THE BIOLOGY OF PARASITISM AND ULTRASTRUCTURAL DEVELOPMENT OF MEROSPORANGIA IN THE MYCOPARASITE SYNCEPHALIS SPHAERICA

Dissertation for the Degree of Ph. D.

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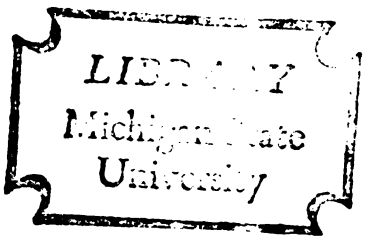

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THE BIOLOGY OF PARASITISM AND
ULTRASTRUCTURAL DEVELOPMENT OF MEROSPORANGIA
IN THE MYCOPARASITE *SYNCEPHALIS SPHAERICA*

presented by

KAREN KLOMPARENS BAKER

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Botany

Everett S. Benke

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Date August 11, 1977

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ABSTRACT

THE BIOLOGY OF PARASITISM AND ULTRASTRUCTURAL DEVELOPMENT OF MEROSPORANGIA IN THE MYCOPARASITE SYNCEPHALIS SPHAERICA

By

Karen Klomparens Baker

Syncephalis sphaerica van Tieghem (Piptocephalidaceae, Mucorales) is a biotrophic, haustorial mycoparasite. Fifty genera of fungi, representing four classes, were tested as potential hosts for the parasite.

Twenty genera of Mucorales supported growth and sporulation of S. sphaerica. A single Ascomycete (a yeast) and two members of the Imperfect Fungi were also parasitized by S. sphaerica. None of the Basidiomycetes tested supported growth of the parasite.

The mode of parasitism of Syncephalis sphaerica was examined and found to be very similar to that described for other species of Syncephalis: large appressoria and extensive, highly branched haustoria. The large appressoria of S. sphaerica were especially conspicuous on the sporophores of the mucoraceous hosts and the haustoria spread throughout the sporophores.

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Axenic culture of the mycoparasite was partially successful, with dense mycelial growth and some sporulation on selected media. The use of filtrates with host exudates as well as the use of killed mycelia to support growth were unsuccessful.

The effects of host nutrition on parasitism and the effects of parasitism on the hosts were examined. S. sphaerica had no adverse effects on the growth and sporulation of any host with the exception of Pilobolus umbonatus. Host nutrition significantly affected the success of parasitism by Syncephalis. As the hosts were grown on increasingly rich media, the success of parasitism by S. sphaerica increased also.

Attempts to induce zygospores in S. sphaerica were unsuccessful.

Transmission and scanning electron microscopy were used to study the development of Syncephalis sphaerica merosporangia. Merosporangia were initiated as simple evaginations of the ampulla wall. Three spores were cleaved out of each merosporangial protoplast by invaginations of the merosporangial plasmalemma. An abscission area was formed in each cleavage zone. A fibrillar spore wall was laid down between the spore plasmalemma and the inner lining layer of the merosporangial wall. Portions of the outer merosporangial wall remained attached to the spore at the time of detachment. During late cleavage, a wall was laid down on the surface of the ampulla at the

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base of each merosporangium forming a scar. Aggregated mature spores remained associated with the ampulla as a liquid spore drop.

THE BIOLOGY OF PARASITISM AND ULTRASTRUCTURAL DEVELOPMENT OF MEROSPORANGIA IN THE MYCOPARASITE SYNCEPHALIS SPHAERICA

By

Karen Klomparens Baker

A DISSERTATION

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Department of Botany and Plant Pathology

1977

To the memory of
Dr. William G. Fields

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INTRODUCTION

Syncephalis sphaerica van Tieghem, a member of the Piptocephalidaceae, is a biotrophic, haustorial mycoparasite. Mycoparasitism has been of interest to scientists since the mid-1800s when the genus Piptocephalis was described by deBary (1865) and the genus Syncephalis by van Tieghem and Le Monnier (1873). Since that time these and various other mycoparasites have been studied by mycologists and plant pathologists.

Dispira cornuta (Zygomycetes) was studied by Ayers (1933, 1935), Barker and Barnett (1973), and Kurtzman (1968); Darluca filum (Deuteromycetes) by Bean (1968), Carling et al. (1976) and many others; Gliocladium roseum (Deuteromycetes) by Barnett and Lilly (1962); Pythium sp. (Oomycetes) by Hendrix and Campbell (1973); Rhizoctonia solani (Deuteromycetes) by Butler (1957); Trichoderma sp. (Deuteromycetes) by Weindling (1932) and Wells et al. (1972); and Tieghemomyces sp. (Zygomycetes) by Binder and Barnett (1973, 1974). Burgeff (1920, 1924, 1930) wrote several extensive reports on mycoparasitism in the Mucorales, particularly on the genera Parasitella and Chaetocladium.

More recently, several review articles have discussed various aspects of the parasitic life habit of both genera in the family Piptocephalidaceae (Barnett, 1963, 1964; Barnett and Binder, 1973; Boosalis, 1964). These workers summarized many of the reports in the literature on the biotrophic mycoparasites as well as describing the necrotrophic mycoparasites. The majority of reports include studies of host range, mode of parasitism and various factors affecting mycoparasites, while little work has been done on the biology of mycoparasitism in nature. Many researchers hope to develop a body of knowledge from work with mycoparasites that can be applied to parasitism and the onset of disease in higher plants.

Mycoparasite-systems offer several advantages over the conventional plant-parasite systems. Generation time for the host is often much less than with the plant systems; less space is required for growth of host and parasite; and environmental and nutritional conditions can be more rigidly controlled than those of conventional systems.

Of the two genera classified in the Piptocephalidaceae (Syncephalis and Piptocephalis), only Piptocephalis has received extensive study. Various species of Piptocephalis have been studied as to their host range and mode of parasitism (Armentrout and Wilson, 1969; Berry and Barnett, 1957; Dobbs and English, 1954; Manocha and Lee, 1971), factors affecting parasitism (Berry, 1959; England, 1969; Shigo et al., 1961), axenic culture (Barnett, 1970;

Manocha, 1975), sporangiospore morphology (Jeffries and Young, 1975, 1976), ultrastructure of infection (Jeffries and Young, 1976) and zygospore formation (Leadbeater and Mercer, 1956, 1957). As a result, much more information is available on the genus Piptocephalis than the genus Syncephalis.

Most reports concerning Syncephalis are descriptive, emphasizing morphology and taxonomy (Indoh, 1962; Ou, 1940; van Tieghem and Le Monnier, 1873; Zycha, 1935; Zycha et al., 1969). Portions of a few investigations include comments on some aspect of the biology of Syncephalis such as, general morphology and zygospore formation (van Tieghem, 1875), merosporangial development (Thaxter, 1897), life habit and zygospore formation (Morini, 1902), and axenic growth (Ellis, 1966). Benjamin (1959, 1966) gives the most thorough coverage of a number of aspects of the biology of Syncephalis in his review of the merosporangiferous Mucorales.

Syncephalis californica has been studied in considerable detail by Hunter and Butler (1975). The mode of parasitism, host range, axenic culture and formation of zygospores of S. californica were reported in that study. S. californica differed from other species of Syncephalis by having a 270° angle of recurvature of the sporangiophore. It produced giant hyphal swellings on its hosts and only parasitized members of the Mucorales.

In recent years, ontogenic studies in the Mucorales have dealt with the development of the zygospores (Gauger, 1961; Hawker and Beckett, 1971; Hawker and Gooday, 1967, 1968; Hocking, 1967, O'Donnell, 1976), septum formation (Benny and Aldrich, 1975) and germination of the sporangiospores (Buckley et al., 1968; Dykstra, 1974; Hawker and McV. Abbott, 1963; Young, 1973; Jeffries and Young, 1975). Only a few studies have followed the entire development of the structures involved in asexual reproduction (Bracker, 1968; Fletcher, 1972, 1973).

