for a time. This was invaluable in decisions that anastomosis had occured between hyphae arising from conidia of two different species.

Anastomoses were observed to occur between Arthrobotrys oligospora and A. superba; between A. oligospora and A. robusta; and between A. superba and A. robusta. Figure 32 shows anastomosis between germ tubes arising from conidia of A. robusta and A. superba.

No new strains were obtained by the procedure outlined in method (a). Anastomosis figures isolated from matings between A. oligospora and A. superba and between A. oligospora and A. robusta yielded colonies which, at an early stage bore some very much enlarged 2-septate conidia (Fig. 33); the vast majority of the conidia were of the A. oligospora type. Some of the enlarged, biseptate conidia were irregular in shape (Fig.34). The enlarged conidia, when subcultured, gave rise to colonies that were indistinguishable from those of A. oligospora, as did single germ tubes arising from these spores.

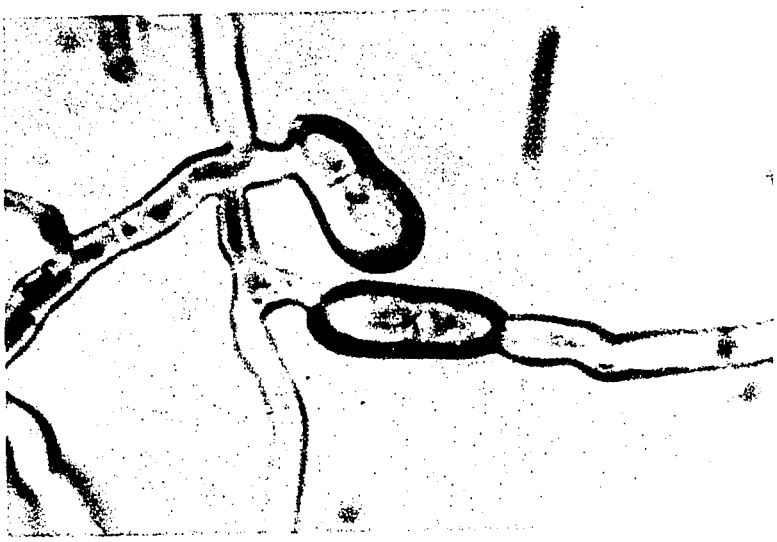


Figure 34. Anastomosis between germ tubes of Armillaria superba and A. robusta.

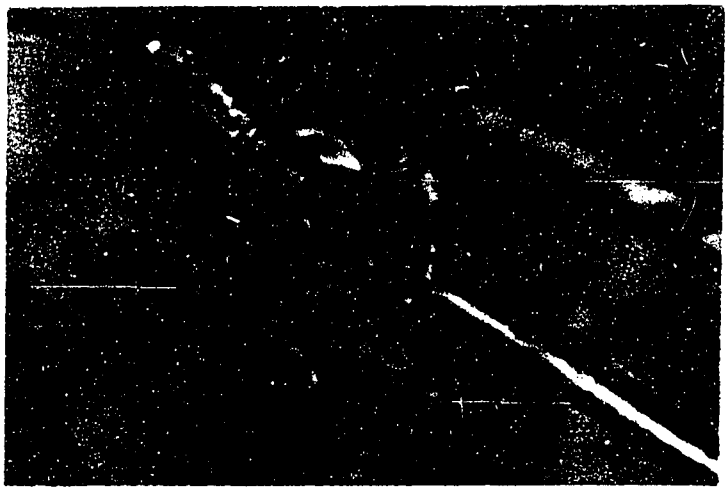


Figure 33. A 2-septate conidium that arose from an anastomosis figure that occured between Arthrobotrys oligospora and A. superba.

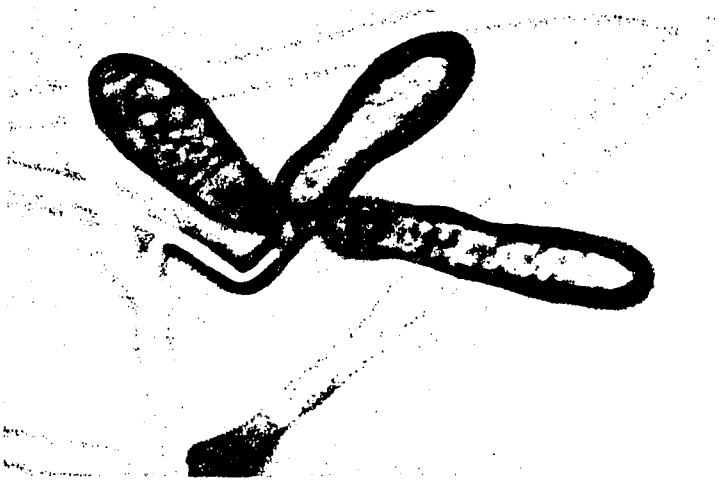


Figure 34. An irregularly shaped conidium that arose from an anastomosis figure that occured between Arthrobotrys oligospora and A. superba.

A new strain, designated Arthrobotrys Strain 24 was obtained from an anastomosis figure that arose from a mating between A. robusta and A. superba. This strain 24 was distinguished from its constituent species by its larger conidia. Its conidiophore was usually not branched and bore from 1 - 5 whorls of closely packed 2-celled conidia. The conidia were 20 - 45u in length and 11 - 17u in width and the septum was towards the middle, the distal cell being the larger. Very occasionally, 2-septate conidia were found. No chlamydospores were observed.

D. DISCUSSION

Three-celled conidia have not been reported for any of the three species used in this study. Their production from anastomosed cells is corroboration that somatogamy did occur, as is the production of the new strain Arthrobotrys 24 from A. robusta and A. superba.

The apparent ease with which anastomosis occurs between A. oligospora, A. superba and A. robusta leads one to believe that although morphological characters permit a distinction between them, these species may in reality be variants of a single species. The failure to obtain new stable strains from matings of A. oligospora and A. superba, or from A. oligospora and A. robusta may have been due to the occurence of nuclear dissociation in the composite strain, the

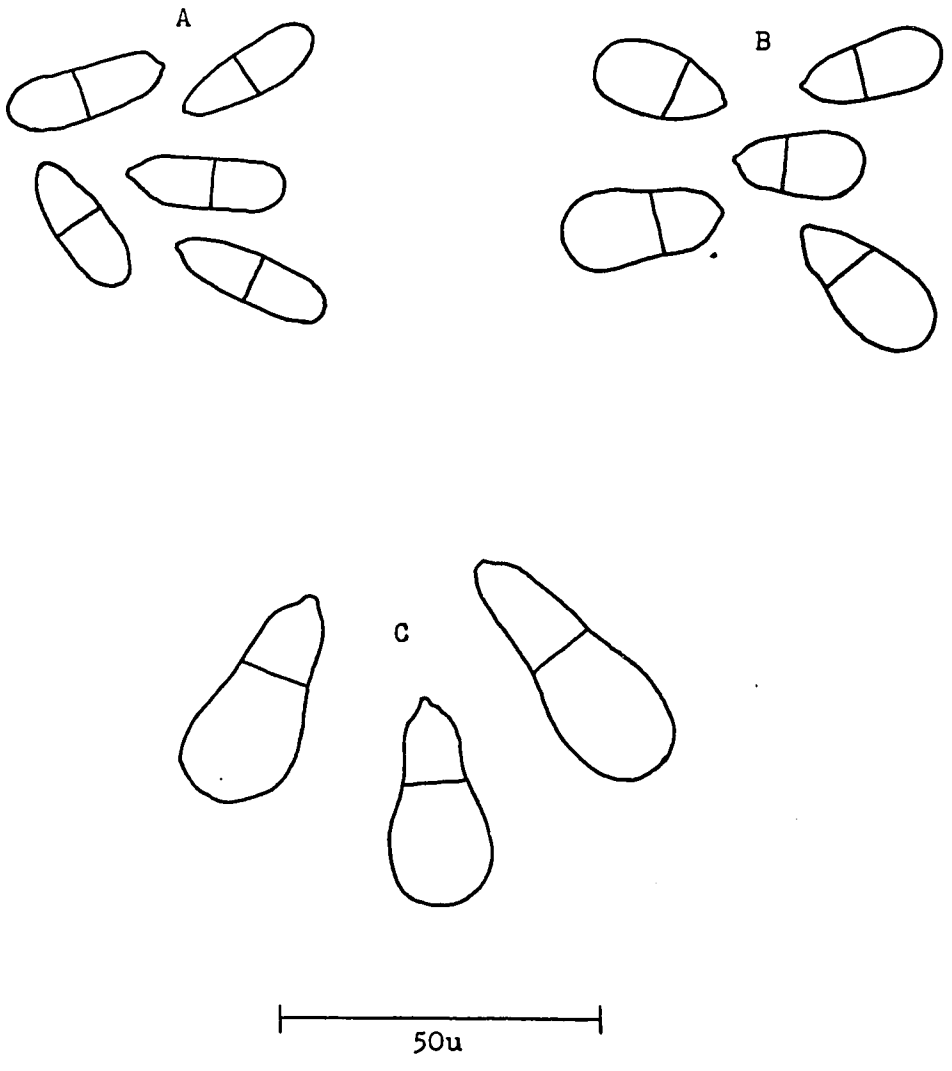


Figure 35. A. Conidia of Arthrobotrys superba (wild type).
B. " " " robusta (wild type).
C. " " " strain 24.

A. oligospora component becoming predominant. Another possibility is that genetic incompatability might exist. However, this does not rule out the possibility that they were variants of one species as intra-specific incompatability is known to exist (Holton and Fischer, 1941; Nelson, 1957).

VI. ELECTROPHORETIC STUDIES ON SELECTED SPECIES AND NEW STRAINS OF NEMATOPHAGOUS FUNGI

A. INTRODUCTION

A criterion, which is still in its infancy as far as fungal taxonomy is concerned and whose merits have yet to be determined, is the use of electrophoretic patterns of soluble proteins. Norris (1966) states, "The use of gel electrophoresis in microbial taxonomy is at present in an exploratory phase and more information is needed before the potential of the method, or the significance of the groupings indicated, may be ascertained. The indications at present are that these techniques have a valuable part to play in the future development of the subject".

Electrophoretic techniques are currently used as aids in the diagnoses of pathological conditions in humans. Smithies (1955), and Dessauer and Fox (1956) have used electrophoretic protein patterns as aids in the taxonomy of animal species. Chang *et al.* (1962), using acrylamide gel as a supporting medium, showed marked differences between the electrophoretic separations of soluble proteins from different species of *Neurospora*. Clare (1963) concluded from starch-gel electrophoresis of proteins from different species and strains of species of *Penicillium* that the technique was a useful aid in the delimitation of fungal species.

B. MATERIALS AND METHODS

1. PREPARATION OF SOLUBLE PROTEIN SAMPLES

The fungi were grown in Czapek Dox broth, amended with 0.1 per cent yeast extract, contained in 500ml Erlenmeyer flasks. The cultures were incubated for 8 days at 25°C on a rotary shaker.

The mycelia were collected by filtering the cultures through a Buchner funnel under suction. They were washed thrice with distilled water and freeze dried.

A freeze dried sample was ground to a fine powder in a pestle and mortar and 0.5gm of the powder transferred to a Teflon tissue grinder with a clearance of 0.004mm. Three millilitres of Tris-citrate buffer at pH 8.6 (0.076M Tris, 0.5mM ethylenediamine tetraacetic acid and 0.005M citric acid) were added to the powder and the tissue grinder operated with the aid of an electric motor for 15 minutes. The suspension was cooled during this operation in order to avoid denaturation of proteins by the heat generated through friction.

The suspension was collected in a centrifuge tube together with the washings resulting from the addition of a further 2ml of buffer to the tissue grinder. The suspension was subjected to centrifugation at 41,000g for 30 minutes. The result was a clear, golden hued supernatant covered with a thin scum. A translucent pellet, thought to be albumin,

was invariably formed against the wall of the centrifuge tube at the base of the compacted fungal debris. Attempts at removing the pellet and re-suspending it in the supernatant were unsuccessful; this was because the fungal debris overlying it was of such a consistency that it could not be separated cleanly from it.

The supernatant was pipetted off but, even with the greatest of care, little fragments of scum were also collected. This necessitated centrifugation for a further 15 minutes, again at 41,000g.

The protein samples deteriorated quickly on storage in the freezer compartment of a refrigerator.

2. BUFFERS

Poulik's (1957) discontinuous borate-citrate buffer system was used.

Starch and polyacrylamide gels were made with Tris-citrate buffer at pH 8.6, of the same composition as the buffer used for extracting the protein samples.

For polyacrylamide gel electrophoresis, borate buffer at pH 8.7 (0.3M boric acid and 0.1M sodium hydroxide) was used in the electrode compartments of the apparatus while for starch gel electrophoresis, better results were obtained with the same buffer at double the ionic strength (0.6M boric acid and 0.2M sodium hydroxide).

3. PROTEIN STAINING AND DESTAINING

Amido black 10B was used for staining the starch and the polyacrylamide gels. The staining solution was prepared, according to the directions of Smith (1960), as follows:

Amido black 10B 1.0gm

Methanol-acetic acid-water (5:2:5)... 100ml

The stain was poured on the gel and after 10 minutes, the excess stain was poured off, the gel washed in water and destaining carried out over a two day period in six changes of a destaining solution made up of methanol-acetic acid-water in the proportion of 5:1:5.

After destaining the starch slices, they were left in 5 per cent acetic acid. This procedure, according to Smith (1960), intensifies the blue of the protein bands and makes the intervening areas porcelain white. The gel also becomes very brittle in the process.

4. STARCH GEL ELECTROPHORESIS

Protein separations were made from the following samples:

i) Arthrobotrys oligospora (wild), A. superba (wild), A. robusta (wild), Strain A. 24.

ii) A. oligospora (wild), A. oligospora 121A,

A. superba (wild), A. superba 146A, A. superba 154B.

iii) A. robusta (wild), A. robusta 146D, A. robusta 152B, Strain A. 24.

The composition of the gel and the conditions of the electrophoretic process were according to Kristjansson (1963):

8.74gm of hydrolysed starch were suspended in 20ml of cold Tris-citrate buffer, and 130ml of the buffer heated to 90°C were added rapidly to it. The suspension was swirled by hand for a minute and, without degassing, was poured into the starch gel tray. A few drops of mineral oil were applied to a sheet of glass and the glass placed oiled side down on the gel tray. Weights were applied.

After one hour, the weights and the glass sheet were removed and the gel cut across its width, 4cm from one end, with a razor blade. The smaller part of the gel was pushed back and pieces of Whatman No. 3 filter paper, 0.75cm x 0.6cm, soaked in a sample of the fungal extract were placed approximately 1cm apart against the exposed cut surface of the larger portion of the gel. The smaller part of the gel was carefully replaced against the larger portion of the gel. The gel tray was covered with Saran Wrap* leaving 2.5cm at either end exposed to allow contact with the filter wicks.

One hundred and sixty five volts were initially applied for 15 minutes. The current was shut off, the paper

*- a product of the Dow Chemical Company, Midland, Mich., U.S .A.

inserts removed, the gel recovered with Saran Wrap and again 165 volts were applied for a further period of 15 minutes. The voltage was then raised to 350 and the current registered was 20mA. The brown borate boundary was allowed to migrate exactly 8cm; this was accomplished in 2½ hours from the time the system was switched to high voltage. The gels were cooled by carrying out the electrophoretic separations in a refrigerator at 5°C.

At the completion of a separation, the gel was sliced by a taut wire supported by a steel wishbone.

5. POLACRYLAMIDE GEL ELECTROPHORESIS

Protein separations were made from the following samples:

i) Arthrobotrys oligospora (wild) - 3 isolates from 3 different localities.

ii) A. oligospora (wild), A. superba (wild), A. robusta (wild), Strain A. 24. These four samples were duplicated on the same gel.

iii) A. oligospora (wild), A. oligospora 121A, A. superba (wild), A. superba 146A, A. superba 154B, A. robusta (wild), A. robusta 146D, A. robusta 152B.

Electrophoretic separation of proteins of A. robusta 116D and 122C were not made because strain 116D was obtained after this experiment had been completed and strain 122C made

such poor growth in yeast amended Czapek Dox broth that enough mycelium for extraction of a protein sample was not obtained.

