Durham E-Theses

A study on an isolate of Syncephalis from Leicester

Bawcutt, R. A.

How to cite:

Bawcutt, R. A. (1966) *A study on an isolate of Syncephalis from Leicester*, Durham theses, Durham University. Available at Durham E-Theses Online: http://etheses.dur.ac.uk/10180/

Use policy

The full-text may be used and/or reproduced, and given to third parties in any format or medium, without prior permission or charge, for personal research or study, educational, or not-for-profit purposes provided that:

- a full bibliographic reference is made to the original source
- a link is made to the metadata record in Durham E-Theses
- the full-text is not changed in any way

The full-text must not be sold in any format or medium without the formal permission of the copyright holders.

Please consult the full Durham E-Theses policy for further details.

Academic Support Office, The Palatine Centre, Durham University, Stockton Road, Durham, DH1 3LE
e-mail: e-theses.admin@durham.ac.uk Tel: +44 0191 334 6107
http://etheses.dur.ac.uk

A Study on an isolate of *Syncephalis* from Leicester

Thesis submitted for the M.Sc. degree of the Faculty of Science, University of Durham,

1966.

R. A. Bawcutt. B.Sc. (Dunelm), M.I.Biol.

DURHAM UNIVERSITY SCIENCE
20 OCT 1966
SECTION LIBRARY

Acknowledgments.

It is with gratitude that I acknowledge my debt to G. Leadbeater, who first introduced me to the genus Syncephalis with all its facets and problems and who generally supervised the course and content of the work. This thesis could never have appeared without his continual encouragement.

My thanks are also extended to P. Pulford and M.Richardson for allowing me to examine their various cultures and isolates and to all, especially C.K. Mercer and W. Morris, for invaluable discussions on problems involved.

I would also acknowledge the help, for some of the translations of original papers, of the following:-
Mrs.R.Szabo (German), C.Parker (Portuguese and Italian) and D.E.Caulton (Russian).

Part I	Introduction	p. iv
Part II	Materials and Methods	p. 30
Part III	Experimental results.	p. 41
Part IV	Discussion	p.108
Appendix I		p.116
Appendix II		p.122
Bibliography		p.132

PART I.

Part I. Introduction.

a. Range of variation of taxonomic features within the genus *Syncephalis* p. 1

b. Validity of some species of *Syncephalis* p. 19

c. Discovery of *Syncephalis* and classification according to ideas on the nature of the asexual apparatus p. 21

d. Ecology of *Syncephalis* p. 26

e. Nature of parasitism of *Syncephalis* p. 27

f. Object of the investigation p. 29

(a) Range of variation of taxonomic features within the genus *Syncephalis*

Since 1873, when the genus was first erected by van Tieghem and Le Monnier (1873), 36 species of *Syncephalis* have been described. The validity of some of these species has been questioned but no authoritative account of the genus exists to resolve such problems.

A list of the species of *Syncephalis* described to date, is given in Table 1.

Table 1. List of species of Syncephalis

S. cordata	van Tieghem & Le Monnier	1873
S. cornu	" "	"
S. asymmetrica	" "	"
S. depressa	" "	"
S. minima	" "	"
S. reflexa	van Tieghem	1875
S. ventricosa	"	"
S. sphaerica	"	"
S. nodosa	"	"
S. fasiculata	"	"
S. intermedia	"	"
S. ramosa	"	"
S. tetrathela	"	"
S. nigricans	van Tieghem	1876
S. furcata	"	"
S. pendula	"	"
S. fusiger	Bainier	1882
S. curvata	"	"
S. cordata var. minor	Schroeter	1886

Table 1. cont.

S. unnamed	Marchal	1891
S. pycnosperma S. wynneae S. tenuis	Thaxter " "	1897 " "
S. glabra	Morini	1902
S. aurantiaca	Vuillemin	1902
S. adunca	Vuillemin	1903
S. bispora (now Syncephalopsis)	Raciborski Boedijn	1909 1959
S. tranzschelii	Naumov	1939
S. nana	Dade	1937
S. tengi	Ou	1940
S. ubatubensis	Viegas & Teixeira	1943
S. truncata	Boedijn	1959
S. fuscata S. obconica S. penicillata S. rapacea	Indoh " " "	1962 " " "

Further, it is to be noted that Thaxter (1897), Povah (1917), Buller (1934), Ginai (1936), Warcup (1951), Benjamin (1959), Drechsler (1961), Indoh (1962) and Leadbeater and Richardson (1965), have all observed or isolated undescribed species of Syncephalis.

The free mycelium of Syncephalis consists of extensively branched hyphae, with triangular, circular or oval anastomoses. The hyphae are very thin, being usually less than 1 μ in diameter. They are not usually septate, although Bainier (1882) reported the presence of cross-walls in S. fusiger and Benjamin (1959) considered them to develop with age.

The mycelium forms an extensive web not only over the surface of the medium but also over the entire host plant and even extends on to the damp surfaces of the containing petri-dish. This web, in turn, gives rise to erect branches. The mycelium is white or yellow in colour and its presence can easily be seen with the naked eye.

The free mycelium is maintained by the extensive mycelial (haustorial) system of the parasite within the host. The entry of the parasite into the host has been described by van Tieghem (1875), Zopf (1888), Buller (1934) and Benjamin (1959). The Syncephalis hyphae in contact with the host often produce irregularly swollen appressoria (Fig. 1A). From the inner surface of the appressoria, one or two haustoria penetrate the host cell-wall and sometimes form further appressoria just inside the host. The haustoria form an extensive internal hyphal system throughout the whole host plant. The haustoria are usually somewhat greater in diameter than the vegetative hyphae. At various points, the internal haustoria emerge and continue growth as the extensive free mycelium.

The free mycelium forms rhizoids either on its aerial parts or where

it comes into contact with the host hyphae or the surface of the medium. A good description of typical rhizoidal development in the genus has been given by Vuillemin (1902). A mycelial branch extends, acquires a diameter larger than normal and dichotomises once (Fig. 1B). One of the branches so formed becomes more developed but remains simple or becomes once branched; the other dichotomises two or more times to form a "rosette". Erect branches of the rosette often elongate to form sporophore(s), whilst the remaining branches constitute the rhizoidal system.

The rhizoids (Figs. 1C-E, 2A-C) vary from 2 - 8 µ in diameter and in many species, but not all, are septate. The rhizoidal system is considered as acting as a nutritive reservoir for the developing sporophore(s).

As the taxonomy of the genus is based mainly on the morphology of sporophore, it is necessary to dwell on its typical form and characteristic variations.

The extremity of a developing sporophore swells to form a spherical to turbinate head (Fig. 1C-E). Within the genus, a diameter range from 9.5 - 18 µ in S.obconica to 100 µ in S. cordata is shown by such heads. Sporophore lengths, also, show a wide range. In S. tetrathela they are 40 - 50 µ long, in S. cordata they can be 3 mm. in length. A sporophore axis can exhibit a uniform diameter, but characteristically it shows basipetal attenuation which can be most marked.

Usually, only one of the rhizoidal branches elongates to form a solitary sporophore. In S. fasiculata, and occasionally in other species, two or more of the rhizoidal branches elongate to form fasicles of

Fig. 1.

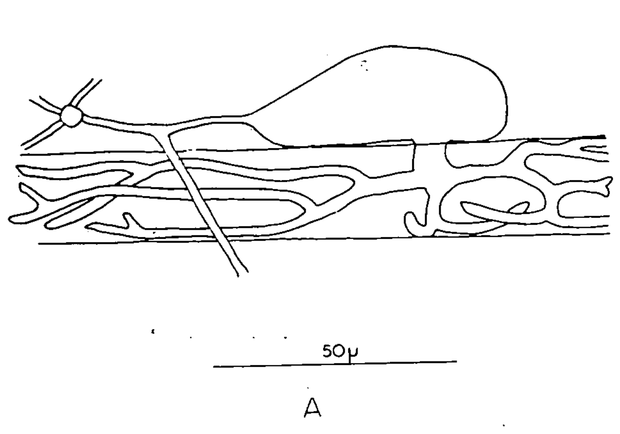
A. Syncephalis sp. Development of appressorium and penetration of host. (Redrawn from van Tieghem 1875)

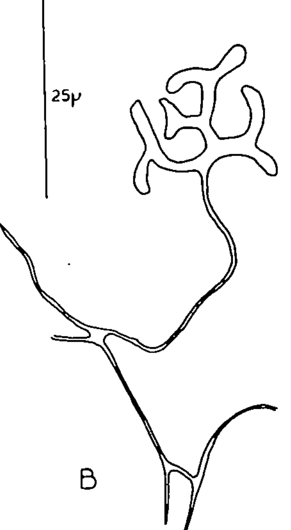
B. S. nodosa. Rhizoidal system (Redrawn from Vuillemin 1902)

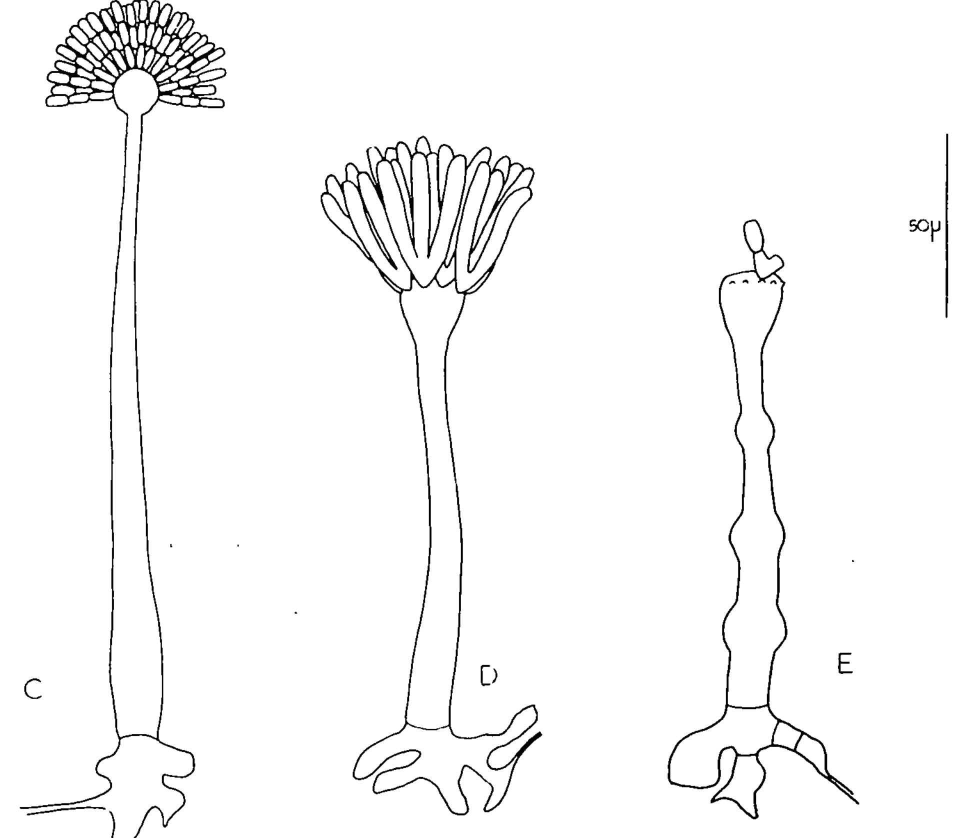
C. S. sphaerica. Sporophore with rhizoids and basal septum. The head is spherical and bears, over 2/3 of its surface, simply inserted, radiating merosporangia (Drawn in section)

D. E. S. nodosa. Sporophore in D. is young and smooth, but at maturity, develops nodes (E). Head is turbinate and bears branched merosporangia in a distal ring.