Specifically, developmental studies on members of the family Piptocephalidaceae have emphasized the ontogeny of appressoria and haustoria and subsequent development of the parasite (Armentrout and Wilson, 1969; Manocha and Lee, 1971; Manocha, 1975; Jeffries and Young, 1975, 1976) as well as the development of zygospores (Leadbeater and Mercer, 1956, 1957; Benjamin, 1959).

Development of merosporangia and merosporangiospores in the genus Syncephalis was first described by van Tieghem and Le Monnier (1873) and more fully by van Tieghem (1875). Later, Thaxter (1897) reported a developmental sequence for the genus. The most recent reviews describing the ontogeny of the asexual reproductive structures in the family were completed by Benjamin (1959, 1966). These studies utilized light microscopy. Benjamin examined six species of Syncephalis and reviewed several other species for germination of the spores and the

morphology of the merosporangia, sporangiophores, and zygospores. Zygospores themselves were uncommon in the species studied. No further information was reported beyond that of Thaxter's for spore development.

While light microscopy provides some useful information on merosporangial ontogeny, it cannot provide conclusive data on cleavage initiation and development, cell wall deposition, or abscission from the apical swelling upon which the merosporangia are borne.

Therefore, there is no in-depth report of merosporangial development in the Piptocephalidaceae in the current literature, even though the classification of the genera in the Mucorales into families is based primarily on the type of asexual reproduction. Such ontogenic information is particularly useful in defining the parameters to be used in distinguishing one family from another in the Mucorales and also in assigning a specific genus to a family.

This dissertation presents a detailed account of the host range mode of parasitism and its effects on the host fungi, and axenic culture of Syncephalis sphaerica, as well as a description of the ultrastructural development of the merosporangia in S. sphaerica using both transmission and scanning electron microscopy.

MATERIALS AND METHODS

Host Range

Syncephalis sphaerica van Tieghem was maintained on *Mucor ramannianus* Moller. Host range studies were conducted using 1.7% Difco corn meal agar with 0.3% sucrose, 0.2% glucose, and 0.1% yeast extract (B agar) as the growth medium. Either spores or mycelium of the host and spores of *S. sphaerica* (collected from mature spore droplets with sterile glass needles) were inoculated together onto the agar medium. Cultures were incubated at 25°C. Each non-mucoraceous host was coinoculated with the parasite a minimum of ten times, while the mucoraceous hosts were inoculated with *S. sphaerica* five times.

Fifty different genera of fungi were tested as possible hosts for *S. sphaerica*. Of these, twenty genera were members of the Zygomycetes (all Mucorales), ten Imperfect Fungi (including four Blastomycetes), sixteen Ascomycetes (including twelve yeasts) and four Basidiomycetes. Each host was examined for support of vegetative growth of the parasite by examining culture plates for the characteristic arachnoid mycelium of *Syncephalis* sp. Parasitism was considered successful on the basis of relatively abundant and normal sporulation of *S. sphaerica*.

Varying degrees of abundance of the asexual reproductive structures were noted also. Abnormal sporulation produced dwarf sporangiophores with fewer merosporangia on the apical swelling.

Mode of Parasitism

Cokeromyces recurvatus or Gilbertella persicaria were used as the hosts to study the mode of parasitism of Syncephalis. Each host was coinoculated on B agar with Syncephalis spores. Agar squares with both host and parasite mycelium were collected every 4-6 hours up to 6 days, placed on glass slides, stained with 0.3% cotton blue in lactophenol and studied with a Leitz compound light microscope. Specimens were examined for evidence of appressoria and haustoria.

Axenic Culture

Axenic culture of S. sphaerica was attempted using B agar, half-strength cornmeal agar, YpSs agar (Benjamin, 1959), Ellis' synthetic medium (Ellis, 1966), Difco PDA, N-minimal medium (N-min) (0.1% KNO_3, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.01% CaCl, 0.01% NaCl, 2% sucrose) and N-complete medium (N-com) (N-min plus 0.1% yeast extract and 0.1% peptone). In all cases either spores or mycelium of Syncephalis sphaerica served as inoculum.

The possible use of filtrates with host exudates as well as the used of killed mycelium as substrates to support growth and sporulation of S. sphaerica were

examined by growing various hosts in either N-min or N-com broth for 6-8 days. The liquid was collected in a sterile flask using a 0.22 μm pore size vacuum filter system (Millipore corporation). Initially, 0.5 ml of each filtrate was dropped on germinated and ungerminated S. sphaerica spores which had been streaked onto plates of B agar. An additional 0.5 ml of filtrate was added to each agar plate for five successive days. Each host filtrate was tested on ten agar plates inoculated with the parasite spores. Plates were examined at two day intervals up to twelve days. Stock liquid broth alone (without the host growth) was used as a control.

Host mycelia were grown in N-com broth culture, removed, washed three times in sterile distilled water and autoclaved for ten minutes at 15 psi. Spores or mycelium of the parasite were inoculated onto the cooled mycelial mat in a sterile petri dish with no medium and examined 8-15 days later for evidence of growth and/or sporulation. This experiment was repeated three times for each host.

Effect of Host Nutrition on Success of Parasitism

In several cases of successful parasitism, further investigations were completed to examine the effects of host nutrition on parasitism. For these studies, a variety of agars were used to support the growth of the host; plain agar (Difco), half-strength Difco corn meal agar (cm/2), and B agar (see host range). Cultures were

incubated at 25°C in the dark and examined 8-10 days after inoculation.

The success of parasitism on each host used for each medium employed was rated on the basis of relative abundance of Syncephalis merosporangia produced. Experiments were repeated five times.

Effects of Temperature on Parasitism

Experiments were conducted to determine the effects of temperature on the success of parasitism. Thamnidium elegans, Mucor ramannianus, and Gilbertella persicaria were used as the hosts. Spores of S. sphaerica, collected with a sterile glass needle, were coinoculated onto B agar with the mycelium of the host. Cultures were incubated in the dark at either 20°C, 25°C, or 30°C. Cultures were examined after 9-10 days of incubation for relative sporulation of the parasite. These experiments were repeated three times.

Induction of Zygospores

Various methods were used to induce the formation of zygospores in S. sphaerica. The methods can be divided into three categories; genetic, nutritional, and environmental.

Genetic: A total of four single spore isolates of S. sphaerica were crossed in various combinations on various media and on various hosts. Attempts to secure isolates from other collections were unsuccessful.

Nutritional: The various agars and hosts used in all other experiments were all observed for zygospores. In addition, Ellis' synthetic medium and Pablum agar (Benjamin, 1959) were used as growth media to try to induce the formation of zygospores.

Environmental: Cultures from the various temperature experiments were observed for zygospores, as well as inoculating separate plates with various hosts plus Syncephalis or plates of Ellis medium with Syncephalis alone and incubating them at 5°C, 15°C, 20°C, 25°C, and 30°C.

Flooding of mature cultures with 5-10 ml of sterile distilled water was also tried.

Light Microscopy

Sporangiophores or mycelium of Syncephalis sphaerica alone or with host mycelium were collected from variously aged colonies growing on agar plates. Where sporangiophores were examined, they were teased apart using sterile glass needles to separate them from any mycelium which could obscure details.

All material was mounted in either clear lactophenol or lactophenol with 0.3% cotton blue and examined with a Zeiss Photomicroscope II. Photographs were taken with a built-in 35 mm camera on Kodak Panatomic X film using differential interference, phase contrast illumination.

Transmission Electron Microscopy

Asexual reproductive structures were harvested by cutting 0.5mm X 0.5mm squares of agar from the edges of colonies in petri dishes. Sporangiophores were clearly visible under a dissecting microscope and could be selectively collected. Various developmental stages were usually present on each square.

The specimens were fixed in a 3% glutaraldehyde-3% acrolein mixture in 0.1M cacodylate buffer on ice for 2 hours. The samples were then washed for 1 hour on ice in 2 changes of 0.1M cacodylate buffer. Post fixation was done in 1% osmium tetroxide in 0.1M cacodylate buffer on ice for 1 1/2 hours with a gradual conversion to room temperature for 1/2 hour. A 1/2 hour wash in 2 changes of 0.1M cacodylate buffer followed. Dehydration was accomplished using ethanol at steps of 25%, 50%, 75%, 95%, and two changes of 100% on ice at 20 minute intervals. The sample was left in 100% ethanol in the cold overnight.