The vertical polyacrylamide gel apparatus was obtained from the E-C Apparatus Corporation, Philadelphia, and the procedure used was essentially the one outlined in their Technical Bulletin No. 133.

The base of the apparatus consists of the outer cooling plate with the lower electrode chamber (the anode) attached. The upper part of the apparatus includes the inner cooling plate with the upper electrode chamber (the cathode). The two parts, when fitted together and held by toggle clamps, form a gel tray between the outer and the inner cooling plates.

Before fitting the two parts together, a sponge strip was inserted into the bottom of the outer cooling plate. Cooling water was allowed to flow so that visibility through the cooling plate would be unimpaired during the pouring of the gel.

An 8 per cent gel was prepared by dissolving 12gm of Cyanogum 41* gelling agent in 150ml of Tris-citrate buffer, adding 0.3ml of 3-dimethylaminoproprionitril catalyst, and filtering the solution. Just prior to pouring the solution into the gel tray, 0.3gm of the second catalyst, ammonium persulphate, dissolved in 2ml of the buffer were added to the well stirred solution.

*- a product of the E-C Apparatus Corp., Phila., Pa., U.S.A.

The apparatus was tilted to an angle of approximately 30 degrees, and the solution carefully poured into the gel tray while gradually lowering the apparatus. The tilting aids in preventing the entrapment of air bubbles between the cooling plates. If any air bubbles formed, they were eliminated by rocking the apparatus from side to side or up and down. The well-former was then inserted into the top of the gel tray.

After about 45 minutes, the excess gel beyond the slot was cut out and discarded. The well-former was carefully removed taking great care not to break the webs between the wells which would allow mixing of samples to occur. This process was facilitated by loosening the gel adhereing to the well-former with a long transfer needle bent in the form of an S. The electrode chambers were filled with borate buffer so that the platinum electrode in the bottom compartment and the wells in the upper compartment were covered. Air bubbles invariably formed in some of the wells and they were removed by gentle manipulation with the fore-finger of the hand.

A few grains of sucrose were dissolved in the samples so that their specific gravity would be higher than that of the buffer. With a narrow bore pipette, the samples were dropped through the buffer into the wells.

Two hundred volts were applied for 90 minutes. The current registered was 105mA or about 8.5mA/cm width of the gel. At the end of this time, under the conditions of the operation, the borate front moved exactly 10cm. To facilitate

observation of the movement of the borate front, a small crystal of bromothymol blue was added to the sample in the first well.

The gel was removed at the completion of the operation, the position of the first sample fixed by cutting out a wedge from the base of the gel at that point, and the gel stained.

Photographs of gels were taken with an Ashai Pentax camera using Kodak Panchromatic Plus-X film. Heightened contrast was obtained by using a red filter (Kodak Daylight Filter No.85, Series 7).

C. RESULTS

Fewer protein bands were obtained with starch gel as a supporting medium than with polyacrylamide gel. Further, the majority of the bands were fuzzy and indistinct even after steeping the gels in 5 per cent acetic acid. Consequently, in the final analysis of results, only those obtained through use of polyacrylamide were taken into consideration; the bands produced were clear and well demarcated.

With wide wells, fewer bands were obtained than with narrow wells containing less of the sample.

There was complete correspondence between the protein patterns of the three A. oligospora isolates from three different localities (polyacrylamide gel separation i).

The wild type of A. oligospora showed 7 protein bands; of A. superba, 10 bands; of A. robusta, 12 bands; and the composite strain A. 24 showed 9 bands (Fig.36). Three of these bands, in all the three wild type species and in the composite strain, were in identical positions.

The variation between protein patterns of strains and their wild types (Fig.37) was almost the same as between the wild type species themselves (Fig.36).

D. DISCUSSION

The close correspondence between the protein patterns of the three wild type isolates of Arthrobotrys oligospora and the differences between patterns of different species, indicate that polyacrylamide gel electrophoresis is a useful tool for taxonomic purposes.

There were distinct differences between the protein patterns of the three species but between 25 and 43 per cent of their bands corresponded. The three protein bands which were in the same positions indicated that the proteins they represented had the same migration velocities and were probably identical.

There were distinct differences between protein patterns of new strains and the wild types from which they

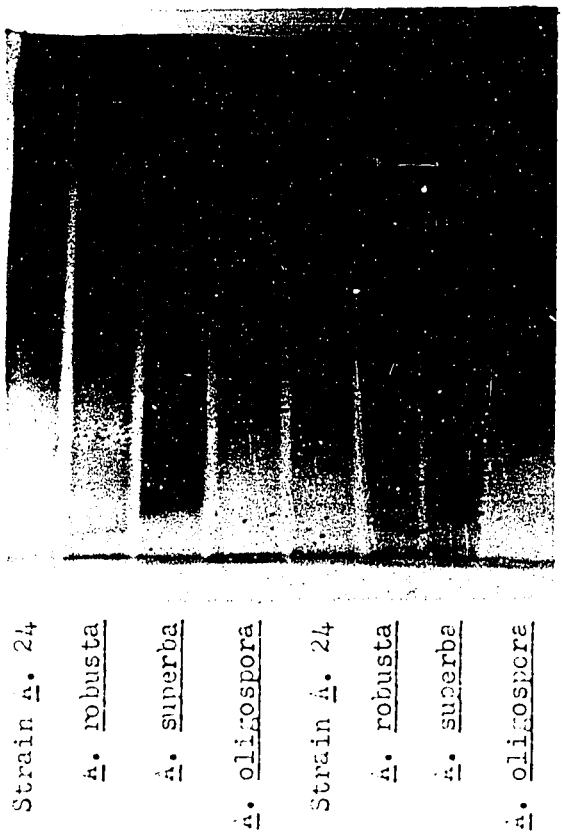


Figure 36. Polyacrylamide gel separation of soluble proteins from mycelial extracts of A. oligospora, A. superba, A. robusta, and the composite strain A. 24.

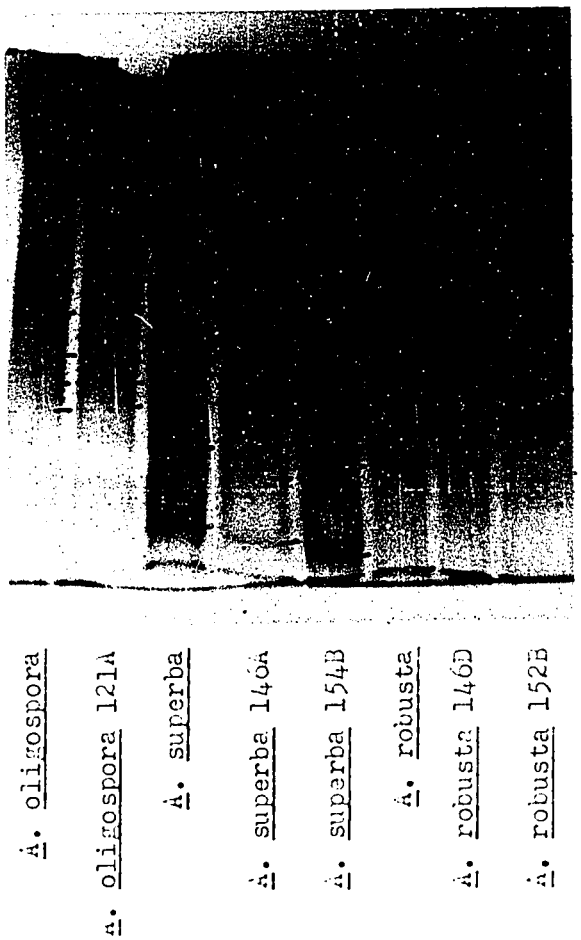


Figure 37. Polyacrylamide gel separation of soluble proteins from mycelial extracts of A. oligospora, A. superba, A. robusta, and new strains selected from these species.

were produced and these differences were almost of the same magnitude as those between the species themselves. It is believed that this constitutes additional circumstantial evidence for considering A. oligospora, A. superba and A. robusta as strains of a single species.

Gel electrophoresis may not be a definitive tool but it may make a valuable contribution in the realm of numerical taxonomy.

SECTION II. STUDIES ON THE TRAPPING ACTIVITY OF SELECTED NEMATOPHAGOUS FUNGI

I. THE EFFECT OF AGE ON TRAPPING ACTIVITY OF NEMATOPHAGOUS FUNGI

A. INTRODUCTION

When a large number of nematodes is added to a culture of a predacious hyphomycete, the fungus usually displays a rapid increase in trapping activity which is maintained for a time and then the activity declines (Duddington, 1957a). Commandon and deFonbrune (1938) investigated the adhesive mechanisms of Dactylella ellipsospora which produces adhesive knobs, and Arthrobotrys oligospora in which sticky networks are formed. In both species, the stickiness increased to a maximum at a certain period in the life of the fungus and then gradually declined. They found a similar optimal age for trapping activity in Stylopage hadra, which is a member of the Zoopagales. In S. hadra the whole surface of the mycelium is sticky.

Couch (1937) was unable to get the constricting rings of Dactylella bembicoides to close by introducing a glass needle into them and stroking them. Duddington (1962a) suggested, as one of the reasons for Couch's failure to obtain closure of the rings by this method, the possibility

that the rings he was working with were old and had lost their ability to react to the mechanical stimulus.

Further knowledge of the effect of age on the trapping activity of the nematophagous hyphomycetes is necessary if they are to be successfully employed in the biological control of plant parasitic nematodes. Hams and Wilkin (1961) pointed out the importance, in the biological control of Heterodera species, of synchronizing the peak of fungal activity with the peak emergence of larvae from cysts. Cooke (1963a) stated that peak predacious activity appeared to occur soon after the fungi were added to soil, and the subsequent decline was often rapid.

B. MATERIALS AND METHODS

The fungus used was Arthrobotrys oligospora and it was cultured on weak Corn meal agar.

Three long rectangular culture plates fitted with lids, whose outside dimensions were 24" x 2" x ¾", were sterilized by immersing them in a 10 per cent solution of Javex* for 12 hours and washing them with sterile water.

One hundred and fifty millilitres of the medium were poured into each dish and a small square of a culture of A. oligospora on weak Corn meal agar was inoculated in the centre.

*- a product of Domtar Consumer Products Ltd., Montreal, Canada.

Rhabditis oxycerca nematodes reared on soil-peanut butter (Thomas, 1963) were washed from the lids of the culture dishes and cleared of any accompanying debris by passing them through a Baermann funnel (Baermann, 1917) using two double thicknesses of facial tissue. The nematodes were washed in ten changes of sterile tap water and the final suspension standardized so that each drop contained approximately 50 nematodes.

The inoculated plate-cultures, with or without nematodes, were wrapped in polythene and incubated at room temperature in dark cupboards which were first sprayed with Kelthane* and then swabbed down with kerosene to kill any mites that may have been present.

C. EXPERIMENTS AND RESULTS

1. TO DETERMINE THE EFFECT OF AGE OF MYCELIUM ON ITS ABILITY TO PRODUCE TRAPS

The lateral growth made by the fungus in each 24 hour period, in the absence of nematodes, was marked off on the bottoms of the long plates with a grease pencil.

At the end of the 24th day, the average lateral growth was 22.03cm. The culture in each plate was divided into 6 segments on each side of the point of inoculation,

*- a product of Rohm and Haas Co. of Canada Ltd., 2 Manse Road, West Hill, Ontario, Canada.

each segment corresponding to the approximate 4cm of growth per four day period. These segments were then transferred to sterile Petri plates and approximately 100 nematodes added to each. Counts were made of the number of traps formed and the number of nematodes captured 96 hours after the nematodes were added.

Two measurements were recorded from each long plate (one on each side of the original inoculum) and the data presented in Table (1) are the averages of 6 readings from 3 plates. The last segment, representing the growth made between the 21st and the 24th day, produced a large number of traps averaging about 1,200 per segment. Traps were first produced by the 23rd day's growth (2 day old mycelium) and then trap production spread on either side of this area. The mycelium which was 5 to 8 days old produced a few traps, while the older mycelium produced fewer, if any, traps. However, when about 500 nematodes were put on each of the older segments 96 hours after the first lot of nematodes was added, trap production occured. It appeared that the threshold for trap induction was low in the young mycelium and high in the old mycelium. Further, the most sensitive part of the mycelium was the portion just behind the growing edge of the colony.

2. TO DETERMINE IF THE STIMULUS FOR TRAP PRODUCTION COULD PASS FROM YOUNG TO OLDER MYCELIUM

After 24 days of fungal growth in the absence of nematodes, approximately 100 nematodes were added just outside the growing edges of the colonies in the long dishes.

Trap production started in that part of the mycelium which was 2 days old, and within 96 hours it had spread all along the mycelium, being most profuse in the younger parts.

3. TO DETERMINE IF A FUNGAL COLONY WOULD CONTINUE TO TRAP NEMATODES IF ITS LATERAL GROWTH WERE UNIMPEDED

A day after inoculating the medium in the 3 long plates, with A. oligospora, approximately 200 nematodes were added to each plate, 100 on each side of the fungal inoculum. Observations were made every 24 hours.

The cultures reached the ends of the plates in an average of 28 days. The mycelium was sparse and did not permeate the medium as it does in the absence of nematodes. The majority of the nematodes were trapped within the first 3 days but those that escaped soon multiplied so that nematodes were available for trapping by the fungus at all times. It was consistently noted that the young mycelium near the extremities of the advancing edges of the colonies in all 3 plates trapped nematodes actively while the traps towards the centre of the colony progressively lost their ability to capture nematodes.

Table 1.

The effect of age on the trapping activity of Arthrobotrys oligospora on weak Corn meal agar incubated at room temperature in the dark.

Age of culture in days	Av. No. of traps formed in 48 hrs.	Av. per cent nematodes captured in 48 hrs.
1 - 4 days	approx. 1,200*	84.1*
5 - 8 "	50.0*	2.9*
9 - 12 "	1.3*	0.0*
13 - 16 "	0.6*	0.0*
17 - 20 "	1.0*	0.0*
21 - 24 "	6.3*	0.2*

*- Average of 6 readings from 3 plates.

II. THE EFFECTS OF ENVIRONMENTAL FACTORS ON TRAPPING ACTIVITY OF NEMATOPHAGOUS FUNGI

A. EFFECT OF HYDROGEN ION CONCENTRATION

1. INTRODUCTION

Much work has been done on the effect of hydrogen ion concentration on growth and sporulation but little attention has been paid to the effect of pH on trap formation and the trapping efficiency of nematophagous fungi. Gorlenko (1956), in his report on the control of root-knot of cucumber by the addition of Trichothecium pravicovi and Arthrobotrys kirghizica to the soil, stated that ammonium carbonate increased the predatory activity of the fungi. Soprunov (1950) had earlier found that a Didymozoophaga sp. developed predatory properties in alkaline and neutral but not in acid media. Tarjan (1961) reported that the adhesive knobs of Dactylella drechsleri were not formed in the absence of nematodes at pH 5.2 but were at other pH values in the range 3.3 to 9.2. The observation of Duddington (1955), that nematode-trapping fungi are less frequent in acid peaty soils although nematodes are plentiful, is also noteworthy.

Hydrogen ion concentration is one soil factor that lends itself to easy adjustment not only in the greenhouse but also on a large scale in the field. Hence, information on

the effect of pH on trapping activity is an important preliminary to the successful employment of nematode-trapping fungi in the biological control of plant parasitic nematodes.

2. MATERIALS AND METHODS

The fungus used was Arthrobotrys oligospora.

Corn meal broth was prepared by filtering a suspension of 40g of Corn meal that had been heated at 70°C for 1 hour in 1.2 litres of tap water and the final volume adjusted to 1 litre. The following quantities of 0.15M Na_2HPO_4 and 0.15M KH_2PO_4 were added to 100ml lots of the broth to obtain the desired pH values:

pH value	0.15M Na_2HPO_4	0.15M KH_2PO_4
5	2.0ml	98.0ml
6	14.0 "	86.0 "
6.5	35.0 "	65.0 "
7	82.0 "	18.0 "
8	96.0 "	4.0 "

One and a half grams of agar were added to each of the media which were then autoclaved at 121°C for 15 minutes. When they had cooled to 40°C, the pH values were adjusted with either 1N NaOH or 1N HCl.

Fifteen millilitres of the medium at each of the desired pH values, were poured into each of 6 Petri plates.