FIG. 1.







sporophores.

In S. fusiger, S.intermedia, S. pendula, S.pycnosperma, S. rapacea, S. sphaerica, S. tenuis, and usually in S. depressa and S. wynneae, the sporophore is separated from the rhizoids by a basal septum (Fig. 1C - E).

All species have simple, undivided sporophores except S. furcata in which it is once divided. Such a dichotomy of occasional sporophores has also been recorded in S. penicillata, S. fuscata and S. depressa (Indoh 1962. Mehrotra and Prasad 1964). S. furcata has been considered (van Tieghem 1876) as a possible link between the simple sporophores of Syncephalis and the highly dichotomised sporophores of Piptocephalis.

S. nodosa has a simple sporophore, which at first is smooth along its length, but which characteristically developes, at maturity, from two to five annular nodes with longitudinal striations. (Fig. 1 D - E).

In the five species, S. cornu, S. nigricans, S. reflexa, S. curvata and S. adunca (the series Cornutae of Vuillemin 1902), the sporophore is variously recurved (Fig. 2 A.B.), whilst S. glabra is intermediate in having an upright sporophore initially which becomes recurved later in development.

A large swelling in the basal region of the sporophore characterises S. ventricosa, while one in the curved neck region is a feature of S. adunca (Fig. 2 B. C.)

Radiating from the head of the mature sporophores are simple or branched merosporangia. Developmentally, these emerge as papillae which gradually elongate to form long, thin cylindrical outgrowths (merosporangia) which, according to species, become variously branched.

Fig. 2.

A. S. cornu. Recurved sporophore. Half the head is covered by simply inserted merosporangia, which grow vertically to form a cylindrical mass.

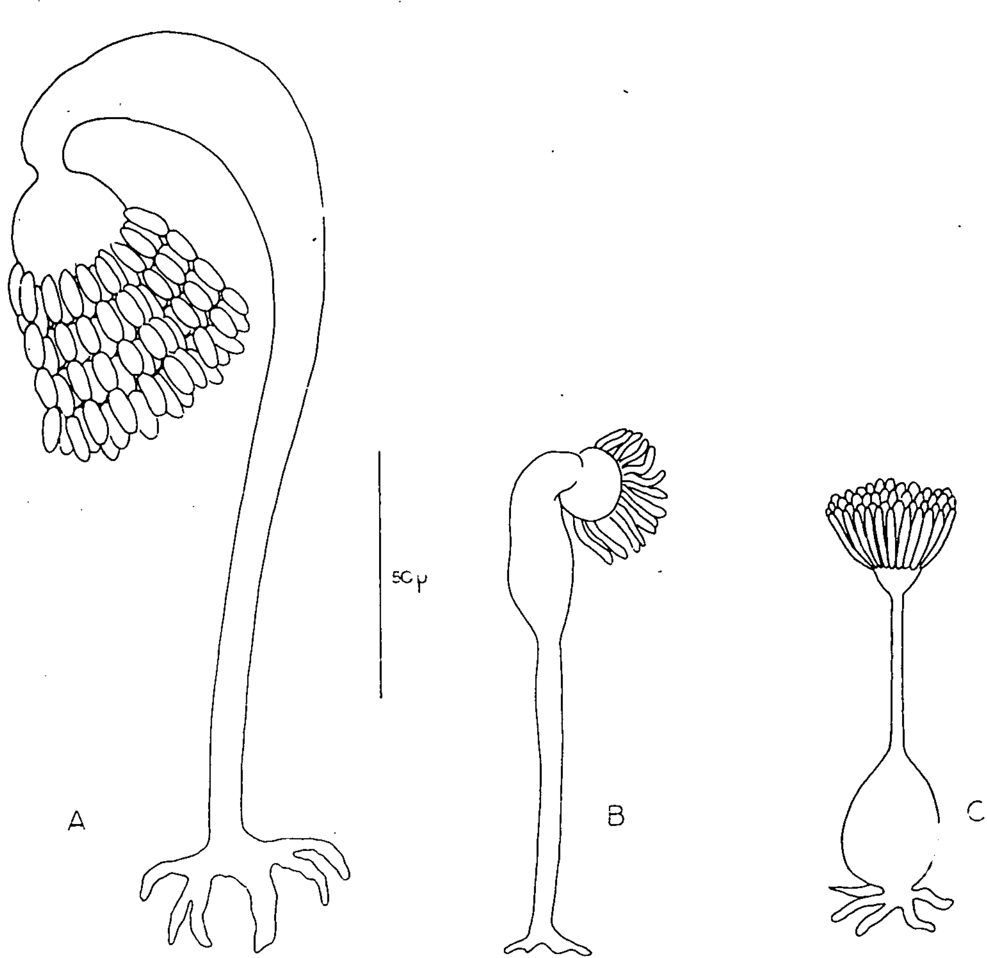
B. S. adunca. Recurved sporophore, with a swelling in the neck region. (Redrawn from Vuillemin 1903).

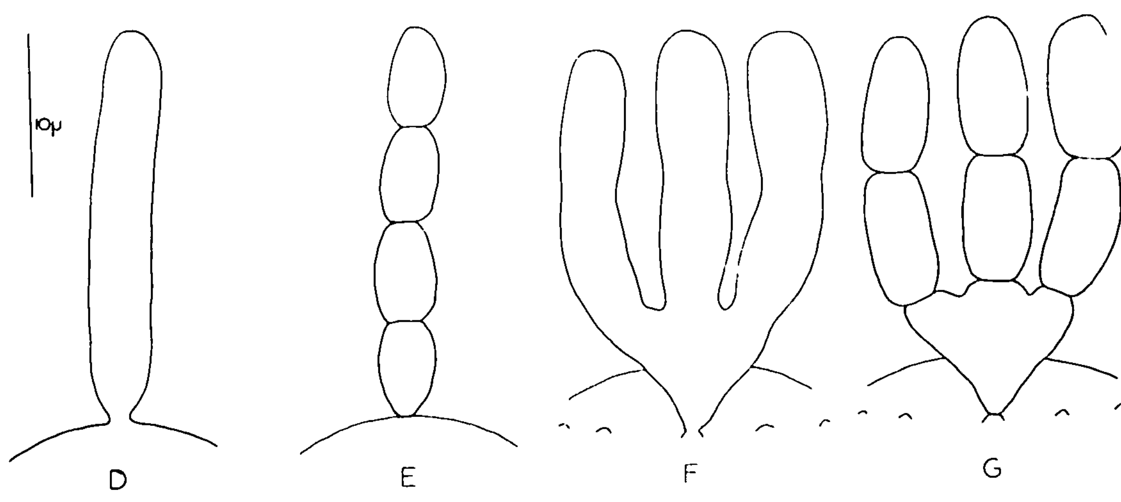
C. S. ventricosa. Upright sporophore, with a swelling in the basal region. (Redrawn from van Tieghem 1875).

D.E. S. cornu. Simply inserted merosporangium (D), forming its complement of uniform spores.

F.G. S. nodosa. Three-branched merosporangium (F), forming spores (G). The spore formed in the basal trunk region is thus of different size and shape from those formed in the rest of the merosporangium. Points of attachment, in a distal ring, of other merosporangia can be seen.

FIG. 2.





These merosporangia, at maturity form chains, or branched chains of spores. In S. adunca, S. cornu, S. curvata, S. furcata, S. nana, S. fuscata, S. nigricans, S. obconica, S. pendula, S. rapacea, S. reflexa, S. sphaerica, S. tenuis, S. tranzschelii and S. ventricosa the merosporangia are simple, being inserted directly on the head of the sporophore (Figs. 1 C. 2 A.D.E. 4 C). The spores produced in each merosporangium are all alike and all capable of germination. Branched merosporangia show some variety. They are sometimes dichotomised to give a short trunk and two elongated apical branches as in S. asymmetrica, S. cordata and S. tetrathela. In S. aurantiaca, S. nodosa, S. minima and sometimes in S. penicillata, two or more branches arise from the trunk (Figs. 1 D.E. 2 F.G.). In all, the trunk is only large enough to accommodate the formation of a single basal spore, which, because of its nodal position is a-typical in form and sometimes in size. Symmetrically cordate, asymmetrically cordate or irregular shapes are normal in such basal spores. (Fig. 3A).

In S. intermedia, S. fasiculata, S. ramosa, S. tengi and S. penicillata, both simple and branched merosporangia occur.

The branched merosporangia of S. depressa and S. penicillata are worthy of mention. (Fig. 3 B.C.) Here, laterally outgrowing merosporangia undergo dichotomies arranged to give a monopodial system consisting of an apparent horizontal portion bearing two to five vertical branches. In each dichotomy, one branch continues horizontally whilst the other becomes vertical. One to four such dichotomies may occur before the horizontal branch turns up vertically at its distal end. The horizontal branch eventually forms from one to three irregular

Fig. 3.

A. *S. nodosa*. Irregular and regular basal spores.

B.C. *S. depressa*. Merorporangia have grown out laterally and then formed vertical branches (B). In C, the lateral branch has formed an irregular spore, whilst normal, regular spores have been produced by the vertical branches. Points of attachment of other merosporangia, in a distal ring, can be seen.

D.E. *S. ramosa*. Dichotomy of merosporangium (D). The spores formed at the points of dichotomy are irregular (E).

F. *S. fusiger*. Basal portion of branched merosporangium is sterile and remains on the head after the detachment of the spore chains. (Redrawn from Bainier 1883).

G. *S. pycnosperma*. Sterile secondary sporophore bearing two merosporangia. (Redrawn from Thaxter 1897).

H. *S. wynneae*. Sterile secondary sporophore bearing twelve merosporangia (Redrawn from Thaxter 1897).

FIG. 3.

10μ

A

B

50μ

C

D

25μ

E

F

25μ

G

50μ

H

spores.

Further complexity is added by the dichotomy of the vertical branches of some of the merosporangia of S. ramosa and S. penicillata, the spores subsequehtly formed at this point being irregular in shape (Fig. 3 D.E.)

Although Benjamin (1959) demonstrated the germination of basal spores in S. nodosa, the capacity of such spores for germination throughout the genus has often been questioned. This is particularly pertinent in considering the condition which arises in, for example, S. fusiger. Here, the basal nodal portions of the merosporangia are sterile and remain attached. (Fig. 3 F.) After the detachment of the spore chains, the sporophore head thus bears a series of sterile cordate appendages. In S. pycnosperma, S. wynneae and S. ubatubensis, the sporophore heads bear distinct and large sterile structures (secondary sporophores, Thaxter 1897), which in turn bear merosporangia. The secondary sporophores of S. pycnosperma support two to four merosporangia each (Fig. 3G), while those of S. wynneae twelve merosporangia each (Fig. 3H). These secondary sporophores remain in position on the head after the spores have become detached.

The question therefore has arisen as to whether or not basal spores, sterile appendages and secondary sporophores represent a continuous series of sterility and enlargement.

The distribution of merosporangia over the surface of the sporophore head varies. In most species, a half or two-thirds of the distal surface bears them (Figs. 1C. 2A); in some others, they arise from the entire surface. S. aurantiaca differs from these latter in that the

crown of the sporophore head is bare of merosporangia. S. nodosa, S. depressa and S. obconica also show bare crowns, but the merosporangia are arranged in distal rings (Figs. 1 E. 2 F.G. 3 B.C. 4 A.) S. tetrathela is quite unusual in having four merosporangia only, arising equidistantly in a distal ring.