The samples were then gradually infiltrated using ERL epoxy resin (Spurr, 1969) using steps of 25%, 50%, and 100% resin at 8 hour intervals with no agitation. Specimens were embedded in a thin layer of fresh ERL resin in aluminum weighing dishes. The resin was polymerized overnight at 65°C. The aluminum was then peeled away from the sheet of polymerized resin, the specimens cut out and the individual samples mounted on resin blocks for sectioning. Ultrathin

sections were cut using a diamond knife on a Porter Blum MT-2 ultramicrotome.

Sections were collected on 300 mesh, uncoated, copper grids and stained for 2 hours in a 1% uranyl acetate in a 100% methanol-70% ethanol mixture (1:3), rinsed and double stained in a 1% aqueous lead citrate for 7 minutes. Sections were examined in a Philips 300 transmission electron microscope operated at 60 kV.

Scanning Electron Microscopy

Samples were collected by cutting squares of agar 1mm X 1mm from the edges of the colonies in petri dishes. Dense populations of sporangiophores were selected with the aid of a dissecting microscope. Various development stages were usually present on each agar square.

Samples were fixed as for transmission electron microscopy or in 5% glutaraldehyde in 0.1M phosphate buffer with postfixation in 1% osmium tetroxide. Dehydration was accomplished using steps of 10%, 20% ..... 90% ethanol with 3 changes of 100% ethanol at 10 minute intervals. Samples were immediately critical point dried using a Sorvall critical point dryer with CO_2 as the carrier gas.

Dried samples were then mounted on double-stick Scotch tape on aluminum stubs. A thin layer of Television Tube Koat (G. C. Corporation) was applied around the edge of the tape and stub. The samples were coated with a

15-20nm layer of gold using a Film-Vac sputter coater and examined in an ISI Super-Mini scanning electron microscope. Photographs were obtained using Polaroid type 105 positive/ negative film.

RESULTS

Syncephalis sphaerica van Tieghem, Ann. Sci. Nat. Bot. Ser. 6, 1:125 (f. 105-109), 1875.

Description of colonies on Mucor ramannianus on B agar: Colonies white, forming a dense white mat over the host mycelium. Vegetative mycelium hyaline, non-septate (except in age), slender, arachnoid. Sporangiophores erect, simple, unbranched, arising singly or in groups from the substrate, 300-370 µm long, slightly tapering, 11-20 µm wide at the base to 8-10 µm wide just below the apical swelling (ampulla). Large rhizoids present at the base of the sporangiophore. Spherical apical swellings 25-30 µm in diameter bearing numerous merosporangia on the upper half. Merosporangia unbranched, containing 3 spores. Spores cylindrical, pale yellow in mass, 8-10 µm X 2.5-3 µm, in a spore droplet at maturity. Zygospores not observed.

Host Range

All species of the order Mucorales which were tested supported both vegetative growth and sporulation of the parasite (Table 1). This included representatives from the following families: Choanephoraceae, Cunninghamellaceae, Mortierellaceae, Mucoraceae, Pilobolaceae,

Table 1.--Host range of *Syncephalis sphaerica* in the class Zygomycetes. Vegetative growth of the parasite is indicated by +. Relative abundance of sporulation of the parasite is indicated by +, ++, or +++.

	Vegetative Growth	Sporulation
Choanephoraceae		
Blakeslea trispora Thaxt.	+	++
Gilbertella persicaria (Eddy) Hesseltine	+	+++
Cunninghamellaceae		
Cunninghamella elegans Lendner	+	++
Mycotypha africans Novak et Backus	+	++
Mortierellaceae		
Haplosporangium bisporale Thaxt.	+	++
Mucoraceae		
Absidia coerulea Bain.	+	++
Actinomucor elegans (Eidam) C. Benjamin et Hesseltine	+	+++
Circinella sp.	+	+++
Mucor genevensis Lendner	+	++
M. hiemalis Wehmer	+	++
M. mucedo (L.) Fres.	+	++
M. ramannianus Möller	+	+++
Phycomyces nitens (Kunze) van Tiegh. et Le Monnier	+	++
Rhizopus stolonifer (Ehrenb. ex Fr.) Vuill.	+	++
Syzygites megalocarpus Ehrenb. ex Fr.	+	++
Zygorhynchus Moelleri Vuill.	+	++
Pilobolaceae		
Pilaira anomala (Ces.) Schröter	+	++
Pilobolus umbonatus Buller	+	++
Radiomycetaceae		
Radiomyces sp.	+	++
Syncephalastraceae		
Syncephalastrum racemosum Cohn ex Schröter	+	++
Thamnidiaceae		
Cokeromyces recurvatus Poitras	+	+++
Helicostylum piriforme Bain.	+	++
Thamnidium elegans Link	+	++

Radiomycetaceae, Syncephalastraceae, Thamnidiaceae. Actinomucor elegans, Cokeromyces recurvatus, Gilbertella persicaria, and Mucor ramannianus appeared to be particularly good hosts for S. sphaerica. Hyphae of the parasite were very dense on these hosts and abundant sporulation occurred after 8 days. Syncephalis sphaerica did not appear to appreciably decrease vegetative growth of those species it parasitized nor did it appear to affect the normal sporulation of any host with the exception of Pilobolus umbonatus. In the latter case, the parasite grew very densely over the host and in a large portion of each plate, aerial hyphae of the parasite encased the sporangia of Pilobolus thereby preventing sporangial discharge.

Of the ten Imperfect Fungi tested, only Aureobasidium pullulans and Sporobolomyces salmonicolor supported vegetative growth and sporulation of S. sphaerica (Table 2). Both vegetative growth and sporulation were very sparse, although the merosporangia of the parasite appeared normal. Trigonopsis varibilis and Candida tropicalis supported very sparse mycelial growth of the parasite, but no sporangia were produced. In these two cases, mycelium of Syncephalis grew less than 2 cm from the point of inoculation.

Four yeast genera from the Ascomycetes tested supported vegetative growth or vegetative growth and sporulation of Syncephalis (Table 3). Vegetative growth

Table 2.--Host range of S. sphaerica in the class Ascomycetes. Vegetative growth and sporulation of the parasite are indicated by +, absence by -. Here + indicates not more than 12 sporangiophores.

	Vegetative Growth	Sporulation
Sphaeriales		
Chaetomium globosum Kunze	-	-
Gelasinospora calospora (Mouton) Cl. et Mir. Moreau	-	-
Neurospora tetrasperma Shear et B.O. Dodge	-	-
Sordaria macrospora Auersw.	-	-
Endomycetales		
Ashbya gossypii (Ashby et Nowell) Guill.	-	-
Cephaloascus fragrans Hanawa	+	-
Dipodascus uninucleatus Biggs	+	-
Endomyces tetrasperma Macy et Miller	+	+
Saccharomycopsis lipolytica (Wickerham et al.) Yarrow	+	-
Eremothecium Ashbyii Guill.	-	-
Hansenula saturnus (Klöcker) Syd.	-	-
Pichia bessyii Kurtzman et Wickerham	-	-
Saccharomyces cerevisiae Hansen	-	-
Schizosaccharomyces octosporus Beijerinck	-	-
Schwanniomyces alluvius Phaff et al.	-	-

Table 3.--Host range of *S. sphaerica* in the Imperfect Fungi. Vegetative growth and sporulation of the parasite are indicated by +, absence by -. Here, + indicates not more than 12 sporangiophores.