All the plates were inoculated in the centre with 2mm squares of a young culture of the fungus on weak Corn meal agar. The plates were wrapped in polythene and incubated at room temperature (about 22°C) on a laboratory table.

After a week, approximately 150 Rhabditis oxycerca nematodes that had been passed through a Baermann funnel (Baermann, 1917) and washed in ten changes of sterile water were added to 3 of the 6 plates at each pH value; the remaining plates were the controls. The addition of nematodes was made after removing and discarding the original block of inoculum, as previous observations had shown that nematodes tend to get into the inoculum block where they are relatively unavailable for trapping.

Visual estimates of the number of traps formed 48 hours after the addition of nematodes, were made by comparing the plate-cultures with standard plate-cultures of the same size (85mm in diameter), containing approximately 1,200 and 2,400 traps, on which the traps had been meticulously counted. The percentage of nematodes captured was calculated after counting the number of nematodes trapped out of the total number of nematodes in a plate. The total number of nematodes varied from plate to plate as some received more than others and further, some nematodes wandered onto the glass walls of the plates and perished.

Trap operating efficiency was assessed by taking

the ratio between the per cent of nematodes captured and the number of traps per square centimeter.

3. RESULTS

The diameters of the colonies were essentially the same at all the 5 pH values (85mm). No traps were produced in any of the nematode-free control cultures.

Figures 38 and 39 show the average number of traps per square centimeter and the average percentage of nematodes captured respectively, at 5 pH values 48 hours after nematodes were added. Trapping efficiency of the colony, or the ratio between the percentage of nematodes captured and the area of the colony, at different pH values is also shown by Figure 39. This is because the area of each colony was essentially the same at all pH values between 5 and 8.

Trap production progressively increased with increasing pH (Fig.38) as did the percentage of nematodes captured (Fig.39). Trapping efficiencies of colonies (Fig.39) and trap operating efficiencies (Fig.40) were lowest at pH 5 and highest at pH 8. The traps produced at pH 5 appeared to have lost their ability to produce the sticky substance that is responsible for nematodes adhereing to them or the substance was not adhesive at this pH value. Nematodes crawled over and through the traps and prolonged microscopic examination did not detect the slightest evidence of stickiness. After 96 hours, there was an increase in the

Figure 38. Average number of traps per square centimeter produced at 5 pH values by 7-day old cultures of A. oligospora on weak Corn meal agar, 48 hours after adding nematodes.

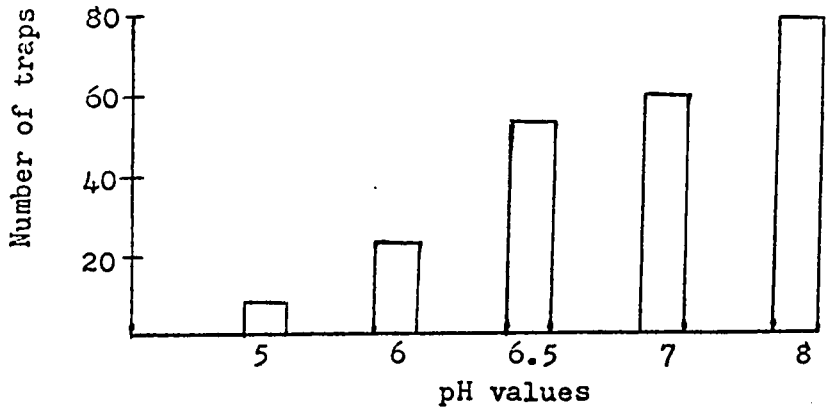


Figure 39. Percentage of nematodes captured by 7-day old cultures of A. oligospora at 5 pH values on weak Corn meal agar, 48 hours after adding nematodes.

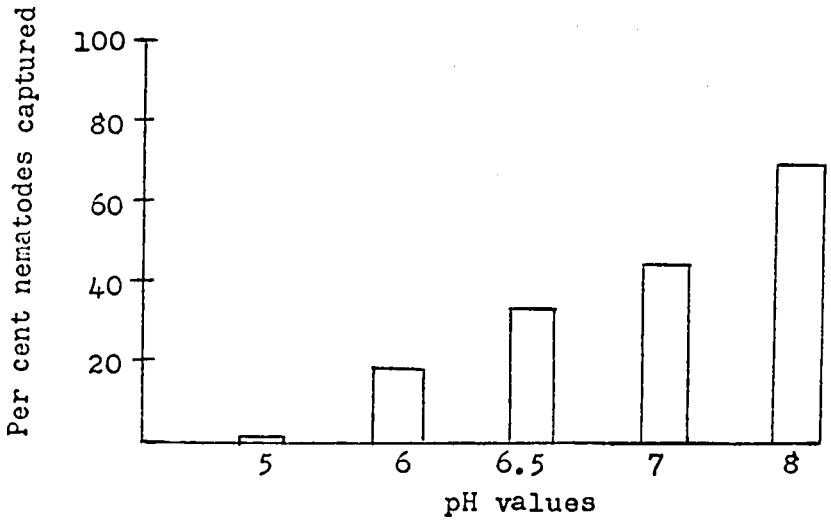
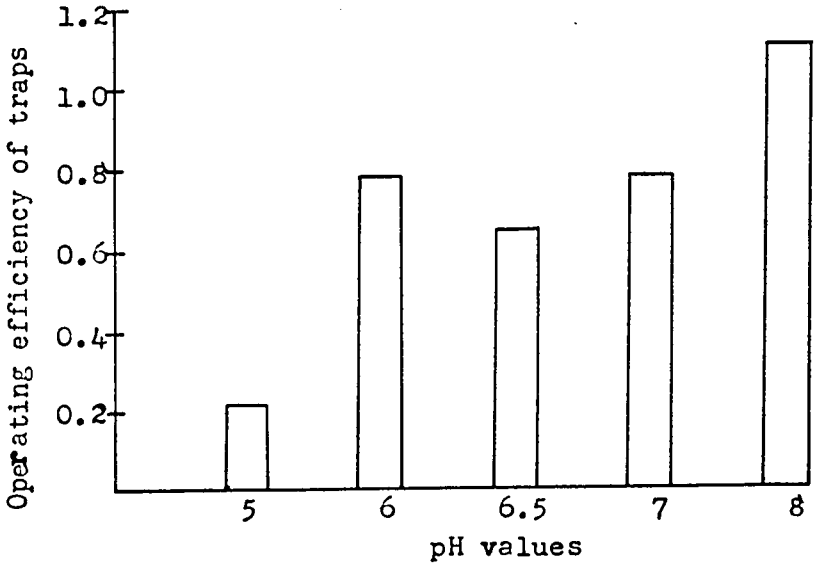


Figure 40. Operating efficiency of A. oligospora traps produced by the fungus at 5 pH values on weak Corn meal agar, 48 hours after adding nematodes.



number of traps and in the percentage of nematodes captured within the pH range 6 to 8 but not at pH 5.

Two drops of 0.15M Na_2HPO_4, the basic salt of the buffer system used, were placed at each of five marked locations on one half of the medium having a pH value of 5 in each of the three Petri dish cultures which received nematodes. Within 10 minutes, nematodes adhered to the traps in those areas to which Na_2HPO_4 was added and trapping continued for a further 24 hours.

4. DISCUSSION

Both trap production and trapping efficiency were higher under neutral to alkaline than under acidic conditions. This may explain the finding of Gorlenko (1956) that better control of root-knot of cucumber by Trichothecium pravicovi and Arthrobotrys kirghizica was obtained by adding ammonium carbonate to the soil. It also agrees with Soprunov's (1950) report of the development of predatory properties by Didymozoophaga sp. in alkaline and neutral but not in acid media.

Duddington's (1955) observation that nematode-trapping fungi are less frequent in acid, peaty soils although their nematode hosts are plentiful, may be explained by their inability to trap nematodes under these conditions.

It is of interest to note that Mankau (1963) found,

from in vitro tests, that the addition of ammonium salts, giving a pH of 7.7 to 8, adversely affected larvae of plant parasitic nematodes but did not affect species which were free living. If this were true under soil conditions, then the addition of ammonium salts together with nematode-trapping fungi would result in more efficient control of plant parasitic nematodes; firstly, through their direct effect on these nematodes and, secondly, by making the soil alkaline and thus increasing the trapping efficiency of the fungi.

B. EFFECT OF TEMPERATURE

1. INTRODUCTION

Tolmsoff (1959) reported that Arthrobotrys superba did not form traps at 10°C and that trapping was greatly retarded at 15°C and 30°C. Tarjan (1961) found that Dactylella drechsleri did not form adhesive knobs on Corn meal agar in the absence of nematodes at temperatures of 70°F and above. He called attention to the importance of cooler temperatures for the formation of traps in pure culture.

Feder (1963) tested at four temperatures, the nematode-trapping efficiencies of five Dactylella species which capture nematodes by three types of mechanisms, constricting rings, adhesive knobs and adhesive networks. He found that in all five species, nematode-trapping efficiencies

were highest at 15°C and decreased markedly as the temperature rose.

These reports are, to a certain extent, conflicting; therefore it was decided to study the effect of temperature on both trap production and trapping efficiency. Feder's (1963) studies did not take into account the number of traps produced at the different temperatures, probably because the species he worked with produce their traps spontaneously in the absence of nematodes.

2. MATERIALS AND METHODS

The fungus used was Arthrobotrys oligospora and it was grown on 15ml amounts of unbuffered weak Corn meal agar, at an initial pH value of 5.6, contained in 90ml Petri plates.

In an earlier experiment, 3 plates of the medium were inoculated with 2mm squares of a young culture of the fungus and incubated for 7 days at each of 6 temperatures, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Rhabditis oxycerca nematodes were added to the plate-cultures at the end of this period. Ninety six hours later, no traps were formed at 30°C and 35°C. The experiment was therefore repeated employing temperature levels 10°C, 15°C, 17°C, 20°C, 22°C, 25°C and 6 replications.

At the end of 7 days, the colony diameters were recorded and approximately 150 nematodes were added to each

plate-culture. The nematodes were added in three equidistant locations just outside the growing edges of the colonies. Nematode-free control cultures at each of the desired temperature values were also employed.

Forty eight hours after adding the nematodes, the numbers of traps produced per square centimeter and the percentages of nematodes captured were recorded. Efficiency of trap operation was assessed by taking the ratio between the per cent of nematodes captured and the number of traps produced per square centimeter. The trapping efficiency of the colony as a whole was determined by taking the ratio between the per cent of nematodes captured and the area of the colony.

Eighteen cultures were incubated at room temperature (22°C) for a week and approximately 150 nematodes added to each culture at the end of this period. Three of these cultures were then incubated at each of the 6 test temperature values.

3. RESULTS

No traps were formed on any of the nematode-free control cultures. The average area of the fungal colonies and the average trap production per square centimeter at each of the 6 temperature levels are presented in Figures 41 and 42 respectively. Trap production per square centimeter was

highest at 17°C and lowest at 10°C. At the other temperatures used, trap production per square centimeter approximated that at 17°C.

The trapping efficiency of the colony (Fig.45) progressively decreased with rise in temperature from 10°C to 25°C while the efficiency of operation of traps (Fig.44) was approximately the same at all 6 test temperatures.

At 10°C the traps were small, of simple construction and seldom compounded (Fig.46). The vast majority were of the single ring type but they appeared to be very efficient - a single small ring lying flat on the substrate could hold a large nematode.

The fungus grown at room temperature and then transferred to the 6 test temperatures after adding nematodes, produced traps at all temperature values between 10°C and 30°C but not at 35°C. However, those traps produced at 30°C were atypical in that they were largely flat and 2-dimensional (lacking the aerial phase) and they proliferated to give large networks with the majority of the individual components not completing their rings but growing out haphazardly (Fig.47).

4. DISCUSSION

The results of this study are in general accord with those of Feder (1963) on Dactylella species. It is therefore possible that trapping efficiency decreases with

Figure 41. Area in square centimeters of 7-day old colonies of A. oligospora at 6 temperatures on unbuffered weak Corn meal agar at pH 5.6, 48 hours after adding nematodes.

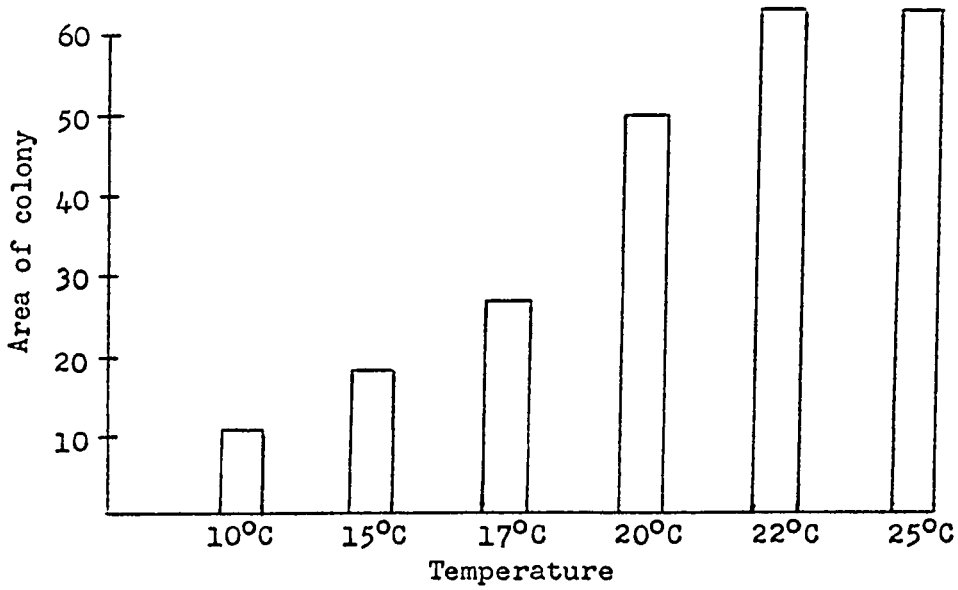


Figure 42. Average trap production per square centimeter by 7-day old cultures of A. oligospora at 6 temperatures on weak Corn meal agar, 48 hours after adding nematodes.

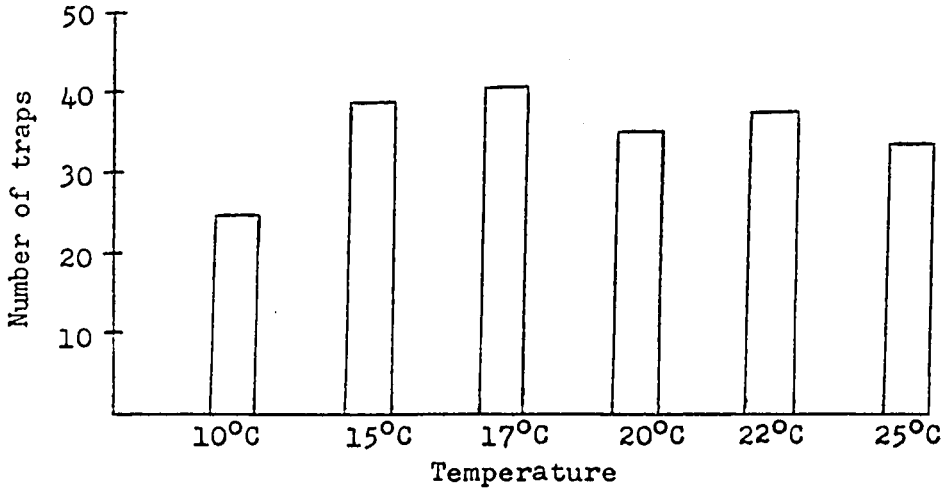


Figure 43. Percentage of nematodes captured by 7-day old cultures of A. oligospora at 6 temperatures on weak Corn meal agar, 48 hours after adding nematodes.