Merosporangia either radiate from the head to form collectively a large spherical mass (e.g. S. sphaerica Fig. 1 C), or all grow vertically to give a cylindrical mass (e.g. S. cornu Fig. 2 A). Because of their unusual peripheral merosporangia, S. depressa and S. penicillata have cylindrical masses of much greater diameter than their sporophore heads (Fig. 4 B). Uniquely, in S. pendula, the merosporangia are gracefully pendulous (Fig. 4 C).

The number of merosporangia developed per head varies considerably, S. tetrathela having the least, four merosporangia per head.

In some species, at maturity, the mass of spores coalesces within a liquid droplet. These species are known as "wet-spored". This has been reported by van Tieghem and Le Monnier (1873) and Oudemans (1897) generally, by Thaxter (1897) for S. wynneae, by Benjamin (1959) for S. cornu, S. nana and S. nodosa and by Ingold (1963) for S. cordata. The spore droplet dries on exposure to air and the spores become cemented to the sporophore head.

The number of spores produced in each merosporangium varies from two in S. fusiger, S. nana, S. tenuis and S. wynneae, to twenty to forty in S. pendula. The spores are generally cylindrical, oval or barrel shaped, although those of S. tetrathela and S. ventricosa are

Fig. 4.

A. S. depressa. Sporophore head showing points of attachment of merosporangia, in a distal ring.

B. S. depressa. Merosporangia growing more or less vertically from the head, in a cylindrical mass.

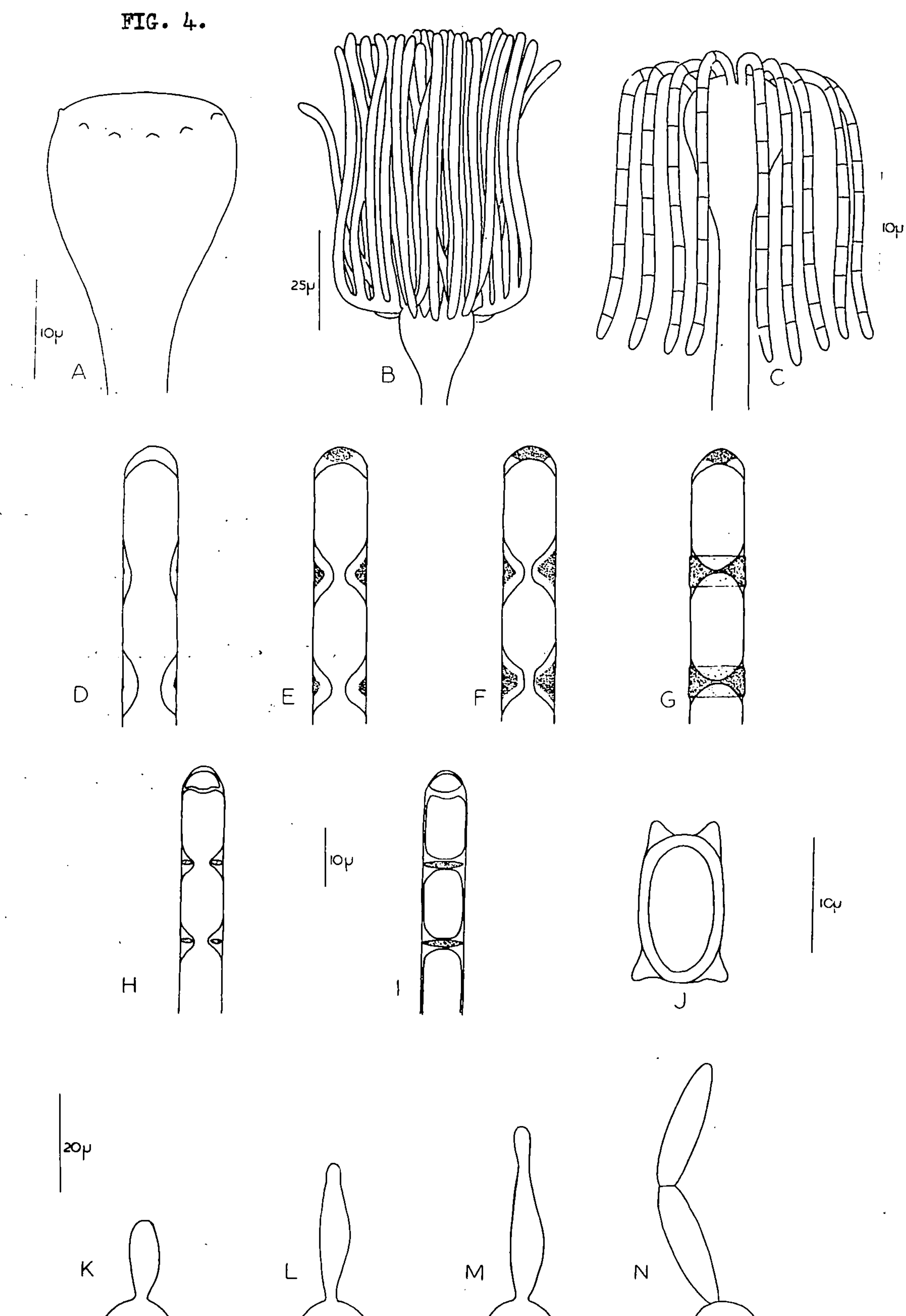
C. S. pendula. Pendulous merosporangia, with simple attachment to the head. (Redrawn from van Tieghem 1876)

D-G. Merosporangia, showing stages in spore formation, in an unknown Syncephalis from Liberian soil (Redrawn from Thaxter, 1897)

H-J. S. pycnosperma. H-I, spore formation.
J, mature spore. (Redrawn from Thaxter 1897)

K-N. S.tenuis. Spore formation by a process of "budding" (Redrawn from Thaxter 1897)

FIG. 4.



spherical and those of S. nana, S. tenuis and S. ubatubensis are fusiform. S. pendula has the smallest spores (4 x 2 μ) and S. tenuis the largest (25 x 7 μ). Within the genus, spores are either colourless, yellow or brown. Full colour is only seen when the spores are viewed in mass.

The steps leading to the formation of spores in the merosporangia of some species of Syncephalis was the subject of a careful study by Thaxter (1897). Thaxter first studied the process in an unknown Syncephalis, somewhat resembling S. cordata and isolated from Liberian soil (Fig. 4 D - G). By progressive centripetal differentiation within the merosporangia, the protoplasts of future spores are segregated by biconcave separation layers of interstitial protoplasm. The segregated protoplasts acquire walls, readily seen between adjacent spores but laterally not readily distinguishable from the merosporangial wall. Each separation layer becomes differentiated so that only peripherally are they stainable with eosin. Further, those portions of the merosporangial wall in contact with the separation layers become raised, probably by deliquescence, above the level of the merosporangial wall elsewhere, so that each merosporangium at this stage is banded in appearance. From Thaxter's diagrams and observations on other Syncephalis species, it can be inferred that the spores would be separated by dissolution of the separation layers and their associated bands of merosporangial walls.

A similar endogenous formation of spores was also noted by Thaxter for S. wynneae and S. pycnosperma, with some notable differences, (Fig. 4 H. I.). Here, the merosporangial walls never show a banded

appearance and do not only undergo dissolution. The separation of spores is achieved by a combination of the dissolution of separation layers and fragmentation of the merosporangial wall. This leads to a distinctive feature whereby cylindrical spores with rounded ends are contained within open-ended cylindrical fragments of the merosporangial wall (Fig. 4 J). However, the process of spore formation in the two-spored merosporangium of S. tenuis was shown by Thaxter to be distinctive. Here, the merosporangial portion eventually forming the distal spore, buds from the merosporangial portion corresponding to the basal spore, but only after the latter portion has become almost fully formed (Fig. 4 K - N).

The descriptions of spore formation in S. nana by Dade (1937) resemble that process in S. tenuis. However, Benjamin (1959) illustrates the spore formation in S. nana more indicative of the normal method of spore septation in the genus.

Not only are there two methods of spore formation, but also two methods by which the spores are fragmented from the sporangial filament. In some species, the merosporangial walls rupture and/or gelatinise locally between each spore, thus separating the spores from each other, but with the merosporangial wall persisting elsewhere around the spores. This persistent and often corrugated wall can be seen in S. adunca, S. asymmetrica, S. intermedia, S. nodosa, S. pycnosperma, S. ramosa, S. sphaerica and possibly in S. cornu and S. tetrathela. In other species, eg. S. penicillata and S. fuscata, the merosporangial wall undergoes almost complete dissolution, leaving faint spots or processes only on the spore wall.

Other methods of asexual reproduction shown by Syncephalis involve the production of stylospores and chlamydospores. That stylospores could be produced by all species of Syncephalis under favourable conditions was stated by van Tieghem (1875), however, only in S. nodosa S. reflexa, S. curvata, S. intermedia and S. glabra have they been observed to date. Stylospores are produced singly, on short stalks, growing perpendicularly to the main axes of slightly thickened, short mycelial lengths, which can be isolated by septa (Fig. 5A). The stylospores are spherical, spiny, 6 μ diameter in S. nodosa and capable of producing the typical Syncephalis mycelium on germination. In S. reflexa, they have also been shown to germinate by means of three to four germ tubes.

Chlamydospores have been described by Bainier (1882) for S. curvata, but only when vigorously parasitising Rhizopus nigricans (Fig. 5 B. C.).

They can be produced either by the aerial mycelium or the mycelium inside the host plant and can be intercalary or terminal. They are spherical, 21 μ in diameter, with a thick wall covered with spines 6 μ long.

Sexual reproduction has been described for S. cornu, S. curvata, S. depressa, S. glabra, S. nddosa and S. reflexa, the process first being described by van Tieghem (1875) for S. cornu. In this species, the end of a thin hypha swells and dichotomises irregularly as though it would later form a sporophore. Two erect, parallel branches enlarge distally into heterogametangia segregated by septa. A zygospore arises as a distal bud from fused gametangia. There is no total involvement of

Fig. 5.