	Vegetative Growh	Sporulation
I. Hyphomycetes		
Aspergillus giganteus Wehmer	-	-
Aureobasidium pullulans (deBary et low) Berhk.	+	+
Gliocladium sp.	-	-
Helicosporium sp.	-	-
Paecilomyces sp.	-	-
Pencillium purpurgenum Fleroff-Stoll	-	-
II. Blastomycetes		
Candida tropicalis (Castellani) Berkhout	+	-
Rhodotorula sp.	-	-
Sporobolomyces salmonicolor (Fisher et Brebeck) Kluyver et van Niel	+	+
Trigonopsis varibilis Schachner	+	-

was very sparse on Endomyces tetrasperma, Saccharomycopsis lipolytica, Dipodascus uninucleatus, and Cephaloascus fragrans with sporulation of the parasite occurring only infrequently on E. tetrasperma. In all these cases, Syncephalis mycelium grew less than 5 cm from the point of inoculation.

None of the Basidiomycetes tested supported vegetative growth of Syncephalis (Table 4).

In each case of successful parasitism, the merosporangiospores of Syncephalis were tested and found to germinate, reinfect M. rammanianus, and reproduce asexually within 10 days. After continuous laboratory culture of 18 months, a reduced ability to parasitize non-mucoraceous hosts was noted.

Mode of Parasitism

No tropism was noted in either host or parasite in the early stages of incubation. Mutual hyphal contact appeared to be by chance.

Appressoria and haustoria were very difficult to locate even though both C. recurvatus and G. persicaria had rather large hyphae. When appressoria were found, usually on aerial portions of the host such as sporangiophores, they appeared larger than the delicate vegetative hyphae of Syncephalis. Haustoria were slender, highly branched and grew in all directions from the appressorium. It was not possible to determine the actual time of

Table 4.--Host range of S. sphaerica in the class Basidiomycetes. Vegetative growth and sporulation are indicated by +, absence by -.

	Vegetative Growth	Sporulation
Agaricales		
Agaricus campestris Fr.	-	-
Pleurotus ostreatus (Jacq.) Fr.	-	-
Schizophyllum commune Fr.	-	-
Volvariella bombycina (Schaeff.) Fr.	-	-

appressorial formation because of the difficulty in locating the infection hyphae.

Axenic Culture

Syncephalis sphaerica produced a dense mat of vegetative mycelium with little aerial hyphae on Ellis' medium. Sporulation occurred 10-12 days after inoculation, but was relatively sparse compared to that occurring on a mucoraceous host. Continuous culture and transfer of S. sphaerica on the synthetic medium resulted in a decreased ability to sporulate, although no decrease was noted in mycelial growth. Reinoculation onto a mucoraceous host resulted in abundant sporulation of the parasite.

On half-strength cornmeal agar, (cm/2) S. sphaerica spores germinated within 24 hours and produced branched germ tubes after 48 hours, but growth of the hyphae was limited to less than 2mm with no sporulation after 10 days. On medium B, spores of the parasite germinated but growth was limited to less than 3mm with no sporulation after 12 days. Growth on N-min, N-com, PDA and YpSs media was similar.

Filtrate from liquid culture was collected from the following hosts; Aureobasidium pullulans, Actinomucor elegans, Gilbertella persicaria, Mucor ramannianus and Sporobolomyces salmonicolor. The filtrate and any host exudates present did not appear to have any effect on the growth of S. sphaerica when spores of the parasite were

treated with the filtrate. Spores of the parasite germinated as readily as on unamended agar, but growth was limited to branched germ tubes of less than 2 mm in length. Neither of the stock liquid media alone appeared to affect the growth of S. sphaerica when applied to the spores in the same manner as the host filtrate. Untreated spores in N-com agar medium were limited in growth to less than 2mm.

Killed mycelium of A. pullulans, A. elegans, G. persicaria, and Mucor genevensis were collected as substrates and inoculated with S. sphaerica spores or mycelium. In each case, some vegetative growth of Syncephalis was noted with colony diameter limited to less than 4mm. No sporulation occurred on any killed host.

Effects of Host Nutrition on Parasitism

The relative abundance of sporulation of Syncephalis sphaerica when its host is grown on various agar media was examined (Table 5). It should be noted that as progressively weaker media (in terms of carbon:sucrose, glucose and nitrogen:yeast extract sources) were used, a corresponding decrease in host growth was observed. As host growth became more luxuriant, the relative abundance of sporulation of the parasite increased. Aureobasidium pullulans showed the most marked change between media: no visible sporulation (although some vegetative growth of Syncephalis) on plain agar, very few sporangiophores on half-strength corn meal agar and more sporulation on B agar. Sporulation was most

Table 5.--Effects of host nutrition on the success of parasitism of Syncephalis sphaerica. Relative abundance of sporulation of S. sphaerica noted by +, ++, or +++, absence of sporulation by -.

	Medium		
	Plain Agar	CM/2	B
Aureobasidium pullulans	-	-/+	+
Cokeromyces recurvatus	+	++	+++
Gilbertella persicaria	+	++	+++
Mucor ramannianus	+	+	+++

abundant on the mucoraceous hosts when they were grown on medium B.

Effects of Temperature on Parasitism

The effects of temperature on parasitism were examined for several host species (Table 6). Here, it must be noted that as the growth of the host varied from temperature (optimum at 25°C), the growth and sporulation of *S. sphaerica* also varied. *Thamnidium elegans* supported less sporulation of the parasite at 20°C than at either 25°C or 30°C. *Mucor ramannianus* showed the least variation in abundance of parasite sporargiophores at the three temperatures, while *Gilbertella persicaria* also showed very little difference.

Induction of Zygospores

All attempts to induce zygospore formation in *Syncephalis sphaerica* were unsuccessful.

Light Microscopy

The arachnoid habit of much of the vegetative hyphae of *Syncephalis sphaerica* was evident in the light microscope (Fig. 1). The morphology of the asexual reproductive structures was examined. Merosporangia were formed on the top half of a swollen sporogenous apex (ampulla) Figs. 2, 3). The long, tapering nature of the sporangiophore was also evident. A single merosporangium with 3 spores was also examined for details of merosporangial

Table 6.--Effect of temperature on the parasitic development of *Syncephalis sphaerica*. Relative abundance of sporulation of *S. sphaerica* denoted by +, ++, or +++.

Host	Temperature		
	20°C	25°C	30°C
Gilbertella persicaria	++	+++	+++
Mucor ramannianus	+++	+++	+++
Thamnidium elegans	+	++	++

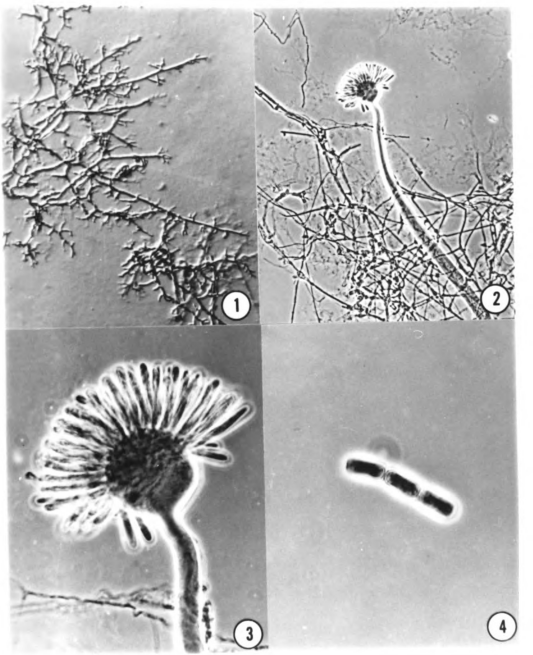
Figs. 1-4. Vegetative and asexual reproductive structures of Syncephalis sphaerica as seen by light microscopy.

Fig. 1. Vegetative mycelium showing arachnoid pattern. X 350.

Fig. 2. Sporangiophore with merosporangia. X 190.

Fig. 3. Ampulla with elongated merosporangia originating on the top half. X 700.

Fig. 4. Single merosporangium cleaved into 3 spores. X 750.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cleavage (Fig. 4). While light microscopy was sufficient to identify Syncephalis to species and to give a general view of asexual reproduction, it did not provide enough detail as to the development of the merosporangia and merosporangiospores.