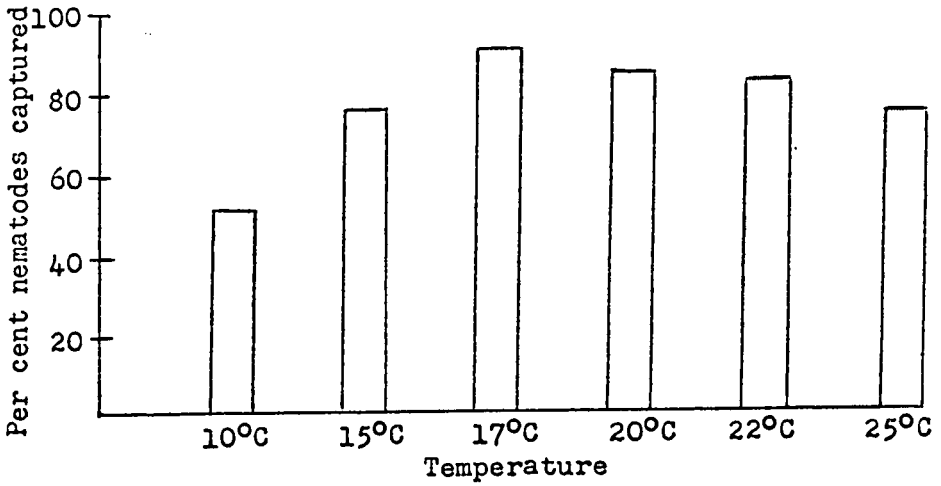


Figure 44. Operating efficiency of A. oligospora traps produced by the fungus at 6 temperatures on weak Corn meal agar, 48 hours after adding nematodes.

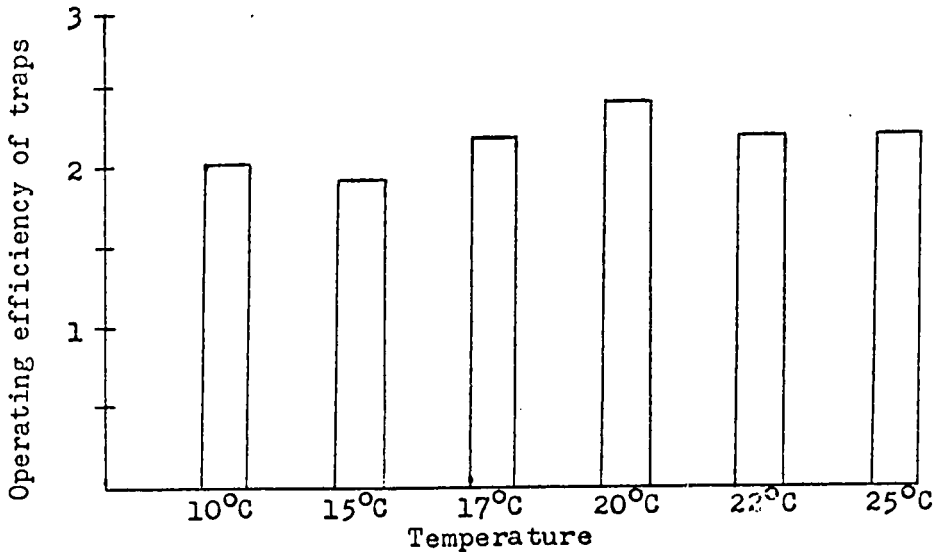


Figure 45. Trapping efficiency of 7-day old A. oligospora cultures at 6 temperatures on weak Corn meal agar, 48 hours after adding nematodes.

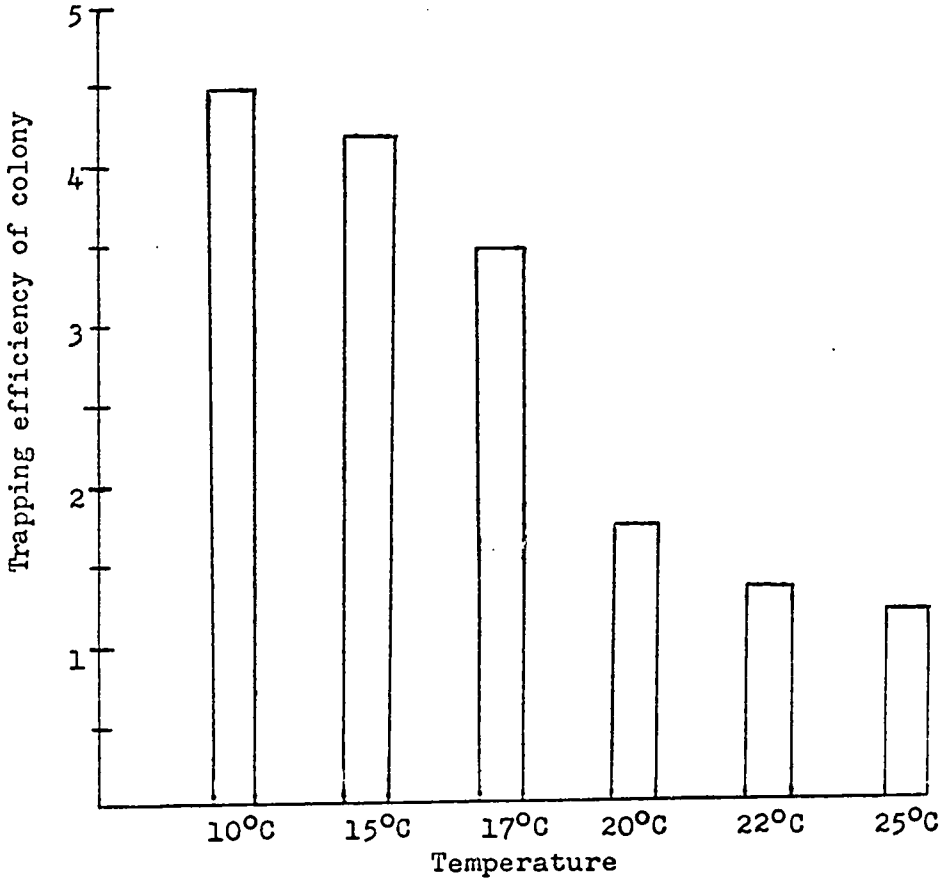


Figure 46. Small, simply constructed traps, lying flat on the substrate, produced by Arthrobotrys oligospora on unbuffered weak Corn meal agar at 10°C, 48 hours after the addition of nematodes.

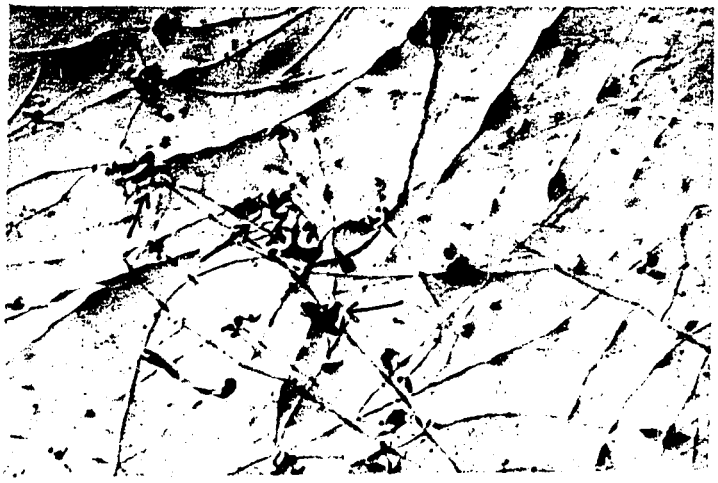


Figure 47. Haphazard construction of Arthrobotrys oligospora traps produced by the fungus when it was grown at room temperature (about 22°C), nematodes added and the cultures transferred to 30°C.

rise in temperature for most, if not all, nematode-trapping hyphomycetes. It is, however, difficult to reconcile these findings with those of Tolmsoff (1959) who found that Arthrobotrys superba did not form traps at 10°C and that trapping activity was greatly retarded at 15°C. Tolmsoff stated that he used Milk agar and Potato dextrose agar to isolate A. superba from soil but did not mention what medium he used for his temperature studies.

Feder (1963) appeared to favour the theory that the lowered motility of the nematodes at low temperatures resulted in fewer escapes from traps to which they adhered, but he did not rule out the possibility that low temperatures lowered the "threshold of reactivity" of the fungus. The results of this study indicate that the lowered motility of nematodes was not responsible for the high trapping efficiencies at low temperatures. Fewer traps were produced at 10°C than at the other temperatures but they were more efficient; thus, increased adhesiveness of the traps or increased production of the adhesive substance was responsible for the high trapping efficiencies at low temperatures.

C. EFFECTS OF DIFFERENT MEDIA AND LIGHT CONDITIONS

1. INTRODUCTION

Much of the work on the effects of culture media

and light conditions on the nematode-trapping fungi has been carried out primarily to determine the conditions best suited for growth and sporulation of the fungi with a view to their production in bulk for biological control of plant parasitic nematodes. Olthof (1962) studied the effects of 12 different media and 3 light conditions on the growth of 4 species of nematode-trapping fungi. He also summarized the previous work done on the subject.

Little work has been done on the effects of culture media and light conditions on trapping activity of the nematode-trapping fungi. Tarjan (1960) tested the trapping activities of five species of nematode-trapping hyphomycetes by adding them to Erlenmeyer flasks containing different types of organic matter and Panagrellus redivivus nematodes. He concluded that Arthrobotrys musiformis was the most active of the five, and unground oat hulls the best organic substrate. Couch (1937) concluded, from experiments on the factors responsible for inducing formation of constricting rings in Dactylella bembicoides, that ring production greatly increased as food supply in the medium was reduced. He also observed that no rings were produced on Potato dextrose agar. Tolmsoff (1959) stated that he was able to consistently recover Arthrobotrys superba from soil when he used Milk agar but he recovered the fungus from the same soil in only one of several thousand Petri plates of Potato dextrose agar.

2. MATERIALS AND METHODS

The 14 media employed in studies on the variability of Arthrobotrys oligospora (page 45) were used. Twelve Petri plates, each containing 15ml of the medium, were inoculated with 2mm squares of a young culture of A. oligospora from weak Corn meal agar. Six cultures on each medium were kept in a dark cupboard and 6 in the light supplied by the normal fluorescent lighting of a laboratory. Incubation was at room temperature which was about 22°C.

After 1 week, the squares of inocula were removed and approximately 150 Rhabditis oxycerca nematodes were added to each of 3 cultures on each medium kept in the light and to 3 of those kept in the dark. The remaining 3 cultures on each medium in the light and in the dark were the nematode-free controls.

The number of traps produced and the percentage of nematodes captured were recorded 24 hours after adding the nematodes.

3. RESULTS

The results, as presented in Table 2, show that traps were formed within 24 hours on Lima bean agar, Rice extract agar, Corn meal agar, Czapek Dox agar, Mycological agar and Oat meal agar but not on Potato dextrose agar, Oxgall

Table 2.

Trapping activities of 7-day old cultures of Arthrobotrys oligospora under 2 lighting conditions on 14 different media, assessed 24 hours after adding nematodes.

Medium	Light			Dark		
	Av.No. of traps		Per cent nematodes captured	Av.No. of traps		Per cent nematodes captured
	Exp.	Control		Exp.	Control	
Lima bean agar	284	0	52.0	35	0	7.8
Rice extract agar	180	7	27.0	0	0	0.0
Corn meal agar	789	0	75.5	283	0	29.9
Czapek Dox agar	558	0	26.2	1620	0	14.8
Oxgall agar	0	0	0.0	0	0	0.0
Wort agar	0	0	0.0	0	0	0.0
Mycological agar	-*	-	-*	-*	-	-*
Oat meal agar	-*	-	-*	-*	-	-*
Potato dextrose agar	0	0	0.0	0	0	0.0
Malt extract agar	0	0	0.0	0	0	0.0
Chlamydospore agar	0	0	0.0	0	0	0.0
Prune agar	0	0	0.0	0	0	0.0
Mycophil agar	0	0	0.0	0	0	0.0
Czapek Dox yeast extract	0	0	0.0	0	0	0.0

*- Traps were formed but counting them and finding the percentage of nematodes captured were difficult because sporulation was very dense.

agar, Wort agar, Malt extract agar, Mycophil agar, Chlamydospore agar and Czapek Dox agar amended with yeast extract even 96 hours after nematodes were added. On Prune agar, no traps were formed either in the light or in the dark in 48 hours but traps were seen 72 hours after adding nematodes in those plates which were in the light and at 96 hours in those that were in the dark. Eventually, more traps were formed on the Prune agar than on Rice extract agar or on Lima bean agar.

With the exception of Czapek Dox agar, more traps were formed and the percentage of nematodes trapped was higher on the cultures incubated in the light than in the dark.

No traps were formed by the fungus cultured on Oxgall agar or on Wort agar but the nematodes died on these media. These two media probably contain substances toxic to nematodes.

On Rice extract agar, traps were formed spontaneously by the control cultures in the light; the average for 3 replications was 7 traps per plate-culture. Further, on Rice extract agar in the light, there was a marked increase in the number of traps within an hour of adding nematodes and in 5 hours the average number was 115; however, after 24 hours the average number had risen to only 180. In the dark, no traps had been formed 48 hours after adding nematodes, but traps were seen when the plate-cultures were next observed, that is 72 hours after nematodes were added.

The highest percentage of nematodes captured occured on Corn meal agar. From this it appears that Corn meal agar is the best of the tested media for studies on trapping efficiencies.

4. DISCUSSION

A consistent finding, except in the case of Czapek Dox agar, was that more traps were produced and the percentage trapping was higher in the light than in the dark. This is an unexpected result as the fungus normally lives and operates in the dark.

The other results are complex and it is difficult to interpret them. Since little is known of the nutritional requirements of A. oligospora, the results obtained cannot be explained on a nutritional basis. However, it appears that a low nutritional status is conducive to trap formation, and Corn meal agar is probably the best of the commonly prepared culture media for studies on the efficiency of trapping by A. oligospora.

D. EFFECT OF NEMATODES OF DIFFERENT GENERA

1. INTRODUCTION

According to Duddington (1955), the predacious

hyphomycetes are polyphagous and show little host specificity, being able to capture a wide range of nematode species. He expressed the opinion that size is the factor which determines whether a nematode is going to be captured or not: a robust fungus captures large nematodes while a delicate fungus can only capture small nematodes. Duddington (1955) also considered the possibility that the degree of striation of the nematode cuticle may play a part in determining whether a nematode is going to be trapped or not.

Commandon and deFonbrune (1938), when stating that a sterile culture filtrate in which nematodes had lived would stimulate trap formation in the nematophagous fungi, omitted to mention the numbers or the species of nematodes they used. Pramer and Stoll (1959) reared Neoaplectana glasseri nematodes in meat infusion broth and found that the sterile culture filtrate induced the formation of traps in Arthrobotrys conoides. When 110,000 nematodes were present in 60-day old cultures, aliquots of the culture filtrates showed maximum activity when diluted 1/5 and slight activity when diluted 1/100; weaker activity was shown by filtrates from cultures containing fewer nematodes. Duddington (1962a) was unable to induce trap formation in A. oligospora and A. robusta with sterile filtrates from cultures of Ditylenchus dipsaci containing up to 256 nematodes per millilitre. Feder et al. (1960) demonstrated that a single dried, freshly killed Panagrellus redivivus nematode induced trap formation in

Dactylella doedycoides. Feder et al. (1963) reported that the sterile filtrate, from washed P. redivivus nematodes that had been boiled for 1 minute, was able to stimulate trap formation in three of four species of Dactylella tested at concentrations varying from one part in ten to one per million.

From this it would appear that, apart from different nematode-trapping fungi having different thresholds for trap induction, the trap inducing substance or substances from P. redivivus are more active than those from N. glasseri and D. dipsaci. Up to the present time, no systematic effort has been made to study the response of a particular nematophagous fungus to different genera or species of nematodes.

2. MATERIALS AND METHODS

Five species of nematodes belonging to 5 different genera were used in this study. They were Rhabditis oxycerca, Meloidogyne hapla, Aphelenchoides sp., Aphelenchus sp. and Mononchus sp. The effects of both adults and juveniles of all species were tested, except for those of M. hapla, the adult females of which are usually immobile.

R. oxycerca nematodes were reared on soil-peanut butter plates (Thomas, 1963); M. hapla juveniles were obtained from recently hatched eggs; Aphelenchoides sp. were reared on a fungus of the genus Alternaria; Aphelenchus sp. on the fungus Pyrenochaeta terrestris; and Mononchus sp.

on R. oxycerca nematodes in plates of Corn meal agar.

The nematodes were washed in 10 changes of sterile water and the different nematode suspensions were standardized so each drop contained approximately 50 nematodes.

Twenty four 50mm Petri plates, each containing 3ml of Water agar, were inoculated with 2mm squares of a culture of Arthrobotrys oligospora from weak Corn meal agar and incubated at 22°C. Water agar was used to reduce the bacterial contamination, and the amount of 3ml was chosen in order to keep the medium so thin that nematodes, notably Aphelenchus sp., could not escape by burrowing into the medium and lying against the bottoms of the culture plates.