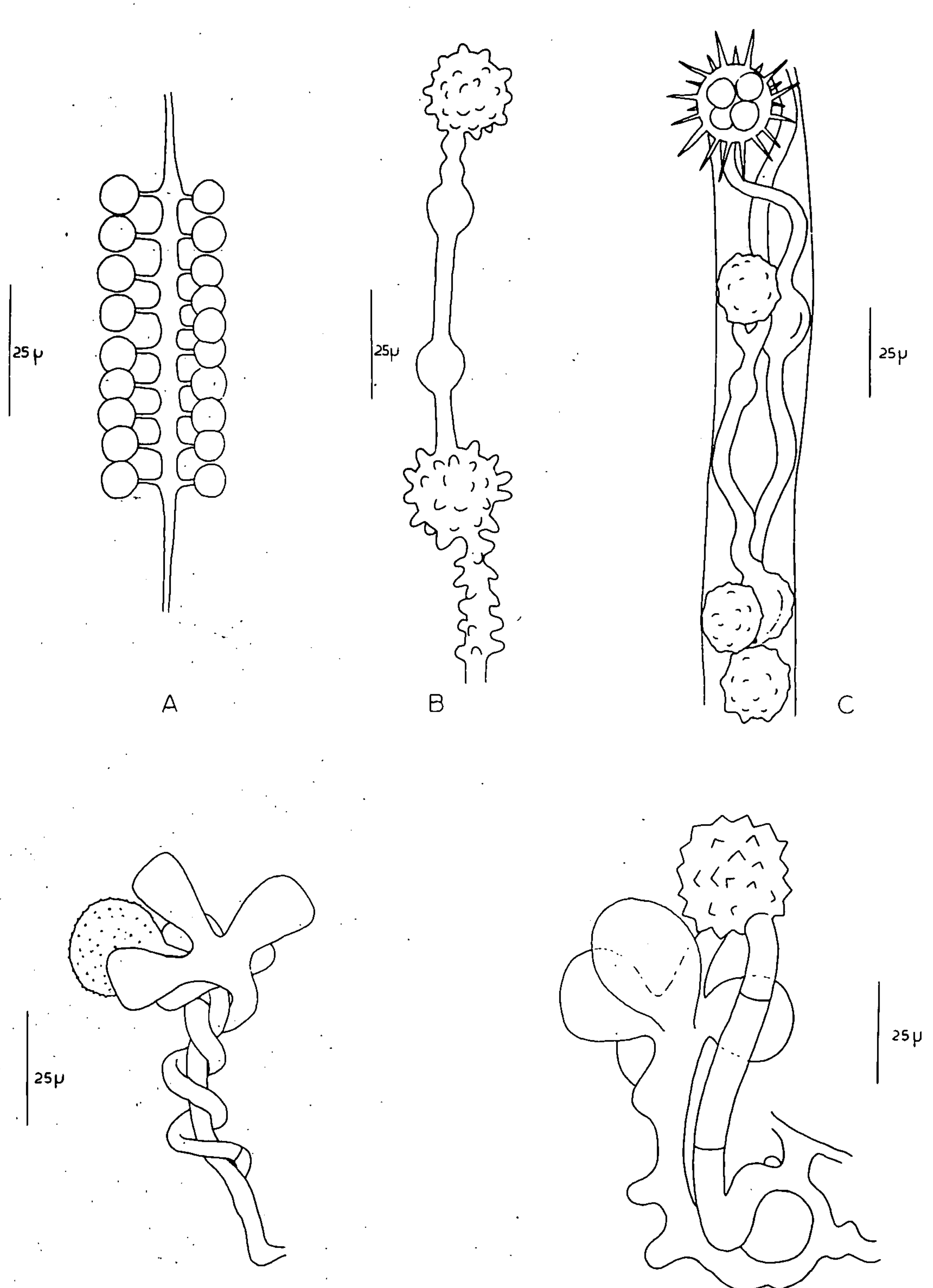
A. *S. reflexa.* Stylospores. (Redrawn from van Tieghem 1875)

B.C. *S. curvata* Chlamydospores produced when parasitising *Rhizopus nigricans*. Formed on aerial mycelium (B), or on internal mycelium (C). (Redrawn from Bainier 1883).

D. *S. nodosa.* Zygospore. One copulating branch spirally twisted around the other, the zygospore budded off from the distally delimited gametangium. Ampoules developed. (Redrawn from Thaxter 1897).

E. *S. curvata.* Zygospore, with ampoules developed at the base of one of the copulating branches. (Redrawn from Bainier 1883).

FIG. 5.



the fused gametangia in zygospore formation, distinct gametangial remnants being left during the segregation of the zygospore by septa arising within the gametangia. The zygospore wall becomes thickened, covered with conical warts and is about 25 - 30 µ in diameter. The walls of the gametangia also become thickened and remain attached to the zygospore after the rest of the mycelium has disintegrated. On germination, a typical sporophore develops directly without mycelial and rhizoidal formation.

Bainier (1882) showed the zygospores of S. curvata to be formed in a like manner but, with the development at maturity, of large ampoules or vesicles at the base of the copulating branches (Fig. 5 E), an observation also noted by Thaxter (1897) for S. cornu and missed by van Tieghem.

An outstanding type of zygospore formation was also first described for S. nodosa by Bainier (1882) and later commented upon by both Vuillemin (1886) and Thaxter (1897). The two copulation branches are of equal diameter, one spirally twisted around the other (Fig. 5 D). In both, the gametangia are segregated distally and, unusually, after fusion, the zygospore is budded from the spiral gametangium at a point near the septum separating the gametangium from the suspensor. Formation in groups of between four and twenty and swollen ampoules also characterise this species. Additional reports by Thaxter (1897) for S. reflexa, by Morini (1902) for S. glabra and by Christenberry (1940) for S. depressa, serve to emphasise the distinctive formation in S. nodosa and that with other zygospores, size, colour and wall architecture are variable features within the genus.

(b) Validity of some species of Syncephalis

The validities of several species of Syncephalis erected have been variously doubted by several authors. Thus, the opinion of Schroeter (1886), supported by Berlese and de Toni (1888) that S. asymmetrica was synonymous with S. cordata var. minor has been opposed by Vuillemin (1902), Zycha (1935), Fischer (1892) and Naumov (1935). Indoh (1962) complicated the issue further by considering S. intermedia, S. ramosa, S. tengi and S. cordata var. minor all to be synonyms of S. asymmetrica.

Again, although clearly separated by Bainier (1883) and Vuillemin (1903), S. curvata and S. cornu were considered synonyms by Schroeter (1886), Berlese and de Toni (1888), Moller (1901) and Indoh (1962), whilst the former was inadmissable either to Zycha (1935) or Naumov (1939). The issue was again made complicated by the opinions of Indoh (1962) and Zycha (1935). The former also considered S. cornu and S. nigricans to be the same, the latter lumped S. nigricans and S. adunca together with S. reflexa, yet Indoh agreed with Zycha's opinion of the synonymity of S. adunca with S. reflexa. Again it is to be noted that van Tieghem (1875) and Bainier (1883) clearly contrasted between S. cornu and S. reflexa.

Von Hohnel (1902) too, expressed an opinion that Matruchot's (1899) Gliocephalis hyalina was none other than a Syncephalis species. His new combination, S. hyalina has been criticised by both Arnaud (1952) and Embree (1963a).

Zycha (1935) doubted the validity of other described species too, admitting only nineteen species out of the twenty-six erected to that date. As well as those mentioned above, S. ramosa was absorbed into

S. intermedia, S. tenuis removed to the genus Spinalia and S.glabra excluded. He suggested that S. minima could be an undeveloped form and that it was not impossible for S. tetrathela to be a stunted form of S. cordata.

Boedijn (1959) removed S. bispora from the genus because of the unique sterile lower portions of the merosporangia, re-naming it Syncephalopsis bispora in the new genus Syncephalopsis.

Indoh (1962) stated that S. tetrathela and S. minima could be incomplete forms of S. depressa and excluded S. ubatubensis on the grounds that it had many similarities with Dimargaris. He also agreed with Zycha (1935) in removing S. tenuis to the genus Spinalia despite the rediscovery of the latter by Krzemieniewska and Badura (1954).

The germination of the sporangiospores has been described for S. cordata by van Tieghem and Le Monnier (1873), for S. cornu, S. fasiculata, S. intermedia, S. sphaerica and S. ventricosa by van Tieghem (1875), for S. nodosa by Benjamin (1959) and for S. rapacea by Indoh (1962). The spores when placed on a nutritive medium swell slightly and put out germ tubes, which soon grow into the typical thin mycelium. Léger (1896) reports that the type of medium does not affect the process at all. In most of these species, two germ tubes are put out, one at each end of the cylindrical spore. This speaks for chemical differences between the end and lateral walls of spores, because S. ventricosa, a species with spherical spores, germinates with germ tubes peripherally disposed.

Van Tieghem (1875) stated that S. cordata, S. nodosa and S. reflexa grow in pure culture as saprophytes without a host and he generalised that all species of Syncephalis are therefore facultative rather than

obligate parasites. Attempts by other workers to confirm van Tieghem's finding have failed (the spores of some species will germinate very readily on many different media, but growth soon stops). Benjamin (1959) did succeed in obtaining a very scanty growth of S. nodosa and a few atypical sporophores of S. cornu after ten days on Potato - Dextrose A. This finding does not invalidate the present day opinion, as expressed by Embree (1963b) amongst others, that the genus Syncephalis is obligately parasitic on its host.

(c) Discovery of Syncephalis and classification according to ideas on the nature of the asexual apparatus

Piptocephalis freseniana was established as an independent fungus by de Bary (1865) who considered its spore chains to be derived from the septation of multinucleate conidia of exogenous origin. He thus erected the conidial family, the Piptocephalidaceae within the Zygomycetes.

In 1873, van Tieghem and Le Monnier erected five species of a new parasitic genus with many similarities to Piptocephalis. In the latter, the tips of dichotomous sporophores possessed terminal head cells, deciduous at maturity, which bore numerous spore chains; in the former, simple sporophores bore, on distal enlargements, spore chains with sterile, deciduous proximal cells. Van Tieghem and Le Monnier considered these latter to be homologous with the deciduous head cells of Piptocephalis (têtes caduques). As these "têtes caduques" were concentrated on one terminal enlargement and not dispersed singly on the terminations of a dichotomous system, the name Syncephalis was coined for the new genus.

In 1875, with eight more species of Syncephalis, some with no sterile proximal cells to their spore chains, van Tieghem reconsidered his former tenets. The former "têtes caduques" of Syncephalis were now considered to be sterile derivatives of basal spores; those of Piptocephalis considered to be the equivalents of the distal enlargements in the Syncephalis sporophore i.e. "têtes fixes" of the latter.

Van Tieghem's studies on spore formation in both Syncephalis and Piptocephalis convinced him that both possessed linear sporangia. Ignoring priority, he placed both in the mucoralean tribe, the Syncephalidées, on the grounds that Syncephalis possessed intermediate characters which linked the Syncephalidées with his other three mucoralean tribes, particularly the Mortierellées.

Bainier (1882), in describing S. fusiger introduced a new specific character, whereby sterile proximal portions of spore chains remained permanently attached to the sporophore head. These he considered to be homologous with the head cells (têtes caduques) of Piptocephalis. He considered this new specific character to warrant the sub-division of the genus into subsections:- Microcephalis, with sterile "branchlets" as in S. fusiger; Monocephalis, without such branchlets; and Calvocephalis with unique zygospore formation as in S. nodosa.

The emphasis on this specific character by Bainier was not adopted by Thaxter (1897) who described two species, S. wynneae and S.pycnosperma with fixed branchlets so prominent as to be called secondary sporophores. Because within the genus, a transitional series of species could be arranged, leading to the condition exhibited by S. wynneae, Thaxter

assigned no taxonomic importance to the possession of secondary sporophores and neither did Vuillemin (1902), but for different reasons.

A division of the genus, on a simple basis was done by Fischer (1892) and Vuillemin (1903), whereby erect and curved species were segregated. Fischer adopted the terms *Rectae* and *Curvatae*, Vuillemin, merely on priority, replaced the term *Curvatae* with *Cornutae*.

However, in 1962, Indoh returned almost to the ideas of Bainier, by distinguishing a group *Syncephalis in the strict sense* from those with secondary sporophores, the *Syncephalis wynneae group*. He thought this necessary on consideration of the ambient relationships of *Syncephalis*, *Piptocephalis* and *Syncephalastrum*. He, too, considered the secondary sporophores to be homologous with the deciduous head cells of *Piptocephalis*. One is reminded too, that Bodijn (1959), because he considered the secondary sporophores of *S. bispora* unusual, erected the new genus, *Syncephalopsis*.