Transmission and Scanning Electron Microscopy

Sporangiophores were initiated as large, unbranched aerial hyphae (Fig. 5). They enlarged at the apex to become clavate in shape (Fig. 6) and finally spherical (Fig. 7). Large rhizoids were present at the base of each sporangiophore.

Merosporangia were initiated as simple evaginations of the wall of the apical swelling (ampulla) Figs. 8, 9). The outer, electron opaque wall layer of the merosporangia appeared to be continuous with the outer ampulla wall layer; and the inner, electron transparent layer of the merosporangia appeared continuous with the inner layer of the ampulla wall (Fig. 10). Merosporangia were initiated only on the upper portion of each ampulla and were formed regularly and simultaneously over this surface (Figs. 8, 9). Fully elongated merosporangia appeared as rod-like structures connected to the ampulla (Figs. 11, 12, 13).

At pre-cleavage, the lipid globules began to accumulate in the zones which subsequently became the sites of cleavage (Fig. 12). Also at this time, an electron opaque wall lining layer was deposited between the

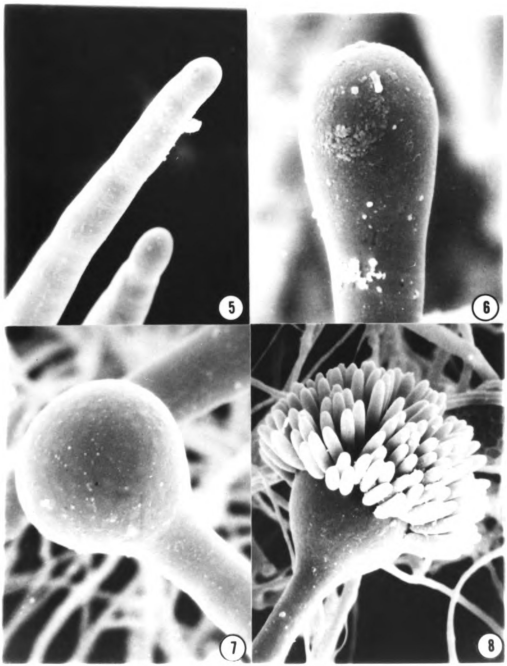
Figs. 5-8. Scanning electron microscopy of the early stages of development in merosporangia in Syncephalis sphaerica.

Fig. 5. Early stage of sporangiophore production showing large, aerial hyphae. X 2,200.

Fig. 6. Early swelling of the sporangiophore apex into an ampulla. X 4,160.

Fig. 7. Fully swollen ampulla. X 3,176.

Fig. 8. Ampulla with merosporangial initials originating on the top half. X 1,760.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Figs. 9-12. Ultrastructural development of merosporangial elongation in Syncephalis sphaerica.

Fig. 9. Ampulla with merosporangial evaginations. X 2,166.

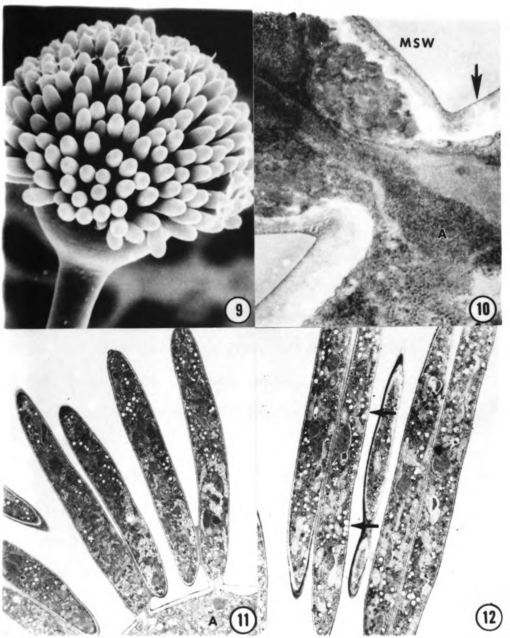
Fig. 10. Single merosporangial initial. Arrow indicates outer ampulla wall. X 48,888.

Fig. 11. Elongated merosporangia. X 3,657.

Fig. 12. Fully elongated merosporangia. Lipid globules accumulated in 2 zones (arrows). X 3,657.

KEY TO LETTERING ON FIGS. 9-32.

A = ampulla, am = amorphous material, L = lipid, MSW = merosporangial wall, PL = plasmalemma, V = vacuole.


MSW
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electron opaque and electron transparent layers of the merosporangial wall (Fig. 14).

Three spores were cleaved out of the merosporangial protoplast simultaneously by invaginations of the merosporangial plasmalemma from the periphery of the merosporangium toward the center (Figs. 15, 16, 17). As the plasmalemma developed centripetally toward the center of the merosporangium forming each cleavage zone, an electron transparent abscission zone was formed between the opposing plasmalemma (Figs. 15, 16). During this cleavage, the cytoplasm isolated between the spore protoplast and the merosporangial wall degenerated. This cytoplasm remained evident into the post-cleavage stage as a mass of amorphous material in each cleavage zone (Figs. 15, 16). Cleavage occurred at the tip of each merosporangium as well as at each base near the ampulla leaving a cap (Fig. 18) and a base of the amorphous material.

Plasmalemmal invaginations continued centripetally from the sides (Fig. 19) of the merosporangium until the membranes fused in the center to complete spore delimitation (Fig. 20). At this time, the abscission zones extended across the merosporangium delimiting three equal-sized spores in a single row (Figs. 21, 22). The electron transparent inner layer of the merosporangium present at merosporangial initiation was not longer discernible at the completion of spore delimitation (Fig. 24).

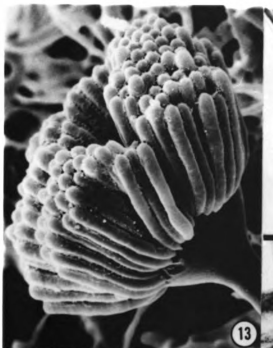
Figs. 13-16. Ultrastructural development of pre-cleavage and cleavage in merosporangia of Syncephalis sphaerica.

Fig. 13. Ampulla with fully elongated merosporangia. X 1,760.

Fig. 14. Merosporangial wall. Arrow indicates electron opaque wall lining layer. X 40,000. Inset shows base of a merosporangium and wall lining layer. X 42,857.

Fig. 15. Single cleavage area of a merosporangium with invagination of the plasmalemma. Arrow indicates abscission zone. X 25,000.

Fig. 16. Detail of cleavage area. Arrow indicates abscission zone. X 60,714.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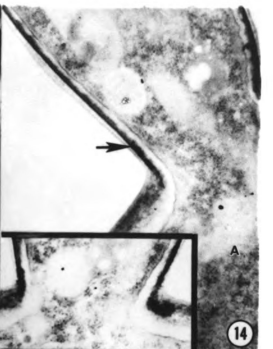
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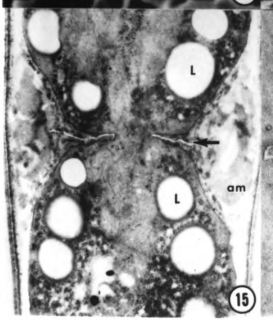
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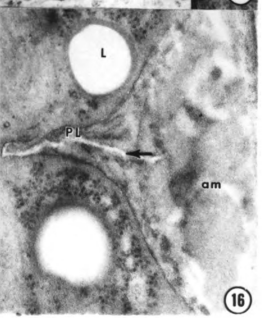
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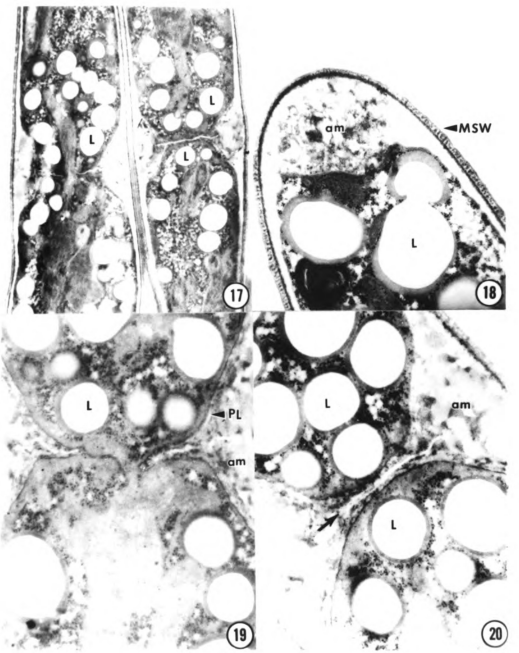
Figs. 17-20. Ultrastructural development of cleavage zones in merosporangia of Syncephalis sphaerica.