When the plate-cultures were 3 days old, approximately 100 nematodes of each species were added to each of 4 cultures. The number of traps formed and the per cent of nematodes captured were recorded 48 hours after adding the nematodes.

3. RESULTS

The most noteworthy observation from the data of Table 3 is the apparent inability of Arthrobotrys oligospora, under the conditions of the experiment, to capture adult mononchs and Aphelenchus sp. as a result of the absence or paucity of trap formation. Four days after the addition of the nematodes, those plate-cultures containing Mononchus adults still produced no traps while those with Aphelenchus sp.

Table 3.

Average number of traps produced and average per cent capture of nematodes of 5 genera by 3-day old cultures of Arthrobotrys oligospora on Water agar at 22°C, 48 hours after adding the nematodes.

Nematodes	Av.No. traps per 50mm plate	Av. per cent nematodes captured
Mononchus sp. (adults only)	0.0	0.0
" (juveniles only)	15.0	7.1
Meloidogyne hapla (juveniles only)	38.0	37.2
Aphelenchus sp.*	2.5	0.0
Rhabditis oxycerca*	764.0	82.1
Aphelenchoides sp.*	1400.0	100.0

*- mixed population of adults and juveniles.

showed a very slight increase in number of traps over that recorded 48 hours after adding the nematodes; the average from 4 cultures was 7 per culture, but nematodes were not captured. Juvenile mononchs induced the formation of a few traps and the percentage captured was low.

The greatest number of traps (an average of 1,400 per plate) and the highest percentage capture (100 per cent) were recorded in plate-cultures to which Aphelenchoides sp. were added. Seventy two hours after the addition of these nematodes, all had been captured and even their integuments had disappeared. The traps induced by Aphelenchoides sp. appeared to be more delicate and less compounded than those induced by R. oxycerca (Figs. 48 and 49).

The traps induced by M. hapla were confined to the two spots where the nematode inoculum fell and the number of juvenile nematodes captured was greater than the number of traps. This latter result may be explained by the fact that the traps were very much compounded and as many as 6 nematodes were captured by one trap (Fig.50). Those worms that escaped from the drops of inoculum, wandered all over the fungal culture but did not induce trap formation in locations other than where the drops fell.

4. DISCUSSION

The results of this study demonstrate the existence

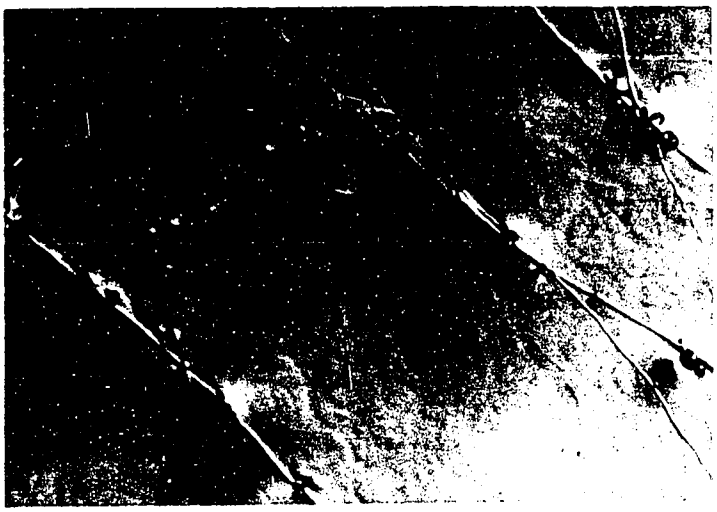


Figure 43. Arthrobotrys oligospora traps induced by Aphelenchoides nematodes.

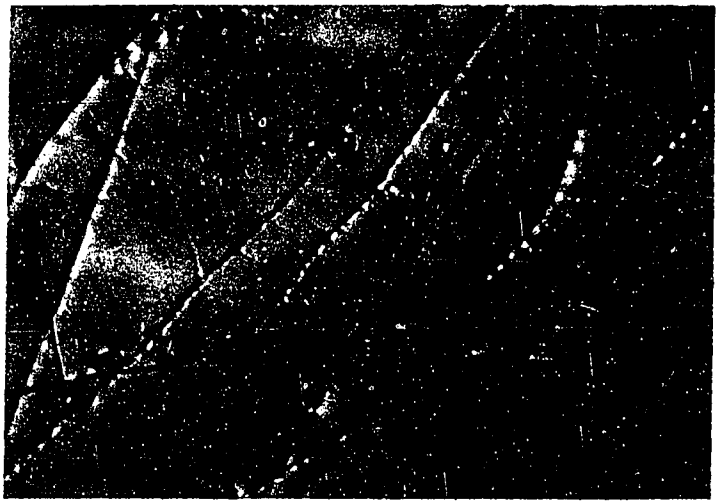


Figure 44. Arthrobotrys oligospora traps induced by Tylolaimus cylindricaudatus nematodes.

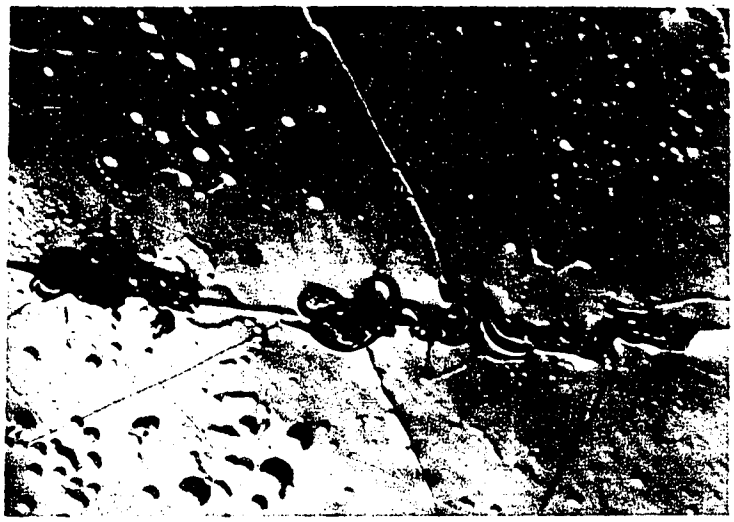


Figure 50. Several Heloidogyne haola juvenile nematodes captured per trap of Arthrobotrys oligospora.

of differential trapping by A. oligospora. Not only do different nematodes stimulate trap production by the fungus in different degrees but they are also trapped to different extents.

Nematode species of the genus Mononchus, as possible agents for controlling plant parasitic nematodes, have attracted more attention than any other soil-inhabiting nematode predators but no experimental work in this direction has been performed (Christie, 1960). The possibility of obtaining control of plant parasitic nematodes through the combined action of Mononchus sp. and nematode-trapping fungi assumes importance as the mononchs are not trapped by the fungi and so there might be synergistic action of the predacious nematode and the predacious fungi to overcome the nematode parasites.

III. DETERMINATION OF THE TRAP INDUCING CAPABILITIES OF NEMATODE AND SERUM PROTEINS

A. INTRODUCTION

Roubaud and Deschiens (1939) carried out simple quantitative tests on the trap inducing capabilities of a number of substances of animal origin. They observed that human serum and human dung induced many traps while rabbit serum and rabbit dung showed weak trap inducing activity. According to Duddington (1962a), this suggests that the trap inducing principle may be a specific protein. Commandon and deFonbrune (1938) stated that the active principle, in water in which nematodes were suspended, was destroyed by boiling. Deschiens and Lamy (1942) reported that the trap inducing substance in guinea pig serum was not destroyed by heating or by treatment with alcohol. It appears, therefore, that the active principles from nematodes and guinea pig serum are different.

Pramer and Stoll (1959), using one extraction procedure, obtained the active principles from human serum and from a sterile nematode-free culture filtrate in which nematodes had been reared; they called the latter "Nemin". Both Nemin and the active principle from human serum were not destroyed by acetone or by a temperature of 100°C for 10 minutes. They stated that this was an indication of the two

substances being similar if not identical.

According to Feder et al. (1963), nemin-like material induces trap formation after drying, boiling, freezing and alcohol treatment just like the semi-specific organizers that induce the formation of organ rudiments in vertebrate embryos. The embryological work of Willier et al. (1955) suggests that steroid compounds and fatty acids are most active. The work of Pramer and Stoll (1959) indicates that proteins are not responsible for trap induction as the proteins, in their sterile nematode-free culture filtrate in which nematodes had been reared, would have been denatured during the drastic treatment the filtrate was subjected to during the process of extracting nemin. However, Kuyama and Pramer (1962) purified and described the properties of a protein which induced trap formation in nematophagous fungi.

B. MATERIALS AND METHODS

The test fungus was Arthrobotrys oligospora and it was grown on weak Corn meal agar.

A fresh sample of hen's serum and a fresh extract of Rhabditis oxycerca nematodes were tested for trap inducing ability by placing drops of the extracts at the periphery of 3-day old cultures of the fungus. Polyacrylamide gel electrophoretic separations of proteins in the two samples were carried out. The procedure, the buffers and the conditions

of the separations were the same as those described on pages 82, 83, 86, 87.

The nematode extract was the supernatant obtained by grinding, in Tris-citrate buffer (0.076M Tris, 0.5mM ethylenediamine tetraacetic acid and 0.005M citric acid) at pH 8.6 and centrifuging at 41,000g for 30 minutes, a freeze dried sample of nematodes that had been reared on soil-peanut butter plates (Thomas, 1963), passed through a Baermann funnel, washed in 10 changes of sterile water and concentrated by settling and decanting.

Eight electrophoretic strips, containing proteins from each sample, were present in a gel. One of the 8 strips was cut out of the gel and the protein bands in it were located by means of staining and destaining procedures. The positions of the protein bands in the remaining 7 strips were thus determined. The proteins from the unstained strips were cut out of the gel and placed at the periphery of 3-day old cultures of A. oligospora on weak Corn meal agar. Pieces of unstained gel containing no proteins (controls) were placed at the periphery of young cultures of the fungus and nematodes were added. The cultures were incubated at 23°C and examined every day for the occurence of traps.

C. RESULTS AND DISCUSSION

Ten protein bands were located in hen's serum (Fig.51) and 2 in the nematode extract (Fig.52). None of

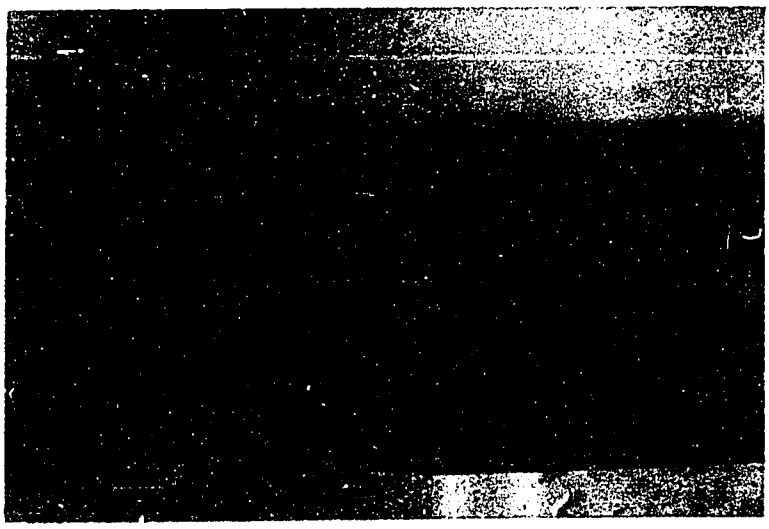


Figure 51. Polyacrylamide gel electrophoretic separation of the soluble proteins in hen's serum.

Figure 52. Polyacrylamide gel electrophoretic separation of the soluble proteins in an extract of Rhabditis oxycerca nematodes.

the proteins induced the formation of traps in A. oligospora although both hen's serum and the nematode extract did so. In the control cultures, traps were formed adjacent to the pieces of unstained gel containing no protein. This indicated that no trap-forming inhibitor was present in the gel.

It appears from this study that the trap inducing principle in hen's serum and in nematode extract is not a protein. However, the possibility exists that two or more proteins working together may induce trap formation.

IV. EFFECT OF THE GENETIC CONSTITUTION OF NEMATOPHAGOUS FUNGI ON THEIR CAPACITY TO FORM TRAPS

A. INTRODUCTION

The work of Feder et al.(1960) suggests that Dactylella doedycoides is heterocaryotic with regards to its capacity to form constricting rings. Because conclusive proof of this was lacking, an investigation was made to determine if a fungus has to be heterocaryotic in order to produce traps.

B. MATERIALS AND METHODS

a) Fungus. Arthrobotrys dactyloides strain 124D, which was selected from the wild type of A. dactyloides (page 63), was used. It produces microconidia and it was considered possible that the microconidia had uninucleated cells, in which case the mycelium arising from one of the cells of a microconidium would be homocaryotic.

b) Staining. Microconidia, produced when the fungus was growing on weak Corn meal agar, were stained by the AzureA - SO_2 method of Huebschman (1952).

c) Germ tube isolations. Germ tubes on 1-day old cellophane cultured microconidia were located with the x50 magnification of a stereoscopic microscope. After verifying, with a

binocular microscope at x200 magnification, that a germ tube was discrete, it was cut with a razor-blade chip knife (Whitney and Parmeter, 1963) and transferred on a piece of the underlying cellophane to a Petri plate of weak Corn meal agar. Sixteen such single germ tube isolations were made. The plate cultures were incubated at 22°C and examined at intervals for the production of rings.

C. RESULTS

About 95 per cent of the stained microconidia showed only 1 nucleus per cell of the conidium (Fig.53). A little over 4 per cent had 2 nuclei in each cell and the remainder showed 3 nuclei in one cell and 2 in the other. More than 3 nuclei per cell were not seen. In those microconidial cells which had 2 nuclei, the proximity of the nuclei to one another suggested that the two had arisen from the division of a single nucleus. (Fig.54).

The sixteen microconidial germ tubes gave rise to mycelium which, in every case, produced rings spontaneously in the absence of nematodes.

D. DISCUSSION

By making several single spore isolations, Feder et al., (1960) were able to distinguish between two types of

Figure 53. Microconidia of Arthrobotrys dactyloides strain 124D showing one nucleus per cell of a conidium. The cytoplasm and nucleus have come out of one cell.

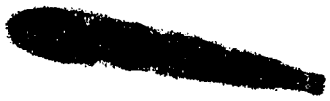


Figure 54. A microconidium of Arthrobotrys dactyloides strain 124D showing two nuclei in each cell of the conidium.

spores in Dactylella doedycoides: type A spores whose mycelium produced rings spontaneously in the absence of nematodes, and type B spores whose mycelium did not do so. Eighty three per cent of spores which arose from type A mycelium retained the type A character on germination in the absence of nematodes, and an additional 4 per cent did so when nematodes were added. Of the spores that arose from type B mycelium, 13 per cent produced rings (type A character) on germination in the absence of nematodes and 30 per cent did so in the presence of nematodes. An explanation that fits these results is that there are at least two types of nuclei, one of which determines the production of rings. In a multinucleate cell of a spore, the absence of this type of nucleus would mean that the resulting mycelium and its spores on germination would not form rings either spontaneously or in the presence of nematodes. If the nucleus which determined ring production were present to the exclusion of the other type of nucleus, then all the spores arising from mycelium of this type of cell would, on germination, form rings whether nematodes were present or not. If both nuclear types were present, the capacity to form rings spontaneously and in the presence of nematodes would be determined by the ratios between them.

The individual cells of microconidia of Arthrobotrys dactyloides strain 124D are predominantly uninucleate. When 2 or 3 nuclei were present in a single cell of a microconidium,

it is believed that they arose from a single nucleus. Consequently, the mycelium arising from a germ tube developing from such a cell is considered to be homocaryotic. The spontaneous production of rings by this mycelium indicates that the fungus does not have to be heterocaryotic in order to produce rings. However, the possibility cannot be ruled out that, in the selection of A. dactyloides strain 124D from the wild type, the nuclear type that determines ring production was selected from a mixture of nuclear types.