Taxonomic treatments of the *Mucorales* subsequent to those of Brefeld (1872) and van Tieghem (1875) are various. Schroeter (1866) followed Brefeld adding *Syncephalastrum* to his *Piptocephalidei* whilst Berlese and de Toni (1888) favoured van Tieghem's treatment except for the inclusion of the *Chaetocladiaceae* as a fifth tribe.

Fischer (1892) presented a much accepted division of the *Mucorales* into sub-orders, with the *Piptocephalidaceae* containing *Syncephalis*, *Piptocephalis* and *Syncephalastrum*, renamed as the *Cephalidaceae* within the sub-order *Conidiophoreae*. This re-arrangement was accepted by both Oudemans (1897) and Lendner (1908). The latter, cognisant of Thaxter's work (1897), included in his *Cephalidaceae*, genera with possible

affinities, such as *Dispira* (van Tieghem 1875), *Sigmoideomyces* (Thaxter 1891), *Spinalia* (Vuillemin 1904) and *Dimargaris* (van Tieghem 1875).

Fischer's arrangement was modified by Naumov (1924) who introduced two further sub-orders and allocated the *Cephalidaceae* to the *Pseudoconidiophoreae*. Further, (1935), having established the monogeneric *Syncephalastraceae* he allowed the *Cephalidaceae* to be trigeneric only with *Syncephalis*, *Piptocephalis* and *Dispira*. However, the *Cephalidaceae* of Zycha (1935) not only contained *Syncephalastrum* but also *Kickxella* (Coemans 1862), *Coemansia* (van Tieghem and Le Monnier 1873) and *Sigmoideomyces*. Notable amongst other authors who have adopted the *Cephalidaceae* is Vuillemin (1902).

Schroeter (1893) and Fitzpatrick (1930) retained the original family name *Piptocephalidaceae* and the latter included within it, not only the three customary genera, but also *Dispira*, *Dimargaris*, *Kickxella*, *Martensella* (Coemans 1863), *Coemansia*, *Spinalia* and *Saitomyces* (Ricker 1906). Notably, the retention of the original family has been adopted too by Hesseltine (1955) and Boedijn (1959).

The content of the family has been simplified, firstly by Linder (1943) who established the *Kickxellaceae* as a new mucoralean family containing *Kickxella*, *Martensella* and *Coemansia*. Secondly Benjamin (1959, 1961) with interesting studies and discoveries of merosporangiferous *Mucorales* established the new *Dimargaritaceae* with *Dimargaris*, *Dispira* and *Spinalia*. His retention of Naumov's *Syncephalastraceae* makes the *Piptocephalidaceae* digeneric, with only the validities of *Sigmoideomyces* and *Saitomyces*, genera excluded by Hesseltine, to be discovered.

A discussion of Syncephalis cannot avoid mention of the divergent views of the homologies of its asexual reproductive structures.

The conidial view-point proposed for Piptocephalis by de Bary (1865) has been upheld for Syncephalis by Brefeld (1872), Fischer (1892), Schroeter (1893), Oudemans (1897), Lendner (1908) and Migula (1910)

The more popular sporangial viewpoint, introduced by van Tieghem (1875) and supported by Thaxter (1897), attracted Bainier (1882), Schroeter (1886), Berlese and de Toni (1888), Marchal (1892), Léger (1896), Mangin (1899), Moreau (1913), Naumov (1924), Fitzpatrick (1930), Zycha (1935), Martin (1940), Christenberry (1940) and Bessey (1961).

The merosporangial concept conceived by Vuillemin (1902) has been adopted by Gaumann (1926), Hesseltine (1955), Boedijn (1959) and Benjamin (1959). Vuillemin (1902) admitted that the homologies of various parts of the sporophore in Syncephalis, Syncephalastrum and Piptocephalis were in doubt, but was equally adamant that all genera bore "merisporocystes". Later in 1922, he explained the similarity of Syncephalis and Syncephalastrum as an effect of convergent evolution. Gaumann's ideas were also apparent in the use of such terms as "extra-matricial partial sporangia" or "extra sporangial deciduous sporangioles". Although it is easy to see in Piptocephalis that Gaumann may be correct in conceiving its head-cells to be evolutionally reduced mother sporangia, it is difficult to fit in Syncephalis, with the more variable features, into such a scheme.

Both Zycha (1935) and Naumov (1939) shrug off the involvement of considerations of homologues by using the terms "basalzelle" and "sterigmate" respectively to mean the head cells of Piptocephalis, the

basal sterile cells of the sporechains and secondary sporophores in Syncephalis. This attitude is well illustrated in Naumov, who displaced Syncephalastrum to the Syncephalastraceae (Acephalae) because it possessed no "sterigmates". In other words, sporangia were directly inserted on large terminal vesicles. Yet species of Syncephalis with similar insertions were considered exceptions within the genus.

(d) Ecology of Syncephalis

Syncephalis occurs quite frequently when dungs and soils are placed on to agars, always in association with other Mucors on which they are parasitic. Species have been found on the dung of Thompson antelope, Japanese antelope, Bactrian camels, Arabian camels, Cows, Deer, Dogs, Foxes, Goats, Hares, Horses, Kangaroos, Mice, Mules, Rabbits, Rats, Sheep and Japanese weasels. These are mainly herbivorous animals.

Syncephalis has been found to be parasitic on species of Chaetocladium, Cunninghamella, Circinella, Absidia, Helicostylum, Mortierella, Mucor, Pilobolus, Rhizopus and Zygorhynchus and even on a Syncephalis itself (Christenberry 1940). All these are Mucorales.

S. wynneae is parasitic on Wynnea macrotis while others have been reported growing in association with species of Agarics including Boletus edulis and B. satanus and with sphagnum moss. S. ubatubensis was found to be parasitic on an unnamed insect-parasitic fungus (Viegas and Teixeira 1943) and S. cordata to be parasitic on the discharged sporangia of Pilobolus crystallinus (Christenberry 1940). S. cornu and S. reflexa have been isolated from soil by the "root-bit" technique by Ramakrishnan (1955) in the company of species of cellulose decomposers and other plant pathogens.

The genus Syncephalis seems to have a world-wide distribution,

species being found in various places in Europe, Africa, North and South America, India and Japan.

(e) Nature of parasitism of Syncephalis

Studies on the physiology of mycoparasitism involving both facultative and obligate mycoparasites have been numerous. Those of Weindling (1932) on Trichoderma lignorum, Drechsler (1938) on Dactylella spermophagum and Trinacrium subtile, Ayres (1941) on Gonatorhodiella, Malencon (1942) on Claudopus byssisedus and Cephalosporum, Warren (1948) on Papulospora, Blyth (1949) on Gonatorhodiella highlii, Boosalis (1956) on Trichoderma spp. and Penicillium vermiculatum and Godfrey (1957) on unnamed parasites of Endogone are mainly descriptive and/or concerned with mutualistic effects. Other such studies, too, are those of Butler (1957) on Rhizoctinia solani, Barnett (1958) on Calcarisporium, Shigo (1958) on Ceratocystis fagacearum and Barnett and Lilly (1962) on Gliocladium roseum.

However, studies by Shigo (1960a) on Gonatobotryum fuscum, Slifkin (1963) on Olpidiopis incrassata, Whalley and Barnett (1963) on Gonatobotrys simplex are concerned with the axenic development of obligate parasites and the conditions affecting the establishment of parasitisation. Shigo noted that different C:N ratios of test media affect the degree of parasitism; a requirement for micro-elements, especially Mn^{++}; and increased metabolic rates in parasitisation system. Shigo obtained the greatest axenic development using test media containing yeast extract, micro-elements and host filtrates. Whalley and Barnett found a similar need for yeast extract and additionally, a water soluble extract derivable not only from host but other fungi as well.

Slifkin observed no axenic development but discussed the various susceptibilities of host Saprolegnia species, to Olpidiopsis, when grown on test media with differing C:N ratios.

Recently, an interest in the mycoparasitism of Piptocephalis has arisen, many species of which produce axenically, dwarf sporophores on a variety of test media. The extent of development of these is a useful guide in axenic studies. Berry and Barnett (1957), Berry (1959), Shigo (1960b), Shigo, Anderson and Barnett (1961) have studied variously. P. virginiana Leadbeater and Mercer (1957) and P. xenophila Dobbs and English (1954) and remarked on the importance of C:N ratios in test media upon the establishment of the parasite and the need for micro-elements such as Mn, and subsidiarily, Fe, Zn and Ca.

For the germination of P. virginiana, Berry and Barnett stated an important rôle for glutamic acid, the carbon source and micro-elements. Host fungi and some others outside the host range providing thermolabile factors, the one influencing spore swelling and the other, the production of germ tubes. This obligate parasite, they stated, must require either a labile host-provided metabolite or must lack one or more enzyme systems. Berry observed that host susceptibilities vary with developmental stages and incubation temperatures and the importance of glutamic acid should be relegated to compounds arising from, during autoclaving, its interaction with other medial constituents. Berry and Shigo, too, observed the restriction of host development by P. virginiana and P. xenophila. Shigo recorded higher respiratory activities in hosts, when parasitised by P. xenophila and Gonatobotryum fuscum.

Additional work on other mucoralean mycoparasites has concerned the

axenic growth of *Dispira* on synthetic media by Ayres (1933); of *Spinellus* by Ellis and Hesseltine (1962), Leadbeater and Richardson (1963) and Watson (1964+1965); of *Rhopalomyces* by Ellis (1963). In all such cases normal growth forms were obtained on appropriate test media.

No recent work has been reported on the axenic development of *Syncephalis*, although Benjamin (1959) recorded scanty growth and atypical sporophores of *S. cornu* on Potato Dextrose A., but remained convinced that the genus, like *Piptocephalis*, is an obligate parasite.

(f) Object of the investigation

An unusual *Syncephalis* was isolated by G. Leadbeater from Leicestershire soil. The purposes of the investigations recorded in this thesis were:-

(a) To study the development, structure and host range of this isolate.

(b) To promote the axenic development of this parasite.

(c) To make a collection of *Syncephalis* isolates for preliminary ecological information and in order to provide material for comparative studies.

Part II.

Part II. Materials and Methods.

(a) Collection P. 32

(b) Isolation of species P. 32

(c) Long term maintenance of cultures P. 33

(d) Infestation by mites P. 35

(e) Continual reculturing P. 36

(f) Obtaining spores of the parasite P. 36

(g) Germination of spores P. 37

(h) Preparation of microscope slides from cultrues P. 38

(i) Nuclear staining P. 38

(j) Measurements P. 39

(k) Host range P. 39

(l) Preparation of culture media P. 40

(m) Drawings and Photographs P. 40

(a) Collection

Small samples of dung and soil were collected in sterile McCartney bottles, Customs sanction being obtained for the importation of the foreign samples.

(b) Isolation of species

Fragments of soil or dung were placed on to the surface of agars in petri-dishes. Potato Dextrose Agar or Corn-steep liquor Agar to which had been incorporated 0.2 gr./litre streptomycin sulphate, were found to allow satisfactory growth of the moulds while inhibiting bacterial contaminants.

The cultures were inverted to prevent condensed moisture from falling on to the surface of the medium. They were left at room temperature (18 - 23°C.) and examined at intervals of a few days with a binocular dissecting microscope (Watson Stereoscopic X6.5 - X140). These cultures and the original soil and dung collections were not kept in the same laboratory as the later pure cultures, so that the chance of contamination by mites accidentally introduced in them, was minimised.