Fig. 17. Two merosporangia. Right merosporangium with complete cleavage and left merosporangium with cleavage nearing completion. X 12,500.

Fig. 18. Tip of merosporangium with complete cleavage plate. X 31,250.

Fig. 19. Cleavage zone with nearly complete cleavage. X 32,500.

Fig. 20. Cleavage completed. Arrow indicates abscission zone. X 31,250.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The electron opaque wall lining layer deposited along the length of the merosporangium at pre-cleavage formed the side walls of each spore and a new electron opaque wall layer was deposited at the end of each spore protoplast near the abscission zone (Figs. 23, 24). A fibrillar, electron transparent spore wall was deposited between the spore plasmalemma and the electron opaque wall layer (Fig. 24). At the completion of wall deposition, each spore was delimited along its length first by the spore plasmalemma, then by the fibrillar electron transparent layer deposited after spore formation, the electron opaque wall lining layer deposited at pre-cleavage, and finally by the outer merosporangial wall. At the ends of each spore, the protoplast was surrounded by a plasmalemma, the fibrillar spore wall layer and finally by the electron opaque layer deposited after spore delimitation (Figs. 23, 24). The merosporangial outer layer, now the outer wall layer along the length of each spore, partially disintegrated giving the spore a wrinkled appearance in the scanning electron microscope and an uneven, membraneous appearance in the transmission electron microscope (Figs. 25, 26).

During late cleavage and into post-cleavage, changes occurred in the ampulla. A wall was laid down on the surface of the ampulla at the base of each merosporangium (Figs. 25, 27). This new wall capped a small protuberance which became the scar left at the time of

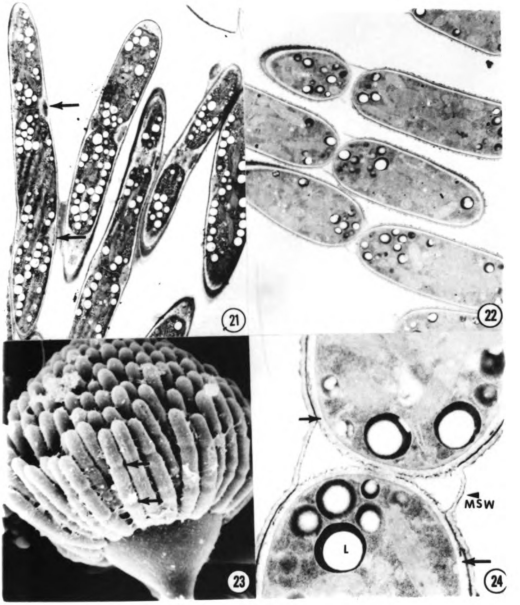
Figs. 21-24. Ultrastructural development of post-cleavage zones in merosporangia of Syncephalis sphaerica.

Fig. 21. Post-cleavage merosporangium with 3 spores. Arrows indicate cleavage zones. X 4,766.

Fig. 22. Portions of post-cleavage merosporangia showing individual spores. X 7,885.

Fig. 23. Ampulla with merosporangia. Arrows indicate cleavage zones. X 2,133.

Fig. 24. Single complete cleavage zone. Electron transparent spore wall layer is formed between the spore plasmalemma and the merosporangial lining layer (arrows). X 28,125.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MSW
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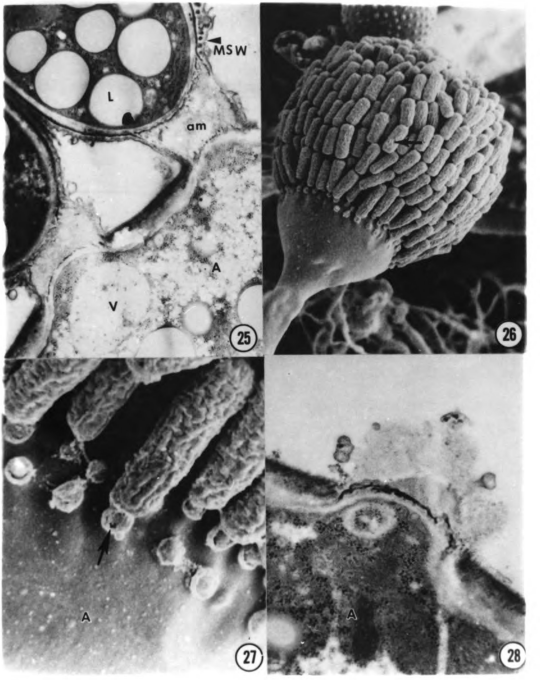
Figs. 25-28. Ultrastructural development of post-cleavage merosporangia and spore droplet formation in Syncephalis sphaerica.

Fig. 25. Basal attachment of merosporangia to ampulla at post-cleavage. X 28,636.

Fig. 26. Ampulla with mature spores. Arrow indicates merosporangial wall fragments. X 1,125.

Fig. 27. Ampulla surface with collar surrounding merosporangial scar (arrow). X 5,625.

Fig. 28. Merosporangial scar on ampulla surface. X. 43,636.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spore detachment. The new wall resembled the end walls of each spore in the merosporangium but did not appear to have its origin in the electron opaque outer ampulla wall. The inner electron transparent wall layer of the ampulla was continuous with the inner wall layer of the scar (Fig. 28).

As the outer merosporangial wall broke down, the basal spore in the row became detached from the ampulla leaving a small collar of merosporangial fragments surrounding the scar (Fig. 27). At this time, the other two spores in the merosporangium became detached from each other at the abscission zones, but usually remained in a row or clump due to adherence of remnant merosporangial wall fragments (Figs. 26, 29). A spike-like projection, most likely remnants of the amorphous substance present in the base of each merosporangium during cleavage, remained attached to the scar area (Figs. 29, 30, 31). At the time of spore detachment, the amorphous material disintegrated in the tip of each merosporangium as well as in each cleavage zone. After spore detachment, the ampulla became filled with a large vacuole and the upper surface remained marked with the merosporangial scars (Fig. 32).

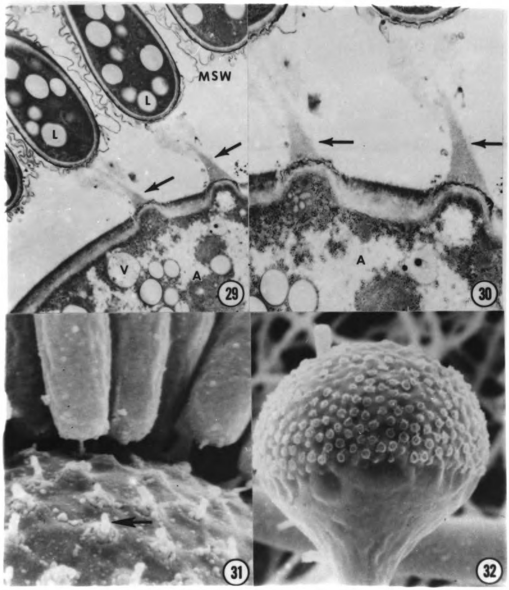
Figs. 29-32. Ultrastructural development of spore detachment in the asexual reproductive structures of Syncephalis sphaerica.

Fig. 29. Surface of ampulla with merosporangial scars and projections (arrows). Note merosporangial wall fragments. X 13,125.