V. THE DESTINY OF TRAP CELLS

A. INTRODUCTION

The constricting ring has attracted more attention than any other type of trapping organ (Duddington, 1962a). This study was undertaken to determine:

i) whether a detached ring-initial would complete the formation of a ring or whether it would grow as an ordinary mycelial hypha

ii) if the individual cells of inflated and non-inflated detached rings could be induced to grow into mycelial hyphae

iii) whether tropisms were responsible for the completion of initiated rings.

B. MATERIALS AND METHODS

Arthrobotrys dactyloides was the fungus used. Conidia of the fungus were grown at 22°C on peeled cellophane squares lying on the surface of weak Corn meal agar in 90mm Petri plates.

Rhabditis oxycerca nematodes were washed from the lids of soil-peanut butter plates (Thomas, 1963) and the nematode wash passed through a Seitz filter. A drop of the sterile filtrate was put on each cellophane culture of the fungus to induce the formation of constricting rings. With

a razor-blade chip knife (Whitney and Parmeter, 1963), 2-celled incomplete rings and inflated and non-inflated 3-celled rings were detached from their parent hyphae and transferred, on pieces of the underlying cellophane, to weak Corn meal agar. One or two cells of some inflated and non-inflated rings were punctured, the rings detached from their parent hyphae, and the unpunctured cell or cells cultured on weak Corn meal agar. All detached rings were incubated at 22°C.

The locations of several incomplete rings on cellophane cultures were marked, by piercing the cellophane in their vicinity, and the cultures were subjected to the influence of the magnetic fields of simple bar magnets or placed upside down and at various angles to determine if magnetotropism or geotropism were responsible for the incomplete rings completing their ring structure.

C. RESULTS AND DISCUSSION

If an incomplete ring was detached at the 2-cell stage of its development and laid on its side, when the terminal cell was curved towards the stalk of the ring, the terminal cell invariably grew towards the stalk to complete the ring; however, if the terminal cell had not begun to curve towards the stalk when the incomplete ring was detached, it invariably grew as an ordinary mycelial hypha (Fig.55). It was observed on two occasions, when an incomplete detached

ring was placed on its end with the terminal cell in the air, that the ring was completed although the terminal cell had not begun to curve towards the stalk of the ring.

The individual cells of detached inflated and non-inflated rings did not grow but when one of the ring cells was punctured, one of the remaining two cells grew to become an ordinary mycelial hypha.

Neither magnetotropism nor geotropism were found to be responsible for initiated rings completing their ring structure.

Plate 39. The terminal cell of a detached incomplete ring of Arthrobotrys dactyloides growing as a mycelial type. The basal cell of the ring stalk is also grown as a mycelial type.

VI. A COMPARISON OF THE POTENTIAL FOR SPONTANEOUS TRAP PRODUCTION BY DIFFERENT CELLS OF A MULTICELLULAR NEMATOPHAGOUS FUNGAL SPORE

A. INTRODUCTION

This investigation was conducted because of a suggestion by Feder (1962) that he had not noticed ring formation in any of his cultures that arose from the middle cell of the 3-celled spores of Dactylella doedycoides.

B. MATERIALS AND METHODS

Dactylella doedycoides was grown at 22°C on Petri plates of weak Corn meal agar. The spores were washed with sterile tap water, placed on peeled cellophane squares lying on the surface of weak Corn meal agar in 90mm Petri plates, and incubated at 22°C. Thirty six hours later, 50 germ tubes arising from the proximal cells and 50 from the distal cells of the spores were cut off and subcultured on Petri plates of weak Corn meal agar. The plate-cultures were incubated at 22°C and the number of constricting rings formed was recorded 72 hours after the germ tubes were subcultured.

C. RESULTS AND DISCUSSION

No germ tubes arose from the swollen middle cells of more than 1,000 spores that were observed. Invariably, 2 and as many as 5 germ tubes arose from each proximal and distal cell of a spore. As few as 2 and as many as 47 rings were formed in the plate-cultures 72 hours after the germ tubes were subcultured. There was no significant difference between the capacities for ring production in the colonies that arose from the proximal and the distal cells of a spore.

SECTION III. OBSERVATIONS ON THE CONTROL OF PLANT PARASITIC NEMATODES BY NEMATOPHAGOUS FUNGI AND PREDACIOUS NEMATODES

A. INTRODUCTION

Following the experiments of Linford and his associates (1937, 1938, 1939) in Hawaii, several attempts have been made to overcome the effects of nematode attack on certain crops by inoculating nematode-infested soil with predacious fungi or by stimulating indigenous predacious fungi in soil to greater trapping activity. Most of these attempts have been directed against root-knot and cyst-forming nematodes (Linford et al., 1938; Linford and Yap, 1939; Deschiens et al., 1943; Hutchinson and Mai, 1954; Dixon, 1954; Shepherd, 1955; Duddington et al., 1956; Gorlenko, 1956; Duddington and Duthoit, 1960; Duddington et al., 1961; Hams and Wilkin, 1961; Mankau, 1961b, 1961c; Olthof, 1962; Duthoit and Godfrey, 1963). Tarjan (1962) attempted the biological control of citrus burrowing nematodes (Radopholus similis).

The majority of these attempts have been unsuccessful, probably because they have all been directed against endo-parasitic nematodes which are within their host tissues and therefore unavailable for trapping by the nematophagous fungi except for the relatively short period of their life outside the host. Thus, the fungi have not been given an adequate

opportunity to prove their worth in controlling nematodes.

Predacious nematodes, especially Mononchus sp., have attracted attention as potential agents of control of plant parasitic nematodes but no experimental work on this aspect of control has been attempted (Christie, 1960). In view of this, and the conflicting reports on the efficacy of nematophagous fungi in controlling plant parasitic nematodes, an investigation of biological control was made.

B. MATERIALS AND METHODS

a) Soil types. Normal greenhouse potting soil was used, the pH of which was adjusted to 8.2 with ammonium carbonate. The soil was contained in 80 three-inch clay pots, half of which was sterilized by autoclaving at 121°C for 2 hours.

b) Host plants. Dent corn variety CO 106, an inbred variety obtained from the Agronomy Department of Macdonald College, was used. Eighty seedlings were selected, 40 in each soil type, and they were grouped into 5 lots according to their heights. Each lot of 16 seedlings was made up of 8 in autoclaved soil and 8 in non-autoclaved soil.

c) Plant parasitic nematode. An Aphelenchus species, which was isolated from the rhizosphere of corn plants growing in the Agronomy plots of Macdonald College, was used. The pathogenicity of Aphelenchus sp. has not been proven but Terry (1965) observed marked stunting and whitening of leaves

of dent corn variety CO 106 within 2 weeks of inoculating sterile sand containing the seedlings with approximately 2000 of the nematodes per 3-inch pot.

d) Predacious fungi. Arthrobotrys strain 24 (an adhesive network former) and Dactylella ellipsospora (an adhesive knob former) were used singly and in combination with one another.

Strain A. 24 was chosen because an earlier experiment had indicated that it was able to compete with the normal flora and fauna of the soil better than the other adhesive network formers available. Cooke (1963a) stated that adhesive knob and constricting ring formers were more efficient nematode predators than the adhesive network formers and for this reason D. ellipsospora was used. Further, Linford and Yap (1939) were successful in controlling root-knot of pineapple with D. ellipsospora.

e) Predacious nematode. Adult nematodes of the genus Mononchus were used, as studies on host specificity (page 122) disclosed that they neither stimulate trap production in the predacious fungi, nor are they trapped when traps are produced as a result of the presence of other nematodes. It was considered possible that a synergistic action of the predacious fungi and the predacious nematode would result in better control than just the fungi alone. The Mononchus sp. used in this experiment was originally obtained as a contaminant in the greenhouse where it had decimated a whole population of Hemicycliophora similis nematodes.

f) Treatments. The fungi were added to the pots of autoclaved and non-autoclaved soil containing 2-week old seedlings, alone and in combination with one another and with the predatory nematode, one week before the plant parasitic nematodes were added. The 8 treatments, designated by the symbols T_1 to T_8, are shown in Table 4; they shall, hereafter, be referred to by their symbols. T_1 was the uninoculated control and T_2 was the plant parasitic nematode alone. Both T_1 and T_2 pots received 10ml of an autoclaved fungal suspension to compensate for any manurial effect that may have occured in the other pots that received like amounts of live fungi. When both fungi were added together (T_5 to T_8), 5ml of each fungus were used. Approximately 2,500 surface sterilized plant parasitic nematodes were added to the pots which received treatments T_2 - T_8. Treatments T_6 - T_8, in addition, each received 20 adult predacious nematodes. All inoculations were performed with sterile wide-mouth pipettes at a depth of approximately 2cm in the soil in 3 locations around the seedlings.

g) Preparation of fungal and nematode inocula. Conidial and mycelial preparations of the fungi were obtained by growing the fungi for 1 week at room temperature on Oatmeal agar (prepared from Difco Bacto powder) and in shake cultures of 0.1 per cent yeast extract amended Czapek Dox broth respectively. As D. ellipsospora grows slower than Arthrobotrys strain 24, twenty plate-cultures and ten liquid shake cultures of the

former were used to obtain the inoculum against ten and five, respectively, of the latter. Conidia were flushed from the Oatmeal agar cultures and suspended in 250ml of sterile distilled water. The mycelium was collected from the shake cultures of each fungus and fragmented, in 250ml of sterile distilled water, for 30 seconds in a sterile Waring Blendor. The conidial and the mycelial suspensions of each fungus were combined.

The plant parasitic nematodes were reared for 2 weeks at room temperature on Petri plate-cultures of Pyrenochaeta terrestris. Mass extractions of nematodes were made by the following procedure: a 9" x 11" rectangle of stiff ¼" wire mesh was fitted with ¼" legs and placed in a 10" x 12" enamelled dish. Facial tissues were placed on the wire mesh and the contents of 25 plate-cultures of the nematode spread out on the facial tissues. Water was carefully poured into the dish from one side till its level was just above the wire mesh. Twenty four hours later, the water was collected in 100ml graduated cylinders and allowed to stand for 12 hours, by which time most of the nematodes had settled to the bottom. The top 70ml of water in each cylinder were decanted and the concentrated nematode suspensions combined. The nematodes were surface sterilized and rid of their accompanying debris by passing them through Baermann (1917) funnels containing a bactericidal-fungicidal non-nematocidal solution instead of water. The sterilizing

solution (Mountain and Patrick, 1959) had the following composition: ethoxyethyl mercury sulphate 4ppm, dihydrostreptomycin sulphate 1000ppm, and 30ppm of aureomycin in hydrochloric acid. After 24 hours in the sterilant, the nematodes were washed 5 times in sterile distilled water. The final nematode suspension was standardized so each millilitre contained approximately 500 nematodes.

The predacious nematodes were reared for 4 months on Rhabditis oxycerca nematodes contained in Petri plates of weak Corn meal agar. The nematodes were extracted through Baermann funnels using Mountain and Patrick's (1959) sterilant in place of water. The nematodes were left for 24 hours in the sterilant, and adult Mononchus sp. were picked by hand, with the use of a dental canal pulp file, and put into sterile distilled water.

h) Design of the experiment. The 80 pots of the experiment containing 2 soil types and 8 treatments in each of 5 replicate blocks were completely randomized (Fig.56). The pots were placed on Petri plates to avoid contact with the gravel of the greenhouse bed.

i) Greenhouse conditions. For the 2 month period of the experiment following inoculation of the soil with parasitic nematodes, the temperature of the greenhouse fluctuated between 72°F and 91°F. The pots were watered daily with 40ml of tap water except on very warm days when they were watered twice a day with 30ml amounts each time. Splashing

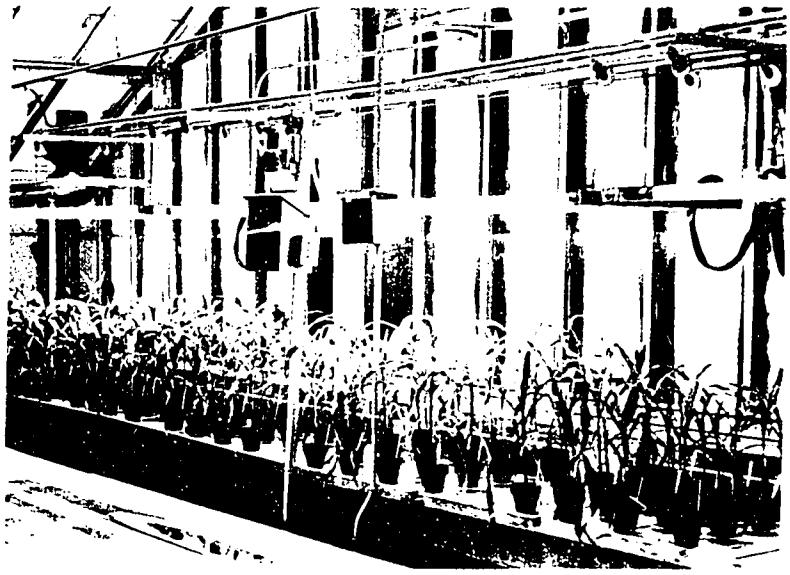


Figure 50. Greenhouse experiment on control of Aphelenchus nematodes on dent corn variety CO 105 by nematophagous fungi and a predacious nematode.

was avoided during the watering. At 14 day intervals, each pot received 10ml of a weak solution of a liquid fertilizer.

j) Data. At the end of the experiment, the soil around the roots of each plant was carefully washed off, the roots blotted between pads of paper towels, and the whole plants weighed to the nearest decigram. The roots of those plants which received the T_2 treatment were stained with lactophenol-acid fuchsin and cleared in unstained lactophenol (McBeth et al., 1941). They were examined with a stereoscopic microscope at x50 magnification for evidence of pathogenicity by Aphelenchus sp.

k) Recovery of the fungi. Soil from each treatment was plated on weak Corn meal agar to reisolate the two fungi used. Rhabditis oxycerca nematodes were added to the plates to encourage emergence of the fungi (Shepherd, 1955).

C. RESULTS

Average fresh plant weights of the 5 replicates in each of the 8 treatments and 2 soil types are presented in Table 5. The original data are presented in Table 4 and the analysis of variance in Table 6.

Application of Duncan's (1955) multiple range test (Table 5) showed that T_1, the control, was significantly ($P < .05$) greater than T_2 (plant parasitic nematodes only). However, T_1 was not significantly different from the other

Table 4.

Fresh weights of dent corn plants (variety CO 106) grown in 2 soil types with 8 treatments.

Soil types	Treatments*							
	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8
Autoclaved	28.0ø	16.1	18.7	18.2	24.7	21.9	24.2	23.4
	18.5	13.0	25.3	18.7	18.9	21.8	24.5	19.0
	20.0	18.6	24.8	20.5	16.5	26.8	21.0	18.6
	23.7	19.7	25.5	14.6	18.7	27.5	18.7	20.8
	26.9	9.1	9.1	15.1	17.5	12.2	23.3	21.5
Non-autoclaved	17.4	11.2	9.5	10.7	10.2	12.9	15.5	10.7
	9.5	9.7	14.2	16.5	14.9	11.5	15.0	11.4
	8.6	10.8	12.1	8.0	11.7	10.1	9.6	17.1
	14.4	7.5	5.8	11.3	12.4	8.6	10.4	13.6
	13.1	5.6	9.6	4.9	9.1	14.0	12.2	7.6

*- T_1 = Autoclaved fungi only (Control).

T_2 = *Aphelenchus* sp. + autoclaved fungi.

T_3 = " + *Arthrobotrys* strain 24.

T_4 = " + *D. ellipsospora*.

T_5 = " + " + *Arthrobotrys* strain 24.

T_6 = " + *Mononchus* sp. + *Arthrobotrys* strain 24.

T_7 = " + " + *D. ellipsospora*.

T_8 = " + " + " + *Arthrobotrys* strain 24.

ø- Averages from 5 replications.

Table 5.

Average fresh weights of dent corn plants (variety CO 106) grown in 2 soil types with 8 treatments.