Species of Syncephalis located in these cultures were immediately transferred to petri-dishes of corn-steep liquor Agar. The developing sporangia of young species of Syncephalis radiate outwards in a distinctive manner, but if they are not located at this early stage they soon become indistinguishable from many other small mucors and imperfects because of the formation of the spore droplet. Two methods were used for this process:- (a) A gross transfer of mycelium, sporophores and spores of the parasite together with those of the host, using a sterile platinum wire.

(b) Taking spores of the parasite with a small platinum loop moistened with distilled water and culturing them together with the spores of various hosts known to be parasitised by Syncephalis.

The gross transfer method (a) was found to be more certain of success, knowing that the particular host is parasitised by the, as yet unknown, Syncephalis and so was generally adopted.

The pure cultures (a pure culture refers to a culture of Syncephalis and any one host) were then examined to determine the identity of the Syncephalis and its original host.

The pure cultures remained in such a condition for two or three weeks as to allow their use for experimental purposes and for re-culturing to form new stocks. They were stored at room temperature in a large glass aquarium with a loosely fitting lid, in an atmosphere of "Shellspark" vapour to discourage mites. (see later note)

In general, the species were grown on the host on which they were first found, but if, during the host-range determination, another more suitable host was found, that too was used in duplicate cultures.

During the course of this work, a single specimen of each of Syncephalis 3, S. cornu, S. ramosa and S. nana, six specimens of S. nodosa and three specimens of S. depressa were maintained in pure culture for comparative studies. S. nana (Dade) was obtained from the C.M.I. and S.3 donated by G. Leadbeater.

(c) Long term maintenance of cultures

Many specimens of Syncephalis and of other moulds were maintained in a culture collection. Four different treatments were employed:-

(a) Slopes of Corn-steep liquor Agar, Potato Carrot Agar or Dung

Agar in 25ml. McCartney bottles were inoculated with spores of Syncephalis and host, the screw caps being slightly loose to allow for diffusion of respiratory gases. If the culture from which the spores were to be obtained was very vigorously parasitised, a gross transfer was found to be sufficient. Slopes of Agar in boiling tubes, plugged with cotton wool were used for the first part of this work, but apart from being time-consuming in preparation, tended to dry out rather quickly. The cultures were stored in cardboard boxes (30 x 25 x 17 cms.) divided inside into compartments. The lids were fitted with a transparent window of "Polyglaze", allowing the cultures to grow in daylight. In one of the internal compartments, was placed a small bottle of "Shellspark" tractor vaporising oil (T.V.O.) with a half inch lamp wick passing through a split cork and protruding about one inch above it. This maintained a vapour to discourage the attraction of mites to the cultures (Dade 1960). These cultures were kept at room temperature and recultured at intervals of six to nine months.

(b) Slopes were prepared as above, but containing slightly less medium. When these cultures had grown satisfactorily, sterile liquid paraffin (decanted from separate bottles to reduce the chance of contamination) was poured over them so that the mycelium was completely covered (Dade 1960). These were stored as above.

(c) Freeze drying. A concentrated suspension of host and parasite spores was obtained by shaking a small quantity of sterile skimmed milk over a young and vigorously growing culture in a McCartney bottle. Small quantities of this suspension were transferred to ampoules and lyophilised using an Edwards "Speedivac" Centrifugal freeze dryer, Model 5PS. On

completion, viability tests were made to ascertain whether or not it had survived the treatment. If the spores had survived, the ampoules were brought into the collection, being stored cool and dark.

(d) Dried Soil. McCartney bottles containing 10-15 ccs. of air-dried, sieved soil were sterilised by prolonged autoclaving. About 1 ml. of a concentrated suspension of host and parasite spores in distilled water was added to each bottle. The bottles were shaken to mix the spores well with the soil and then placed in a desiccator, over silica-gel. When the mixture was completely dry, the lids were screwed down. They were kept in a cool, dry place. This follows a method described by Foster (1949).

Viability tests were made at intervals with the four treatments and showed that the spores of Syncephalis 3 were still viable after 15 months on Agar slopes, 20 months on oiled slopes, 14 months lyophilisation and 16 months in dried soil. The lyophilised spores grew vigorously on testing, possibly even better than in normal sub-cultures.

(d) Infestation by mites

An infestation of the cultures by mites occurred during this work. Mites are considered to kill the fungi by eating them, but more harm would appear to be done by the transferring of spores from one culture to another as the mites penetrate each one in turn (Dade 1960). New cultures were made by spore transfer from the infected cultures, which were subsequently autoclaved. While the exposure of the contaminated cultures to the toxic vapours of p-dichloro-benzene killed the mites, it did not seem to affect their eggs, which hatched out a few days later and continued the infection. Collections of soils and dungs and their first

cultures were not brought near the pure cultures, which were kept in an atmosphere of "Shellspark" vapour, in another laboratory.

(e) Continual reculturing

In a number of species, continual re-culturing on the same type of medium over two or three years, caused certain taxonomic characters to alter. This could be prevented by changing the medium from time to time, particularly using ones poor in soluble sugars (Dung Agar and Tap water Agar to which was incorporated small pieces of sterile dung). On these impoverished media, *Syncephalis* grew slowly but vigorously to its maximum observed dimensions and formed its full complement of spores. Constant reference to the first cultures, under oil, lyophilised or in dried soil, was made to check the constancy of taxonomic features in the current cultures.

(f) Obtaining spores of the parasite

Syncephalis 3 is a small fungus and it was difficult to obtain its spores free from those of the host. Using hosts with small sporophores or those whose sporing was inhibited by growing them in the dark, had been tried, but with little success. A reliable method was evolved by growing the *Syncephalis* with its original *Absidia orchidis* host on Dung Sucrose Agar. This medium greatly depressed the aerial growth of the *Absidia* without affecting the parasite, which spread out beyond the host and formed sporophores standing straight out from the medium well clear of the host. The spores could then be picked off with the small platinum loop with not much chance of detaching host spores. This method was used successfully for *S. nodosa* also and would appear to be of use for many of the small species. No difficulty was experienced with the larger species,

S. depressa and S. ramosa, whose spores could be detached from the sporophores which grew well above the host mycelium, on any normal medium

(g) Germination of spores

To observe the process and conditions governing spore germination, two methods were used.

(a) A small drop of a selected agar medium was allowed to solidify on a coverslip and then inoculated with spores. The coverslip was inverted over a glass or metal ring cemented to a microscope slide, a drop of water being placed in the bottom of the resulting "van Tieghem cell" (Duggar 1909). The complete slides were placed in petri-dishes containing damp filter paper and after incubation were observed microscopically. However, the medium was rather thick and so observations using the high-power objective were difficult to make. Additionally, there might have been a lack of oxygen in the enclosed cells.

(b) The method finally adopted was to place the spores directly on to the surface of the solid medium in a petri-dish, although the chances of contamination were increased. It was rather uneconomic in materials but ensured that lack of oxygen was not a limiting factor.

By these methods, the effects of different nutrients and of variations in temperature and pH. were determined. The pH. of the media was adjusted by the addition of dilute hydrochloric acid or dilute sodium hydroxide or by the use of buffer solutions. The media and buffer solutions were made up separately at double strength, autoclaved and mixed together when cool. The final pH. value of the solid media was determined with narrow-range indicator papers.

(h) Preparation of microscope slides from cultures.

A coverslip was smeared with a thin layer of "Durofix" (The Rawlplug Co.) and gently pressed on to a selected portion of a culture growing in a petri-dish, so that the mycelium adhered to it. It was left for about half a minute, during which time a drop of cotton-blue stain in lactophenol* (or other stain) was placed on a microscope slide. The coverslip was removed from the culture and carefully placed on to the stain. The Durofix film preserves the original position of the fungi and prevents the spores from becoming detached, while being quite permeable to stains and other fixing and washing solutions. These slides could be made permanent by blotting up any surplus stain and ringing the coverslip with nail varnish (Dade 1960).

Another method was used for the preparation of temporary slides. Small squares (1 cm. sq.) of boiled, sterile cellophane, were laid on the surface of the medium in a petri-dish and inoculated with host and parasite spores. After incubation, the cellophane squares, which had become overgrown with the fungal association, were removed and mounted, either in stains, or in water. This method also gave minimal disturbances to the positions of the fungi.

No differences were detected between dimensions measured with water as a mountant and those where lactophenol was used.

(i) Nuclear staining

To follow the nuclear sequences leading to spore formation and spore germination, the following method was used. A Durofix film was made on

---

* see appendix II for method of preparation.

a coverslip and pressed on to the specimen as explained above. The coverslip and film was immersed in Schaudinn's fixing solution,* for 10 minutes, washed with water, hydrolised in 5N. hydrochloric acid for 10 minutes, washed and mounted in a drop of basic fuchsin* for about 20 minutes, until stained. The mount was irrigated with water to wash out the stain from the cytoplasm leaving the nuclei visible. This was a quick modification of a method used by de Lamater (1948).

(j) Measurements

All measurements from microscopic preparations were made with a Watson Image Shearing eye-piece, to within 0.1 µ. Average values for various dimensions were calculated with a Diehl VSR 18/1 calculating machine.

(k) Host range

Syncephalis spores were placed on to the surface of a medium on which they were known to germinate e.g. Dung Sucrose Agar, and incubated for a few days to see if any contaminants were present and to allow the spores to germinate as far as possible. Spores of various fungi were added and, at intervals of a few days, for three weeks, the cultures were examined with a binocular dissecting microscope to determine the degree, if any, of the parasitism. Parasitism was considered not to occur if at least three attempts were negative. Results appear in Appendix I.

---

* See appendix II for method of preparation.

(l) Preparation of culture media.

Where possible, naturally occurring materials were used for preparing culture media (i.e. dung, potatoes, carrots). When microscopic observation through the medium was necessary, they were filtered before hand, but normally, as long as they were well macerated, filtration was not necessary.

Culture media were sterilised at 10 - 15 lbs/sq.in. for 15 - 30 minutes. Any thermolabile compounds were sterilised by Seitz filtration and incorporated into the cooling medium where necessary.

(m) Drawings and Photographs

Drawings were made in indian ink on good quality 22" x 30" paper. They were photographed on 35 mm. Ilford Micro-Neg Pan film, developed in D-76 and printed on Kodagraph Projection paper P84, developed in D-163. Photomicrographs were made on 35 mm. Kodak Panatomic X film, developed in D-76 and printed as above.

PART III.

Part III. Experimental results.

Section 1. Morphology and Ecology of *Syncephalis* p. 43

(a) Description of *Syncephalis 3* p. 43

(b) Discussion of taxonomic position of *Syncephalis 3*. p.57

(c) Ecology of *Syncephalis* p. 60

(d) Other observations on *Syncephalis* p. 65

Section 2. Germination of spores and axenic growth of *Syncephalis*. p. 68

(a) Introduction p. 68

(b) Swelling of spores on germination p. 69

(c) Degree and amount of spore germination p. 69

(d) pH. and temperature range for spore germination p. 71

(e) Germination of spores on introductory test media p. 74

(f) Germination of spores on defined media p. 79

(g) Effects of host secretions and extracts on spore germination p.102

(h) Summary p.106

Section 1. Morphology and Ecology of Syncephalis

(a) Description of Syncephalis 3.

The spores of Syncephalis 3 inoculated alone on to various agars, either remained ungerminated or produced a tiny germ tube at each end of the spore. These germ tubes, only 1 - 2 µ long, often dichotomised into two very short branches.