Fig. 30. Detail of ampulla surface with scars and projections (arrows). X 27,461.

Fig. 31. Surface of ampulla with merosporangial scars. Arrow indicates projection from center of scar. X 8,000.

Fig. 32. Ampulla surface after spore detachment showing merosporangial scars on top half. X 1,600.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DISCUSSION

Unlike other members of the Piptocephalidaceae which have been studied (with the exceptions of Piptocephalis xenophila parasitizing Penicillium waksmani and other Deuteromycetes (Dobbs and English, 1954) and Syncephalis wynneae on Wynnea macrotis (Thaxter, 1897)) Syncephalis sphaerica can parasitize some non-mucoraceous hosts. Based on the abundance of parasite sporulation, however, these non-mucoraceous hosts did not appear to be as suitable as the mucoraceous hosts. Parasitism of non-mucoraceous hosts was limited to one mycelial yeast and one pseudomycelial Blastomycete; Endomyces tetrasperma and Sporobolomyces salmonicolor, respectively, and the hyphomyceteous Aureobasidium pullulans yeast-like colony. The preference for a mycelial growth habit in the host may reflect the mode of parasitism of Syncephalis species, i.e., appressoria and extensive, highly branched, intracellular haustoria.

In contrast, Piptocephalis species produce rather delicate, restricted haustoria (Benjamin, 1959), However, no other speices of Piptocephalis or Syncephalis have

been found to parasitize mycelial or cellular yeasts. The ability to produce very limited vegetative growth, but failure to sporulate on some of the cellular yeast forms, such as Saccharomycopsis lipolytica, may also reflect the necessity of a mycelial growth habit in the host to adequately support the extensive haustoria of S. sphaerica. However, S. sphaerica did produce vegetative hyphae on other mycelial yeasts such as Cephaloascus fragrans and Dipodascus uninucleatus without sporulating. The mycelial growth habit, then, is not the prime consideration for success of parasitism and sporulation. There must be some factor or factors which the parasite requires of the host for completion of its reproductive cycle.

Syncephalis californica (Hunter and Butler, 1975) is the only other member of the genus Syncephalis which has been extensively studied as to its host range. Here, only members of the Mucorales were found to be susceptible to parasitism. Although S. californica conforms more closely to the definition of the characteristics of the family Piptocephalidaceae than those observed by this author for S. sphaerica, there are 44 or more species of Syncephalis described in the literature (Hunter and Butler, 1975) which should be tested before conclusions are drawn as to the host range of the genus in general.

The only host which S. sphaerica appeared to adversely affect was Pilobolus umbonatus. This is most likely related to the method of forcible discharge of

sporangia found in Pilobolus. The aerial hyphae of Syncephalis which encased the sporangia and the sporangiophores of Pilobolus prevented discharge of the sporangia and presumably altered the completion of the natural life cycle of Pilobolus. It is not known whether S. sphaerica affects Pilobolus in this same manner in a natural situation. However, Buller (1934) did report that several genera, including several species of Syncephalis, do parasitize Pilobolus in the wild. Buller also reported observations on the parasitism of Pilobolus sp. by Syncephalis nodosa. Here, as with S. sphaerica, the parasite encased the sporangiophore of Pilobolus stopping any further development of the host.

The mode of parasitism of S. sphaerica is similar to that described by Benjamin (1959) as being typical of the genus. It differs from observations of the parasitism of hosts by Piptocephalis sp. in that S. sphaerica will attack aerial portions of the host, especially sporangiophores. Piptocephalis sp. also generally demonstrate a marked tropism toward host hyphae (Armentrout and Wilson, 1969; Berry and Barnett, 1957), whereas, no such tropism was observed in S. sphaerica. S. sphaerica produces larger appressoria than is reported for species of Piptocephalis. Hunter and Butler (1975) also report large appressoria for S. californica. Unlike S. californica, however, S. sphaerica did not produce the giant hyphal swellings reported by Hunter and Butler.

On the basis of the ability to produce limited growth and sporulation on Ellis' medium, S. sphaerica, as well as other species of Syncephalis reported by Ellis (1966), might best be classified as facultative hyperparasites. Components of the Ellis' medium have not been analyzed to determine which factor(s) promotes the growth and sporulation of the parasite away from the host. However, the Ellis' medium does not appear to be as complete a nutrient base as a mucoraceous host, as demonstrated by a gradual decrease in sporulation with continual culture, in contrast to the culture on a living mucoraceous host. Hunter and Butler (1975) reported similar results for S. californica on the Ellis' medium.

In comparison to S. sphaerica as well as several other species of Syncephalis discussed by Ellis (1966), several reports have also described the limited ability of Piptocephalis virginiana and Piptocephalis sp. to grow axenically (Berry and Barnett, 1957; Leadbeater and Mercer, 1957; Benjamin, 1959). In these species, limited mycelium developed and occasionally dwarf, abnormal-appearing sporangiophores were produced on certain synthetic media. However, no further growth in the absence of a host was possible. In contrast, in these studies, first generation axenic spores of S. sphaerica germinated and produced mycelium. Sporulation was drastically decreased and was usually absent, however, in the second generation of axenic culture.

Manocha (1975) reported morphological and biochemical differences in the axenic-culture spores of P. virginiana when compared to spores produced from parasitic culture. In addition to basic morphological differences in the sporangiophores, the axenic culture spores of P. virginiana had decreased amounts of various lipids and either failed to germinate or to produce hyphae upon germination. S. sphaerica parasitic culture spores contained the characteristic terminal lipid globules found in P. virginiana, however, axenic culture spores also appeared to contain terminal lipid globules (in light microscopy) and did germinate and produce hyphae.

Absence of a diffusible growth factor or factors from host mycelium in the liquid culture employed is similar to that reported by Berry and Barnett (1957) for P. virginiana. Hunter and Butler (1975), however, did report successful but erratic growth of S. californica on autoclaved mycelium of Rhizopus oryzae. Except for vegetative growth on certain media, S. sphaerica, as well as all species of Piptocephalis studied by others, appears to require a living host mycelium in order to grow and reproduce for long periods of time. With further research, it may be possible to define a more complete synthetic medium to compensate for any deficiences present in artificial media and to maintain cultures of Syncephalis sphaerica and others without a living host.

S. sphaerica demonstrated a greater abundance in production of sporargiophores, interpreted as increased success of parasitism, when hosts were grown on increasingly carbon-rich media. This behavior is similar to that reported by Berry (1959) for Piptocephalis virginiana, and by Shigo et al. (1961) for P. xenophila. Shigo et al. also reported that the amount of available nitrogen in the medium greatly affected the parasitic activity. A high carbon concentration must be accompanied by an increase in nitrogen to prevent nitrogen starvation of the host. In these studies with S. sphaerica, the increased carbon was accompanied by the addition of yeast extract (to provide nitrogen) with a corresponding increase in sporulation of the parasite.

Barnett (1970) observed that Tieghemomyces parasiticus, Dispira cornuta, D. simplex, Dimaragaris verticillata, and D. bacillispora all utilized glycerol as the source of carbon, but were unable to utilize glucose. He suggested that the parasites may use intermediates of carbon which may only be found in an active host metabolism. This may explain the lack of ability in these parasites to grow axenically.

Although specific carbon sources or C/N ratios were not examined in this study, a basic change was noted in the success of the parasitic activity of S. sphaerica when hosts were grown on various media. Species of Syncephalis have not been examined as thoroughly as species

of Piptocephalis as to specific sources of carbon which they can utilize. Further research may provide more conclusive information on the effects of host nutrition on parasitism.

The effects of temperature on parasitism are very difficult to interpret. The parasitic development of S. sphaerica varied greatly with each host used at each temperature. Thamnidium elegans was used as a host for P. virginiana by Berry (1959) with nearly the same results as reported here for S. sphaerica. Berry reported that T. elegans was more susceptible to parasitism at 25°C than at lower temperatures. T. elegans also appeared to be more susceptible to parasitism by S. sphaerica at 25°C than at lower temperatures. Neither M. ramannianus or G. persicaria showed any appreciable increase or decrease in susceptibility with temperature change.