Soil types	Treatments								Mean	
	T_1^*	T_2	T_3	T_4	T_5	T_6	T_7	T_8		
	23.4	15.3	20.7	17.4	19.3	22.0	22.3	20.7	20.1	$\bar{X}$
Autoclaved	1.9	1.9	3.2	1.1	1.4	2.7	1.1	0.9		SE
	18.0	28.2	34.3	14.6	16.6	27.7	11.2	9.7		CV%
	12.6	9.0	10.2	10.3	11.7	11.4	12.5	12.1	11.2	$\bar{X}$
Non-autoclaved	1.6	1.1	1.4	1.9	1.0	1.9	1.2	1.6		SE
	28.6	26.7	31.4	41.7	18.8	37.7	21.6	28.9		CV%
Soil types considered together	18.0	12.2	15.5	13.9	15.5	16.7	17.4	16.4		

T_1^*	T_7	T_6	T_8	T_5	T_3	T_4	T_2
18.0	17.4	16.7	16.4	15.5	15.5	13.9	12.2

*- Control.

Table 6

Analysis of variance of data presented in Table 4

Sources	df	M.S.	F	P .05	P .01
Sub-groups	15				
Treatments	7	37.38	2.65*	2.15	2.95
Soil types	1	1590.61	112.80**	3.99	7.04
Treatments x Soil types	7	7.57	0.54	2.15	2.95
Error	64	14.10			

Standard error of treatment means 1.7

treatments (T_3, T_4, T_5, T_6, T_7, T_8). As regards the soil types, fresh weights of the plants grown in autoclaved soil were significantly ($P < .01$) greater than those grown in non-autoclaved soil.

Examination of the stained roots did not show any of the parasitic nematodes. Arthrobotrys strain 24 was recovered from every pot to which it was added while Dactylella ellipsospora was recovered only from autoclaved soil and not from non-autoclaved soil.

D. DISCUSSION

From the results it is apparent that the nematodes of Aphelenchus sp. significantly retarded the growth of corn variety CO 106 although they were not recovered from the roots of the plants to which they were added. It is possible that the nematodes produce a toxin. Further study on this aspect of the Aphelenchus-corn relationship is indicated.

Addition of the fungi singly and in combination with one another gave an increase in plant weight over the treatment containing the plant parasitic nematode alone, which was significant at the 5 per cent level. Control of the parasitic nematode was not significantly improved by superimposing the predacious nematode treatment on the predacious fungal treatments.

It is not known why the corn plants grown in auto-

claved soil weighed more than those in non-autoclaved soil. Autoclaving may have released or otherwise made available to the plants in that soil more nutrients than were available to the plants in the non-autoclaved soil; or autoclaving may have eliminated some unknown parasitic or inhibitory organism from the soil.

GENERAL DISCUSSION AND CONCLUSIONS

The spores of Arthrobotrys oligospora, A. superba, A. robusta and A. dactyloides were shown to have multinucleate cells. New stable strains were selected from these species and it was presumed from this evidence that the fungi were heterocaryotic.

Circumstantial evidence from anastomosis experiments and from electrophoretic studies indicated that A. oligospora, A. superba and A. robusta were probably strains of a single species.

It is not known what relationship the selection of new strains in the laboratory bears with the frequency of their occurence in nature. On the basis of the system of classification that has been used, where minor morphological characters were used for delimiting species of nematophagous fungi, one would be justified in calling each of the new strains a new species and workers encountering them as isolates from the soil would be tempted to do just this. It is possible that several of the named species of nematophagous fungi are in reality strains of a fewer number of species.

If the limits of variation of morphological characters were determined, this would minimize the problems created by their use. A comparative study of adequate numbers of diverse populations of species from different geographic areas may be of help in this connection.

Trapping activity of A. oligospora was shown to be affected by the age of the mycelium, type of medium the fungus was grown on, light conditions, temperature, hydrogen ion concentration, and genus of nematode present. The young mycelium, near the periphery of the fungal colony, required less stimulation from nematodes to produce traps than did the older mycelium in the centre of the colony. In general, trapping efficiency increased with increasing hydrogen ion concentration or decreasing temperature over the range tested. More traps were formed by the fungus in the light than in the dark and it was shown that nematodes of different genera induced different degrees of trap production and were trapped to different extents.

The effects of hydrogen ion concentration and temperature on trapping efficiency are noteworthy, as these two environmental factors can be controlled in a greenhouse. A combination of slightly alkaline soil and low temperature may result in effective control of plant parasitic nematodes by nematophagous fungi under greenhouse conditions. Testing of the nematophagous fungi for trapping activity against ectoparasitic nematodes of several genera is necessary as a preparatory step to their future employment in the control of these nematodes. Differential trapping activity may also make a contribution to the numerical taxonomy of species.

Individual proteins, separated from hen's serum and nematode extract did not induce the formation of traps in A.

oligospora.

Homocaryons produced by A. dactyloides strain 124D produced traps spontaneously in the absence of nematodes indicating that the fungus does not have to be heterocaryotic in order to produce traps. However, the possibility exists that, in the process of selecting strain 124D from the wild type of A. dactyloides, only the nuclear type which determined trap production was selected from a mixture of nuclear types.

The individual cells of the constricting ring traps of A. dactyloides could be induced to grow as ordinary mycelial hyphae and no significant difference was found to exist between the potentials for trap production by mycelium arising from the terminal and proximal cells of 3-celled Dactylella doedycoides spores; the middle cells of the many spores examined were not observed to germinate.

An Aphelenchus sp. on dent corn variety CO 106 was controlled by D. ellipsospora (an adhesive knob former) and A. strain 24 (an adhesive network forming strain that arose from an anastomosis figure that occured between A. robusta and A. superba) when used singly and in combination with one another. This experiment indicated that the adhesive network formers are as efficient in controlling nematodes as the adhesive knob formers.

Predacious nematodes were used for the first time in an experiment on nematode control. When predacious nematodes were added to the fungal treatments, they did not improve control of Aphelenchus sp.

SUMMARY

1. The morphologies of Arthrobotrys oligospora, A. superba, A. robusta, A. dactyloides and Dactylella ellipsospora were studied under standard conditions, primarily so that they would serve as a basis of comparison with new strains developed from them.

2. The vegetative mycelia and the conidia of the wild types of A. oligospora, A. superba, A. robusta and A. dactyloides were stained to ascertain the nuclear condition of the cells as a preliminary to the study of heterocaryotic phenomena in these fungi. Conidial cells, terminal cells of conidiophores, hyphal tips and the vast majority of hyphal cells were found to be multinucleate.

3. The effect of environment on variability of A. oligospora was studied. The aim was to produce new stable strains of the fungus by altering the environment. The results indicated that the fungus, if heterocaryotic, had a nuclear ratio that was not easily altered.

4. Four new stable strains of A. robusta, 2 of A. superba, 1 of A. oligospora and 2 of A. dactyloides were selected from their respective wild types by successive isolation and subsequent culturing of individual intercalary cells. The selection of so many strains in a relatively short period of time provides circumstantial evidence for the existence of heterocaryosis in these fungi.

5. Anastomoses were observed to occur between the wild types of A. oligospora, A. superba and A. robusta. Anastomosis figures, from cellophane cultures of anastomosing fungi, were cut out and cultured. A new stable strain was obtained from an anastomosis figure that occured between hyphae of A. superba and A. robusta. Anastomosis figures that arose from the other matings, when cut out and cultured, initially gave rise to some enlarged 3-celled conidia mixed with normal conidia of A. oligospora. The 3-celled conidia, when subcultured, developed into colonies that were indistinguishable from those of wild type A. oligospora. The apparent ease with which anastomosis occured between the three species suggested that they were probably strains of a single species.

6. Electrophoretic separations of the soluble proteins from mycelium of the wild types of A. oligospora, A. superba and A. robusta and from new strains of these species were carried out. There were distinct differences between the protein patterns of the three species but the positions of between 25 and 43 per cent of their protein bands corresponded. Differences between protein patterns of the new strains and the wild types from which they were selected were of about the same magnitude as the differences between the species themselves. This also suggested that the three species were variants of a single species.

7. The effect of physiological age of A. oligospora mycelium on the trapping activity of the fungus was studied.

Traps were first produced by the mycelium that was 2 days old and the stimulus then spread throughout the colony along uninterrupted mycelial hyphae. Young traps, towards the periphery of a colony, captured nematodes efficiently while the older ones towards the centre were losing their adhesiveness.

8. In general, low temperatures and pH values towards alkalinity enhanced the trapping efficiency of A. oligospora. No traps were formed at 30°C and 35°C.

9. Weak Corn meal agar was found to be the best of 14 media tested for studies on the trapping activity of nematophagous fungi. Light affected trap production in A. oligospora, more traps being produced in the light than in the dark. No traps were produced on Potato dextrose agar, Malt extract agar, Mycophil agar, Chlamydospore agar, and Czapek Dox agar amended with yeast extract.

10. The effect of nematodes of different genera on the trapping activity of A. oligospora was tested. It was found that nematodes of some genera induced the formation of traps by the fungus while others did not.

11. The majority of the 2-celled microconidia of A. dactyloides Strain 124D had one nucleus per cell of the conidium. When 2 or 3 nuclei were present in a single cell of a conidium, they were presumed to have arisen by division from a single nucleus. The homocaryotic mycelium derived from a single cell of a microconidium produced traps

spontaneously in the absence of nematodes.

12. The proteins from hen's serum and fresh nematode extract were separated by polyacrylamide gel electrophoresis. The individual proteins contained in the unstained gel did not induce the formation of traps in A. oligospora.

13. The destiny of detached, incomplete ring traps and individual cells of non-inflated and inflated rings of wild type A. dactyloides was studied. Detached incomplete rings, during their early stages, could be induced to grow as ordinary mycelial hyphae. The cells of complete and near-complete rings could be induced to grow as mycelial hyphae by puncturing one of the three cells in a ring.

14. The potentialities for spontaneous constricting ring production, by hyphae arising from individual cells of 3-celled spores of Dactylella doedycoides, were tested. The middle cell of a spore was not observed to germinate and there was no significant difference between the number of rings produced by hyphae arising from proximal and distal cells of a spore.

15. Nematodes of the genus Aphelenchus on dent corn variety CO 106 were controlled by the fungi Arthrobotrys Strain 24 and Dactylella ellipsospora when used singly and in combination with one another. The superimposition of predacious nematodes of the genus Mononchus on the fungal treatments did not significantly improve control of the parasitic nematode.

CLAIMS OF ORIGINAL WORK

1. Mycelial and conidial cells, of 4 species of nematophagous fungi, were shown to be multinucleate.
2. Ten new strains of 4 species of nematophagous fungi were selected from their wild types.
3. Anastomoses between 3 wild type species of the genus Arthrobotrys were produced.
4. Electrophoretic techniques were used as aids in the taxonomy of the nematophagous hyphomycetes.
5. The effects of environmental factors on the trapping efficiency of Arthrobotrys oligospora were studied.
6. Nematodes of different genera were shown to stimulate trapping activity by A. oligospora to different extents.
7. The destiny of incomplete rings and complete inflated and non-inflated rings of A. dactyloides was studied.
8. Proteins from hen's serum and from nematode extract, separated by electrophoresis, were tested for ability to produce traps in A. oligospora.
9. The effect of the genetic condition of A. dactyloides on trap production was studied.
10. Dactylella ellipsospora and a new strain of Arthrobotrys, Strain A. 24, were found to protect dent corn variety CO 106 against Aphelenchus sp. when used singly and in combination with one another.

ACKNOWLEDGEMENTS

The author acknowledges the debt of gratitude he owes his Director of Research, Professor R.H. Estey of the Department of Plant Pathology, for his unflagging interest and judicious guidance during the course of this study and the preparation of the manuscript. Thanks are also due to Professor W.E. Sackston and Professor R.L. Pelletier for their helpful suggestions and advice.

The author is especially grateful to the Canadian Commonwealth Scholarship and Fellowship Committee for the award of a scholarship, which made this work possible, and to the External Aid Office for administering the scholarship. He is also grateful to the Kenya Education Department for giving him leave of absence to pursue these studies.

REFERENCES

Baermann, G. 1917

Eine einfache Methode zur Auffindung von Anchylostomum - (Nematoden) - Larven in Erdproben.

Geneesk. Tijdschr. Ned. - Ind. 57: 131-137.

Bessey, E.A. 1950

Morphology and Taxonomy of Fungi.

The Blakiston Company, Philadelphia, Toronto. 791p.

Buxton, E.W. 1954

Heterokaryosis and variability in Fusarium oxysporum f. gladioli (Snyder and Hansen).

J. Gen. Microbiol. 10: 71-84.

Buxton, E.W. 1960

Heterokaryosis, Saltation and Adaptation. In Plant Pathology, An Advanced Treatise, Vol. II, Ch. 10: 359-405. (Eds.) Horsfall, J.G. and A.E. Dimond.

Academic Press, New York and London.

Chang, L.O., A.M. Srb and F.C. Steward. 1962

Electrophoretic separations of the soluble proteins of Neurospora.

Nature, London, 193: 756-759.

Christie, J.R. 1960

Biological Control - Predacious Nematodes. In Nematology, Ch. 46: 466-468. (Eds.) Sasser, J.N. and W.R. Jenkins.

Univ. of N. Carolina Press, Chapel Hill.

Clare, B.G. 1963

Starch-gel electrophoresis of proteins as an aid in identifying fungi.

Nature, London, 200: 803-804.

Commandon, J. and P. deFonbrune. 1938

Recherches experimentales sur les champignons predateurs de nematodes du sol. Conditions de formations des organes de capture. Les pieges garrotteurs. Les glaux ou pieges collants.

Compt. Rend. Soc. Biol. Paris 129: 619-625.

Commandon, J. and P. deFonbrune. 1939

De la formation et du fonctionement des pieges de champignon predateurs de nematodes. Recherches effectuees a l'aide de la micromanipulation et de la cinematographie.

Compt. Rend. Acad. Paris 208: 304-305.

Cooke, R.C. 1961

Agar disc technique for the direct observation of nematode-trapping fungi in the soil.

Nature, London, 191: 1411.

Cooke, R.C. 1962a

The ecology of nematode-trapping fungi in the soil.

Ann. Appl. Biol. 50: 507-513.

Cooke, R.C. 1962b

The behaviour of nematode-trapping fungi during the decomposition of organic matter in the soil.

Trans. Brit. Mycol. Soc. 45: 314-320.

Cooke, R.C. 1963a

The predaceous activity of nematode-trapping fungi added to soil.

Ann. Appl. Biol. 51: 295-299.

Cooke, R.C. 1963b

Ecological characteristics of nematode-trapping hyphomycetes. I. Preliminary studies.

Ann. Appl. Biol. 52: 431-437.

Cooke, R.C. 1964

Ecological characteristics of nematode-trapping hyphomycetes.

Ann. Appl. Biol. 54: 375-379.

Cooke, R.C. and C.H. Dickinson. 1965

Nematode-trapping species of Dactylella and Monacrosporium.

Trans. Brit. Mycol. Soc. 48: 621-629.

Cooke, R.C. and B.E.S. Godfrey. 1964

A key to the nematode-destroying fungi.

Trans. Brit. Mycol. Soc. 47: 61-74.

Corda, A.C.J. 1839.

Pracht - Flora europaeischer Schimmelbildungen.

Leipzig und Dresden.

Couch, J.N. 1937

The formation and operation of the traps in the nematode-catching fungus Dactylella bembicoides Drechsler.

J. Elisha Mitchell Sci. Soc. 53: 301-309.

Curry, J. 1949

A droplet culture method for fungus isolations and a storing method for epidermophytosis.

Brit. J. Derm. 61: 54-58.

De Lamater, E.D. 1948

Basic fuchsin as a nuclear stain for fungi.

Mycologia 40: 423-429.

Deschiens, R. and L. Lamy. 1942

Sur les facteurs determinants l'apparition des pieges chez les Hyphomycetes predateurs de nematodes.

Compt. Rend. Acad. Sci. Paris 215: 450-452.

Deschiens, R., L. Lamy. and E. Vautrin. 1943

Essais pratiques de prophylaxie de l'Anguillulose des vegetaux par l'emploi d'Hyphomycetes predateurs.

Compt. Rend. Acad. Sci. Paris 216: 539-541.

Dessauer, H.C. and W. Fox. 1956

Characteristic electrophoretic patterns of plasma proteins of orders Amphibia and Reptilia.

Science 124: 225-226.

Dixon, S.M. 1954

A contribution to our knowledge of predacious fungi with special reference to their use in the biological control of plant pathogenic nematodes.

Ph.D. Thesis. Univ. of London.

Dollfus, R. Ph. 1946

Parasites (animaux et vegetaux) des Helminthes.

Encyclopedie Biologique XXVII, Paul Lechevalier, Paris. 481p.