If, however, a host was present, many of the Syncephalis spores in contact with, or within a few microns of, the host mycelium, germinated to further stages and often penetrated the host. Host influence on the germination of parasite spores was confined to its immediate proximity. In many cases, spores in parallel contact with host hyphae but also with terminal germ tubes, germinated only to those stages realisable in the absence of a host. The host, here, failed to exert a directional influence on the terminal germ tubes, even within a range of 1 - 2 µ. Successful parasitisation occurred with spores either in angled contact with, or disposed at an angle in close proximity to, the host hypha. Such an orientation permitted terminal germ tubes to grow towards the host.

Germ tubes penetrated the host cell wall directly, without the production of ampoules or vesicles which characterise other species (Fig. 6 A.B.) Once inside the host, an intracellular hypha developed very quickly, with the minimum of branching, unlike, once again, other species which produce internal ampoules immediately after host entry.

The process of germ tube formation and initial host penetration left parasite spores almost fully vacuolated (Fig. 6 A.B.). It was

Fig. 6.

A. S.3 Penetration of parasite germ tube into a host hypha. Spore is vacuolated.

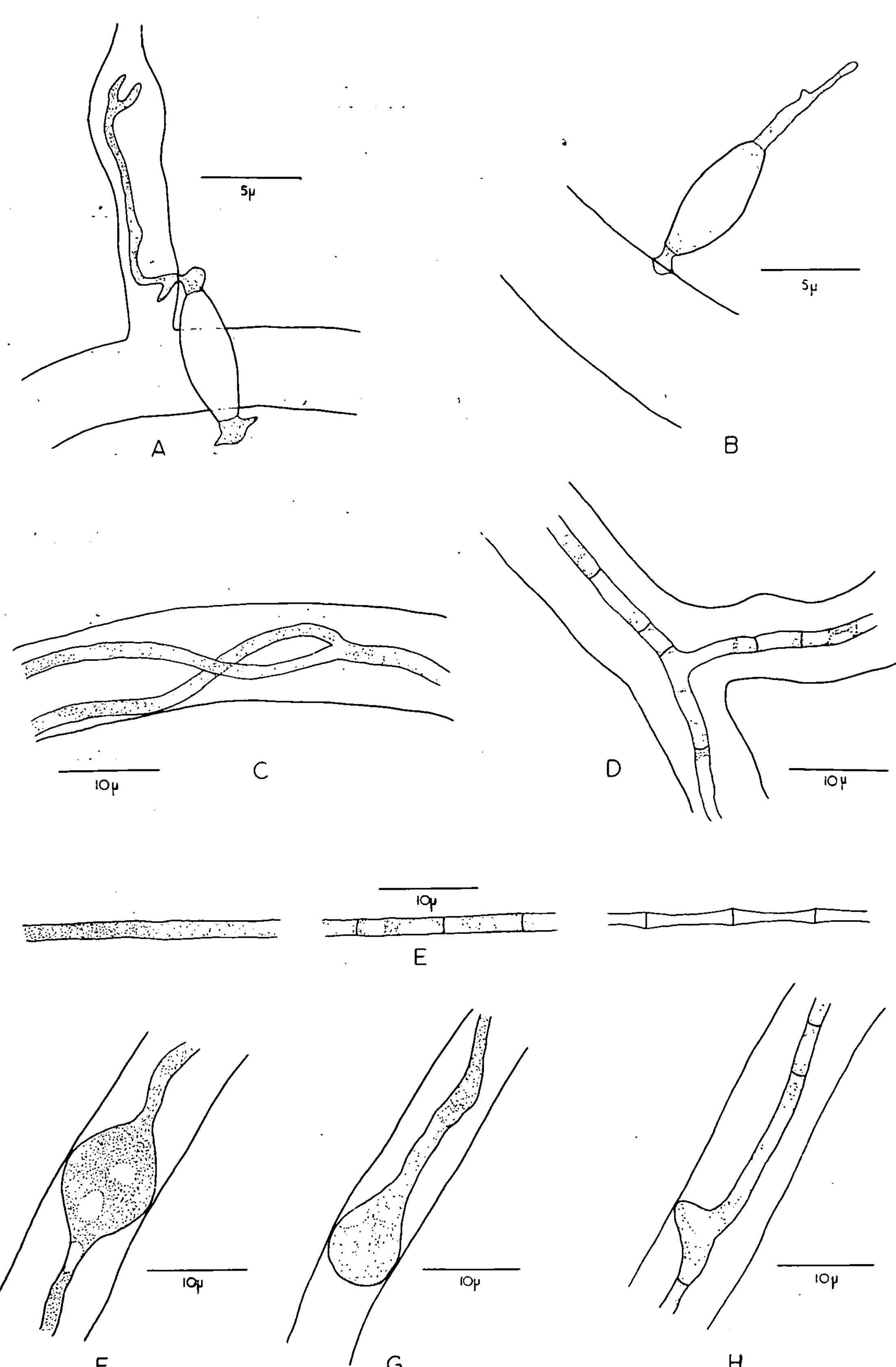
B. S.3 Penetration of host by one germ tube and development of parasite hypha, externally, from the other. Spore vacuolated.

C.D. S.3 Branching of intracellular hyphae.

E. S.3 Stages in vacuolation and septa formation of intracellular hyphae.

F - H. S.3 Swellings developed by intracellular hyphae.

FIG. 6.



often noticed that further penetration permitted the re-assertion of the protoplasmic condition in these vacuolated spores and the development of germ tubes not involved in penetration, to form an external mycelium capable at any stage of effecting further penetrations of the host (Fig. 6B).

The intracellular hyphae grew throughout the host mycelium, even as far as the terminal septa of the sporophores. Such hyphae occasionally branched, either to form two or more parallel hyphae, or to coincide with the branching of the host (Fig. 6 C.D.) Only cross-walls impeded their quick invasion. Protoplasmic and 1 - 2 μ in thickness when young, these intracellular hyphae, with age became progressively vacuolated to contain only thin lining layers of cytoplasm around central continuous vacuoles. This latter process was accompanied by the deposition of rigid septa, which, coupled with the collapse of hyphae walls elsewhere, resulted in a characteristic appearance to aged intracellular hyphae (Fig. 6E). Regular and irregular swellings characterised further, these internal hyphae (Fig. 6 F - H).

The remarkable feature observed, was the departure of intracellular hyphae or their branches, by direct penetration of host walls (Fig. 7A) Such points of exit were occasionally marked by irregular or regular ampoules (Fig. 7B). Hyphae or hyphal branches, on departure developed variously to give matrical, surface or aerial mycelium or indeed, gave rise to sporophores directly. (Fig. 8D).

In other words, free parasitic mycelium can arise from either the development of free germ tubes of parasitic spores which have successfully penetrated the host, or from exit hyphae arising from intra-

Fig. 7

A. S.3 Departure of intracellular hypha from host.

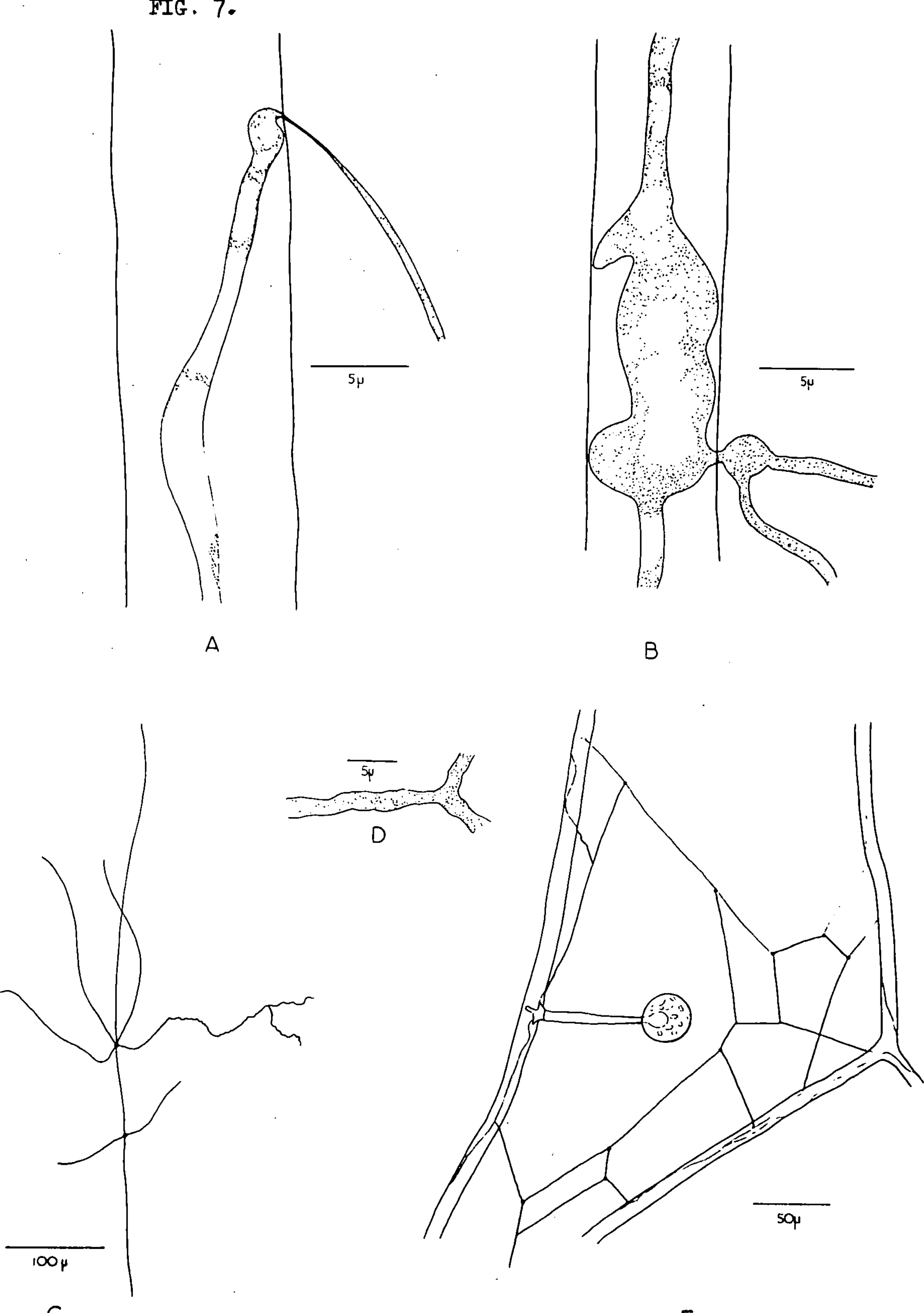
B. S.3 Departure of intracellular hypha, prefaced by formation of ampoules.

C. S.3 Aerial mycelium. One of lateral branches has contacted the surface of the medium.

D. S.3 Surface mycelium.

E. S.3 Web-like investment of the host by parasite mycelium. One Syncephalis sporophore is shown, clamped to host hypha by the rhizoids.

FIG. 7.



- cellular hyphae.

The submerged and surface mycelium possessed hyphae, approximately 2 μ in diameter, richly protoplasmic, cyclotic, highly branched, sometimes anastomosed, but becoming vacuolate and septate with age. (Fig. 7D). Large areas of culture media were covered by such a mycelium, even well beyond the confines of a host colony.