Light microscopy did not prove to be satisfactory in studying the development of the merosporangia in S. sphaerica. Based only on light microscopy, the development of S. sphaerica spores appears to be similar to that described by others for various species of Syncephalis (Bainier, 1883; Thaxter, 1897, van Tieghem, 1875, Vuillemin, 1902). However, essential to any discussion of spore development are such things as cleavage apparatus formation and development, and spore wall development. Electron microscopy was much more valuable for studying the ontogeny of merosporangiospores in S. sphaerica.

The cleavage process in merosporangia of Syncephalis sphaerica differed from that of Syncephalastrum racemosum as described by Fletcher (1972). Fletcher described cleavage in S. racemosum as being random, thereby giving a variety of spore numbers and sizes in each merosporangium. The cleavage apparatus itself consisted of "furrows or loops or rings" which fused to delimit the spore protoplasts. In S. sphaerica, cleavage occurred regularly so as to always produce three spores and cleavage itself was an invagination of the merosporangial plasmalemma, rather than a fusion of component parts.

Thaxter (1897) described these differences in spore development between Syncephalis sp. and Syncephalis pycnosperma and Syncephalastrum racemosum based on light microscopy. His developmental sequence for these two species of Syncephalis is remarkably similar to what has been reported here for S. sphaerica. Thaxter reported that the merosporangial protoplast was divided into spores by "gradually narrowing isthmuses," with walls forming after cleavage. The portion of the merosporangium between each spore was termed the "intermediary zone" which Thaxter reported as being deliquescent at the time of spore wall deposition. This zone also functioned in spore separation.

S. sphaerica is somewhat similar to Syncephalastrum racemosum, Thamnidium elegans (Fletcher, 1973) and Gilbertella persicaria (Bracker, 1968) in the formation

of the inner spore wall. The fibrillar, electron transparent spore wall in S. sphaerica is formed in post-cleavage between the spore plasmalemma and the electron opaque lining layer of the merosporangium, much like T. elegans and G. persicaria. However, in S. sphaerica, the lining layer is not derived from the cleavage apparatus as it is in T. elegans and G. persicaria, but is deposited uniformly within the merosporangial wall at pre-cleavage. Subsequent to spore formation in S. sphaerica, as in Syncephalastrum racemosum, the electron transparent wall layer of the merosporangium which was confluent with that of the ampulla was no longer discernable. However, unlike S. racemosum, the electron transparent wall layer in the ampulla appeared to remain intact.

The development of Piptocephalis species can be compared to that described here for S. sphaerica. Leadbeater and Mercer (1957) described the development of the merosporangiospores of Piptocephalis virginiana using light microscopy and Dobbs and English (1954) described the process of P. xenophila. Several basic differences occur between spore development in species of Piptocephalis and that described here for S. sphaerica.

One difference, visible with the light microscope, is that Piptocephalis species have dichotomously branched sporangiophores, whereas most species of Syncephalis have unbranched sporangiophores. Piptocephalis sp. have little or no terminal enlargement and merosporangia are borne on

small branches. S. sphaerica has merosporangia borne directly on terminal enlargements, although some species of Syncephalis have merosporangia borne on small branchlets on apical swellings (Benjamin, 1959). In those species of Piptocephalis with terminal enlargements, the enlargement is deciduous; whereas in Syncephalis, the enlargements are not.

Comparison of cleavage development between Piptocephalis species and S. sphaerica is difficult because no electron microscopic studies of Piptocephalis, the two genera appear to have similar methods of cleavage development. Leadbeater and Mercer (1957) described narrow transverse hyaline zones separating the merosporangia of P. virginiana into spore protoplasts. This may be analogous to the centripetal growth of the plasmalemma in S. sphaerica which would form a narrow hyaline zone as the abscission layer formed.

Perhaps the most striking difference between the development of spores in species of Piptocephalis and Syncephalis is the manner of the spore wall formation. Again, although electron microscopic evidence is not available for Piptocephalis, based on light microscopy for that genus, Leadbeater and Mercer described a thick deposit of wall material on each side of the cleavage zone in P. virginiana. This is in remarkable contrast to S. sphaerica which has no such deposit at the ends of the spores; and which in contrast actually has very little

spore wall deposition at the ends of the spores. This difference in spore wall deposition may account for the fact that S. sphaerica, as well as other species of Syncephalis, exhibit polar germination of spores; whereas polar germination of spores has never been observed in species of Piptocephalis (Benjamin, 1959).

The amorphous material occurring in the cleavage zones of Syncephalis sp. was reported by Thaxter (1897) as non-stainable regions in the "intermediary zones" which disappeared with spore wall formation. Marchant (1975) also reported the occurrence of the degenerating material during early conidium formation in Fusarium culmorum, but it was unclear as to whether this material had any function in spore dispersal. Benny and Aldrich (1975) reported an abscission vacuole formed below the septum in the pesudophialide of Linderina pennispora and postulated that the mottled appearance of this vacuole and part of the septum itself might have indicated a mucilaginous nature which could function in the spore droplet dispersal mechanism.

S. sphaerica produces a spore droplet at maturity. Although the amorphous material in the cleavage zones, perhaps originating from the electron transparent wall layer which disintegrates, appeared to be mostly disintegrated at the time of spore wall formation, the remaining material may be hygroscopic in nature and thereby aid in spore droplet formation. Fragments of the outer merosporangial wall are persistent around the spores and

may also be of a mucilaginous nature. Fletcher (1972) reported the occurrence of an amorphous material surrounding the chain of spores in the merosporangia of Syncephalastrum racemosum. S. racemosum is dry-spored at maturity, but the spores usually remain in rods when dispersed (Ingold and Zoberi, 1963).

Here, again, the differences in the method of the development of the spores in Piptocephalis and Syncephalis may account for the differences in the appearance of the spores at maturity. S. sphaerica has a wet-spore droplet at maturity whereas Piptocephalis virginiana is dry-spored. Further electron microscopic studies of both dry-spored and wet-spored species of Piptocephalis may allow more definite conclusions to be drawn as to the differences in the methods of each type of spore droplet formation.

Finally, a most conspicuous feature in the developing merosporangia and merosporangiospores of S. sphaerica is the presence of the numerous lipid globules. Lipid globules were especially evident at the sites of cleavage in the merosporangia. It may be possible that the lipids provided some type of energy source for cleavage development. However, no evidence to support this theory has been presented.

The study of various aspects of the biology of a mycoparasite, here Syncephalis sphaerica, may provide some insight into parasitism or at least an easily controlled experimental model for studying the host-parasite

relationship and those factors which affect it. Examination of the ultrastructural development of the merosporangia of S. sphaerica provides information which can be used in further defining the generic and familial taxonomic categories to which Syncephalis sphaerica belongs. It also provides information on a type of sporangiospore development not previously reported for the order Mucorales.

SUMMARY

1. Syncephalis sphaerica parasitized all genera of Mucorales tested. S. sphaerica also parasitized Endomyces tetrasperma, Sporobolomyces salmonicolor, and Aureobasidium pullulans. None of the Basidiomycetes tested supported growth of the parasite.
2. The mode of parasitism of S. sphaerica was similar to that described for other species of Syncephalis. Large appressoria and extensive, highly branched haustoria were formed.
3. Axenic culture of the mycoparasite was partially successful, with dense mycelial growth and some sporulation on selected media.
4. S. sphaerica had no adverse effects on the growth and sporulation of any host with the exception of Pilobolus umbonatus.
5. No zygospores were induced in S. sphaerica.
6. Merosporangial development was studied with the transmission and scanning electron microscopes. Three spores were cleaved out of each merosporanigal protoplast by invaginations of the merosporangial plasmalemma. The completed spore had 2 wall layers

with parts of the outer merosporangial wall attached to the lateral spore walls. A wall was laid down on the surface of the ampulla at the base of each merosporangium forming a scar. Spores remained associated with the ampulla at maturity as a liquid spore droplet.

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