Drechsler, C. 1933

Morphological diversity among fungi capturing and destroting nematodes.

J. Wash. Acad. Sci. 23: 138-141.

Drechsler, C. 1937

Some Hyphomycetes that prey on free-living terricolous nematodes.

Mycologia 29: 447-552.

Drechsler, C. 1941

Predacious fungi.

Biol. Rev. of the Cambridge Phil. Soc. 16: 265-290.

Drechsler, C. 1950

Several species of Dactylella and Dactylaria that capture free-living nematodes.

Mycologia 42: 1-79.

Duddington, C.L. 1949

A new predacious species of Trichothecium.

Trans. Brit. Mycol. Soc. 32: 284-287.

Duddington, C.L. 1951a

Two new predacious Hyphomycetes.

Trans. Brit. Mycol. Soc. 34: 598-603.

Duddington, C.L. 1951b

Further reports of British predacious fungi. II.

Trans. Brit. Mycol. Soc. 34: 194-209.

Duddington, C.L. 1951c

Dactylella lobata, predacious on nematodes.

Trans. Brit. Mycol. Soc. 34: 489-491.

Duddington, C.L. 1955

Fungi that attack microscopic animals.

Bot. Rev. 21: 377-439.

Duddington, C.L. 1956

The predacious fungi: Zoopagales and Moniliales.

Biol. Rev. of the Cambridge Phil. Soc. 31: 152-193.

Duddington, C.L. 1957a

The Friendly Fungi.

Faber and Faber, London. 188p.

Duddington, C.L. 1957b

The predacious fungi and their place in microbial ecology.

Symposia of the Society for general Microbiology VII. Microbial Ecology 218-237.

Duddington, C.L. 1958

Fungi help to rid the soil of eelworm.

International Sugar J. 60: 31-32.

Duddington, C.L. 1960

Biological Control - Predacious Fungi.

In Nematology, Ch. 45: 461-465. (Eds.) Sasser, J.N. and W.R. Jenkins.

Univ. of N. Carolina Press, Chapel Hill.

Duddington, C.L. 1962a

Predacious Fungi and the Control of Eelworms.

In Viewpoints in Biology, Vol. I, Ch. 4: 151-200. (Eds.) Carthy, J.D. and C.L. Duddington.

Butterworths, London.

Duddington, C.L. 1962b

Predacious fungi and nematodes.

Experientia, Basle 18: 537-543.

Duddington, C.L. and C.M.G. Duthoit. 1960

Green manuring and cereal root eelworms.

Plant Path. 9: 7-9.

Duddington, C.L., C.O.R. Everard and C.M.G. Duthoit. 1961

The effect of a predacious fungus and organic matter on cereal root eelworms in oats.

Plant Path. 10: 108-109.

Duddington, C.L., F.G.W. Jones and F. Moriarty. 1956

The effect of a predacious fungus (Dactylaria thaumasia) and organic matter upon the soil population of beet eelworm, Heterodera schachtii Schm.

Nematologica 1: 344-348.

Duddington, C.L., F.G.W. Jones and T.D. Williams. 1956
An experiment on the effect of a predacious fungus (Dactylaria thaumasia) upon the soil population of potato root eelworm Heterodera rostochiensis Woll.
Nematologica 1: 341-343.

Duncan, D.B. 1955
Multiple range and multiple F tests.
Biometrics 11: 1-42.

Duthoit, C.M.G. and B.E.S. Godfrey. 1963
Effect of green manure and predaceous fungi on cereal root eelworm in oats.
Plant Path. 12: 18-19.

Dyer, A.F. 1963
The use of lacto-proprionic orcein in rapid squash methods for chromosome preparations.
Stain Technology 38: 85-90.

Ei, B.N. and S.A. Alakhverdyants. 1962
Preparations with spores of predacious fungi in the control of larvae of pathogenic nematodes. (In Russian).
Izvestiya Akademii Nauk Turkmenskoi S.S.R., Seriya Biologicheskikh Nauk 4: 81-83.

Ei, B.N., S.A. Alakhverdyants and Z.M. Karimov. 1961
An improved biological method for the application of predatory helminthophagic fungi in ancylostomiasis. (In Russian).
Izvestiya Akademii Nauk Turkmenskoi S.S.R., Seriya Biologicheskikh Nauk 1: 70-71.

Estey, R.H. and T.H.A. Olthof. 1965
The occurence of nematophagous fungi in Quebec.
Phytoprotection 46: 14-17.

Feder, W.A. 1962
Personal communication.

Feder, W.A. 1963
A comparison of the nematode-capturing efficiencies of five Dactylella species at four temperatures.
Mycopathologia et Mycologia Applicata 19: 99-104.

Feder, W.A., C.O.R. Everard and C.L. Duddington. 1960
Heterokaryotic nature of ring formation in the predaceous fungus Dactylella doedycoides Drechsler.
Science 131: 922-924.

Feder, W.A., C.O.R. Everard and L.M.O. Wootton. 1963
Sensitivity of several species of the nematophagous fungus Dactylella to a morphogenic substance derived from free-living nematodes.
Nematologica 9: 49-54.

Flax, M. and A.W. Pollister. 1949
Staining of nucleic acids by Azure A. (Abstr.)
Anat. Rec. 105: 56-57.

Fresenius, G. 1852
Beitrage zur Mykologie.
Frankfurt am Main. p. 1-38.

Geitler, L. 1949
Schnellmethoden der kern-und chromosen-untersuchung.
3. Aflage. Wien, Springer.

Gorlenko, M.V. 1956

Predatory fungi and their utilization in nematode control. Nematologica 1: 147-150.

Gossop, G.H., E. Yuill and J.L. Yuill. 1940

Heterogenous fructifications in species of Aspergillus. Trans. Brit. Mycol. Soc. 24: 337-344.

Grove, W.B. 1886

New or noteworthy fungi. J. Bot. 24: 129-137; 197-206.

Hams, A.F. and G.D. Wilkin. 1961

Observations on the use of predacious fungi for the control of Heterodera spp. Ann. Appl. Biol. 49: 515-523.

Hansen, H.N. 1938

The dual phenomenon in imperfect fungi. Mycologia 30: 442-455.

Hansen, H.N. and R.E. Smith. 1932

The mechanism of variation in imperfect fungi: Botrytis cinerea. Phytopathology 22: 953-964.

Haupt, A.W. 1930

A gelatin fixative for paraffin section. Stain Technology 5: 97-98.

Holton, C.S. and G.W. Fischer. 1941

Hybridization between Ustilago avenae and Ustilago perennans. J. Agric. Res. 62: 121-128.

Huebschman, C. 1952

A method for varying the average number of nuclei in the conidia of Neurospora crassa.

Mycologia 44: 599-604.

Hutchinson, S.A. and W.F. Mai. 1954

A study of the efficiency of the catching organs of Dactylaria eudermata (Drechs.) in relation to Heterodera rostochiensis (Wr.) in soil.

Plant Dis. Reptr. 38: 185-186.

Jinks, J.L. 1952

Heterokaryosis - a system of adaptation in wild fungi.

Proc. Roy. Soc. B. 140: 83-99.

Juniper, A.J. 1956

A study of predacious fungi, with particular reference to those occuring in dung.

Ph. D. Thesis. Univ. of London.

Kristjansson, F.K. 1963

Genetic control of two pre-albumins in pigs.

Genetics 48: 1059-1063.

Kuyama, S. and D. Pramer. 1962

Purification and properties of a protein having nemin activity.

Biochim. biophys. Acta 56: 631-632.

Laan, P.A. van der. 1956

Onderzoekingen over schimmels, die parasiteren op de cysteinhoud van het aardappel-cystenaaltje (Heterodera rostochiensis Wollenw.). (English summary).

Tijdschr. PlZiekt. 62: 305-321.

Laan, P.A. van der. 1964

Guard our friends, destroy our competitors.

Nematologica 10: 57-60.

La Cour, L. 1941

Acetic orcein. A new stain fixative for chromosomes.

Stain Technology 16: 169-174.

Lamy, L. 1943

Intensite et vitesse relatives de la formation des despositifs capteurs chez les Hyphomycetes predateurs des nematodes.

Compt. Rend. Soc. Biol. Paris 137: 337-339.

Langeron, H. and R. Vanbreuseghem. 1952

Precis de Mycologie, rev. ed., Ch. 4: 127-147.

Masson.

Lawton, J.R. 1957

The formation of constricting rings in nematode-catching Hyphomycetes grown in pure culture.

J. Exptal. Botany 8: 50-54.

Linford, M.B. 1937

Stimulated activity of natural enemies of nematodes.

Science 85: 123-124.

Linford, M.B. and F. Yap. 1939

Root-knot nematode injury restricted by a fungus.

Phytopathology 29: 596-609.

Linford, M.B., F. Yap and J.M. Oliveira. 1938

Reduction of soil populations of the root-knot nematode during decomposition of organic matter.

Soil Science 45: 127-140.

Lohde, G. 1874

Einige neue parasitische Pilze.

Tageblatt der 47. Versammlung deutscher Naturforscher und Aerzte in Breslau: 203-206.

Mankau, R. 1961a

Antagonisms to nematode-trapping fungi in soil (Abstr.)

Phytopathology 51: 66.

Mankau, R. 1961b

The use of nematode-trapping fungi to control root-knot nematodes.

Nematologica 6: 326-332.

Mankau, R. 1961c

An attempt to control root-knot nematode with Dactylaria thaumasia and Arthrobotrys arthrobotryoides.

Plant Dis. Reptr. 45: 164-166.

Mankau, R. 1962a

Soil fungistasis and nematophagous fungi.

Phytopathology 52: 611-615.

Mankau, R. 1962b

The effect of some organic additives upon a soil nematode population and associated natural enemies.

Nematologica 7: 65-73.

Mankau, R. 1963

Effect of organic soil amendment on nematode populations. (Abstr.)

Phytopathology 53: 881-882.

Mankau, R. and O.F. Clark. 1959

Nematode-trapping fungi in Southern California citrus soils.

Plant Dis. Reptr. 43: 968-969.

Maupas, E. 1915

Sur un champignon parasite des Rhabditis.

Bull. Soc. Hist. Nat. Afr. Nord 7: 34-49.

McBeth, C.W., A.L. Taylor and A.L. Smith. 1941

Note on staining nematodes in root tissue.

Proc. Helminth. Soc. Wash. 8: 26.

McLean, R.C. and W.R. Ivimey Cook. 1958

Plant Science Formulae.

Macmillan and Co. Ltd., London and New York. 205p.

Mountain, W.B. and Z.A. Patrick. 1959

The pathogenicity of Pratylenchus penetrans.

Can. J. Bot. 37: 459-470.

Muller, H.G. 1958

The constricting ring mechanism of two predacious hyphomycetes.

Trans. Brit. Mycol. Soc. 41: 341-364.

Nelson, R.R. 1957

A major gene locus for compatability in Cochliobolus heterosporus.

Phytopathology 47: 742-743.

Norris, J.R. 1966

Taxonomic applications of gel electrophoresis.

In Final Notice and Abstracts for Meeting in London of the Society for General Microbiology, 1966.

Olthof, T.H.A. 1962

Studies on certain nematopagous fungi.

Ph.D. Thesis. Macdonald College of McGill University.

Olthof, T.H.A. and R.H. Estey. 1963

A nematotoxin produced by the nematophagous fungus Arthrobotrys oligospora Fresenius.

Nature, London 197: 514-515.

Olthof, T.H.A. and R.H. Estey. 1965

Relation of some environmental factors to growth of several nematophagous hyphomycetes.

Can. J. Microbiol. 11: 939-946.

Paasuke, M. 1962

Vorlaufige Mitteilung zur Bekampfung von Pratylenchus penetrans durch Raubpilze.

Acta Agriculturae Scandinavia 12: 135-147.

Peach, M. 1952

Aquatic predacious fungi. II.

Trans. Brit. Mycol. Soc. 35: 19-23.

Peach, M. 1954

Contributions to our knowledge of aquatic fungi.

Ph.D. Thesis. Univ. of London.

Poulik, M.D. 1957

Starch-gel electrophoresis in a discontinuous buffer system.

Nature. London 180: 1477-1479.

Pramer, D. and N.R. Stoll. 1959

Nemin: a morphogenic substance causing trap formation by predacious fungi.

Science 129: 966-967.

Riley, W.A. 1893

Parasitic and predacious insects in applied entomology.

Insect Life 6: 130-141.

Robinow, C.F. 1957

The structure and behaviour of the nuclei in spores and growing hyphae of Mucorales. I.

Can. J. Microbiol. 3: 771-789.

Roubaud, E. and R. Deschiens. 1939

Sur les agents de formations de dispositifs de capture chez les Hyphomycetes predateurs de nematodes.

Compt. Rend. Acad. Sci. Paris 209: 77-79.

Roubaud, E. and R. Deschiens. 1941

Essais relatifs a la prophylaxie de l'Anguillulose du mouton par l'usage des Hyphomycetes predateurs du sol.

Compt. Rend. Soc. Biol. Paris 135: 687-689.

Shepherd, A.M. 1955

Some observations on the distribution and biology of fungi predacious on nematodes.

Ph.D. Thesis. Univ. of London.

Shepherd, A.M. 1956

A short survey of Danish nematophagous fungi.

Friesia 5: 396-408.

Smith, I.S. 1960

Chromatographic and Electrophoretic Techniques. Vol. II. Zone electrophoresis.

William Heinemann Ltd., London, and Interscience Publishers Inc., New York. 215p.

Smithies, O. 1955

Zone electrophoresis in starch gels: group varations in the serum proteins of normal human adults.

Biochem. J. 61: 629-641.

Soprunov, F.F. 1950

The predatory fungi destroying the larvae of nematodes. (In Russian).

Bull. Turkmen. Fil. Acad. Sci. U.S.S.R. 4: 92.

Soprunov, F.F. 1958

Predacious Fungi: Hyphomycetes and their Application in the Fight Against Pathogenic Nematodes. (In Russian).

Ashkabad, Moscow.

Soprunov, F.F. and Z.A. Galiulina. 1951

Predacious Hyphomycetes from Turkmenistan soil. (In Russian).

Microbiology, Moscow 20: 489-499.

Soprunov, F.F. and Y.Y. Tendetnik. 1960

Data on the practical use of predacious fungi for the control of some eelworm diseases. (In Russian).

Trud. gel'mint Lab. 10: 192-194.

Subramanian, C.V. 1963

Dactylella, Monacrosporium and Dactylina.

J. Indian Bot. Soc. 42: 291-300.

Tarjan, A.C. 1960

Predacious activity and growth of nematophagous fungi on various organic substances. (Abstr.).

Phytopathology 50: 577.

Tarjan, A.C. 1961

Growth characteristics of Dactylella drechsleri n.sp., an adhesive-knobbed nematode-trapping Hyphomycete from Florida.

Mycopathologia et Mycologia Applicata 14: 136-140.

Tarjan, A.C. 1962

Attempts at controlling citrus burrowing nematodes using nematode-trapping fungi.

Proc. Soil and Crop Sci. Soc. of Florida, 21st Annual Meeting. pp 17-36.

Taschdjian, C.L. and E. Muskatblit. 1955

Hyphal fusion between Trichophyton tonsurans variants as an indication of species relationship.

Mycologia 47: 339-343.

Terry, E. 1965

Personal communication.

Thomas, G.B. 1963

Studies of factors affecting certain nematophagous hyphomycetes.

M. Sc. Thesis. Macdonald College of McGill University.

Tolmsoff, W.J. 1959

The isolation of nematode-trapping fungi from Oregon soils.

Phytopathology 49: 113-114.

Whitney, H.S. and J.R. Parmeter. 1963

Synthesis of heterokaryons in Rhizoctonia solani Kuhn.

Can. J. Botany 41: 879-886.

Willier, B.H., P.A. Weiss and V. Hamburger. 1955

Analysis of Development.

W.S. Saunders & Co., Philadelphia and London. 735p.

Zopf, W. 1888

Zur Kenntnis der Infektions-Krankheiten niederer Thiere und Pflanzen.

Nova Acta Academiae Caesareae Leopoldino-Carolinae Germanicae Naturae Curiosorum. Halis Saxonum t. LII, No. 7: 313-376.

Zopf, W. 1889.

Uber einen Nematodenfangender Schimmelpilz.

Biol. Zentr. Bd. VIII, No. 23: 705-707.