The aerial mycelium, already described as arising directly from germ tubes or exit hyphae, or indirectly from the submerged or surface mycelium, formed an anastomosing network of thin hyphae, 0.5 - 1.5 μ diameter. The investment of the host culture by this network was web-like (Fig. 7E). The hyphae of the network showed active cyclosis, were vacuolate, but did not vacuolate or branch so fully as the submerged or surface hyphae. They were of even thickness and developed a few septa with age. The aerial mycelium contained very small nuclei, which were elongated parallel to the edge of the hyphae. Other extremely small particles, stained red with basic fuchsin were also noticed. The anastomoses were, typically, from 2 -3 μ in diameter and also contained nuclei. The hyphae of this aerial mycelium were often closely applied to the external surface of the host hyphae, especially in regions of emergence and re-penetration. It is interesting that host hyphae already parasitised, were not observed to be re-penetrated by these closely applied, external, parasite hyphae. When aerial hyphae contacted medial or container surfaces, they assumed the form of the submerged or surface mycelium (Fig. 7C).

Formation of stylospores, a method of asexual reproduction in some species of <u>Syncephalis</u>, was never observed for <u>S.3</u>, even though cultured

on many hosts and a variety of natural and synthetic media.

The parasitic mycelium, submerged, surface or aerial, prefaced the formation of sporophores, by the development, typical for the genus, of rhizoidal systems.

Rhizoid formation commenced with the growth of short hyphae, 2 µ in diameter and therefore thicker than a normal hypha (Fig. 8A). When 5 - 10 µ long and with a variably increased diameter, such hyphae dichotomised three to five times to give the characteristic Syncephalis rhizoidal system. As implied above, such systems could be found free or in contact with the host, within the medium, on the medial surface or aerially. It was frequently noticed that exit hyphae, immediately on emergence, form rhizoidal systems (Fig. 8D). It was further noted that a rhizoidal system in contact with a host hypha encircled it in a clamp-like embrace (Fig. 8E). Rhizoids were sometimes septate and most continued development by forming sporophores, which typically arose from the first or second branches of the rhizoidal system (Fig. 8B). Noticeably, those sporophores arising from the medium or medial surface, with rhizoids, either free, or clamped to host hyphae, were geotropic, whilst directions of their growth, when aerial, depended on the direction taken by the sporophore initial. No tropic responses were demonstrated with respect to light.

Typically, a single sporophore arose from a single rhizoidal system. Such a system often gave rise to two, or even a fasicle of, sporophores. Supernumary sporophores arose from separate branches of the rhizoidal system (Fig. 8 C).

Mature sporophores were colourless to white, (55 µ) 92 µ (135 µ)

Fig. 8

A. S.3 Rhizoidal system.

B. S.3 Sporophore arising from rhizoidal system.

C. S.3 Two sporophores arising from one rhizoidal system.

D. S.3 Rhizoid, bearing a sphorophore, formed immediately after emergence of intracellular hyphal branch.

E. S.3 Rhizoid, bearing a sporophore, clamped around host hypha and formed by parasitic aerial mycelium.

FIG. 8.

10µ

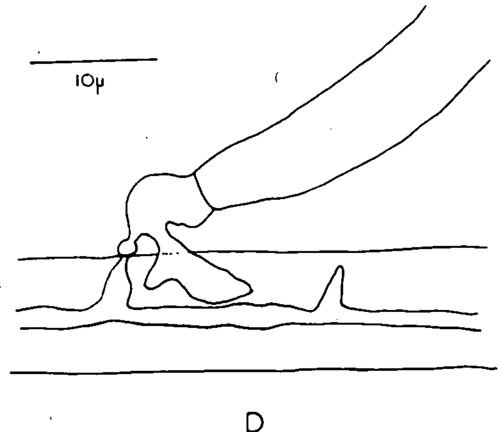
A

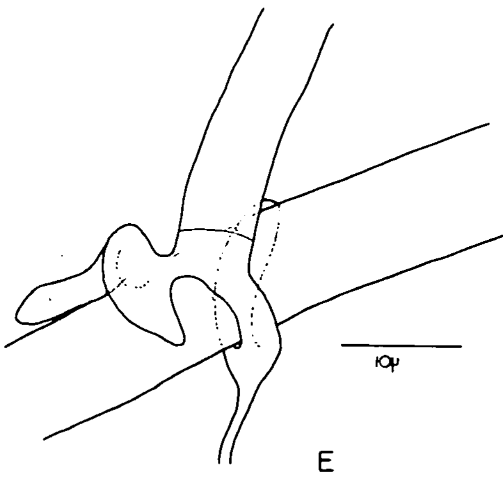
10µ

B

10µ

C





high, (3.0 μ) 5.4 μ (8.0 μ) wide proximally and (2.0 μ) 2.9 μ (4.5 μ) distally. The basipetal tapering was even, ending in a neck which subtended a terminal swelling or head (5.0 μ) 7.6 μ (11.0 μ) in diameter and spherico-turbinate in shape (Figs. 14 B.D. and 9 E).

The sporophore head possessed 15 - 20, eventually bispored, cylindrical merosporangia, (13.0 μ) 19.6 μ (26.0 μ) long and (2.0 μ) 3.0 μ (4.0 μ) wide. The colourless spores produced, were cylindrical to ellipsoidal (6.5 μ) 9.8 μ (13.0 μ) in length and (2.0 μ) 3.0 μ (4.0 μ) in diameter. Spore walls were not completely smooth, but were spotted with small protuberances (Fig. 11A)

S.3 is "wet-spored", that is to say, the head and spores, and sometimes a portion of sporophore as well, are surrounded by a spherical drop of liquid, 35 - 50 μ in diameter, which develops at about the time the merosporangia are fully formed and into which the mature spores are released (Fig. 11 B.C.)

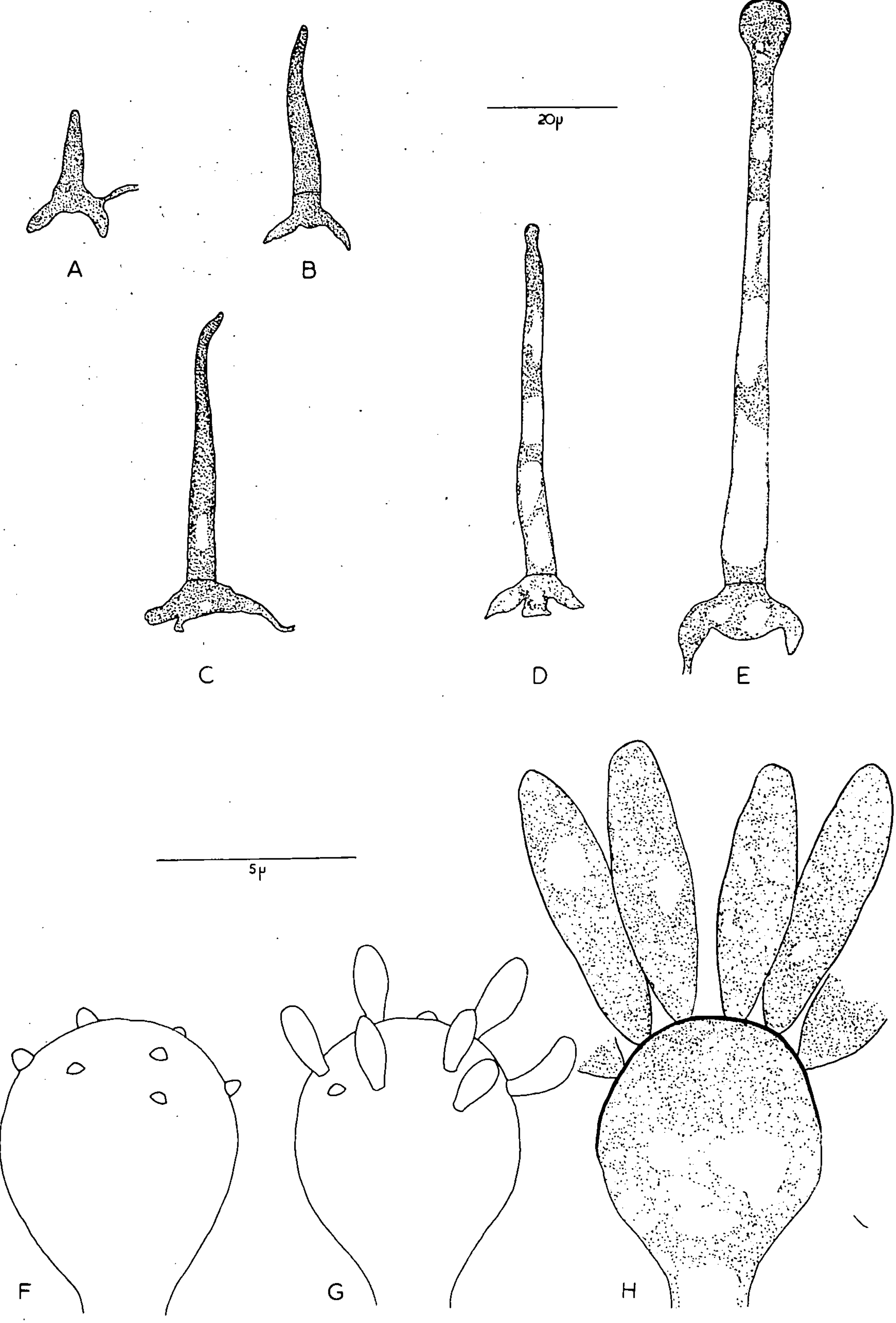
Sporophore initials developed quickly into obclavate structures, showing no other differentiation until two-thirds of their eventual height, when the terminal vesicle or head appeared, attaining its full diameter only with the full growth of the sporophore (Fig. 9A - E) When the sporophore had reached its maximum height, fifteen to twenty small initials developed on the distal half of the head. These merosporangial initials radiated from the head and elongated until approximately 10 μ long by 3 μ wide (Fig. 9F - H). A second stage of elongation resulted in a distal portion with a diameter of 2 μ only (Fig. 10 A - C). These two stages of elongation caused, initially,

Fig. 9

A - E S.3 Development of sporophore from rhizoidal system.

F - H S.3 Early development of merosporangium (H = section)

FIG. 9.



lageniform merosporangia, but at full development, merosporangia were cylindrical with a constant diameter of 3 µ and lengths varying from 13 - 26 µ (Fig. 10 D.E.) This peculiar development of merosporangial initials, gave the impression that the terminal spores bud from basal spores, as described by Thaxter (1897) for S. tenuis. However, a close examination showed merosporangial initials to be cylindrical prior to the formation of spores.

At first, the merosporangial initials radiated evenly out from the head, but when fully elongated, they were encompassed by the spore droplet, which aligned them into a group with all longitudinal axes parallel to the long axis of the sporophore (Fig. 10 F.G.)

Two spores developed within each merosporangium in that manner described by Thaxter (1897) for an unknown Syncephalis from Liberian soil. Thus, protoplasts of the future spores gradually became separated from each other by progressive centripetal differentiation of a separation layer inside the merosporangium. (Figs. 10 H - L. and 13 A) The separation layer itself was differentiated into two portions, one which was stainable with cotton blue, but visible also in water-mounted preparations and which developed early in this process, forming a band across the region of future spore separation, and another portion which was not stainable. The terminal portion of the merosporangium was similarly differentiated, with a stainable cap between the end of the future terminal spore and the tip of the merosporangium. The protoplasts developed walls which could be readily seen between adjacent spores and at the distal end of the terminal spore, but not so easily distinguishable laterally, where in contact with the merosporangial wall. The separation

Fig. 10.

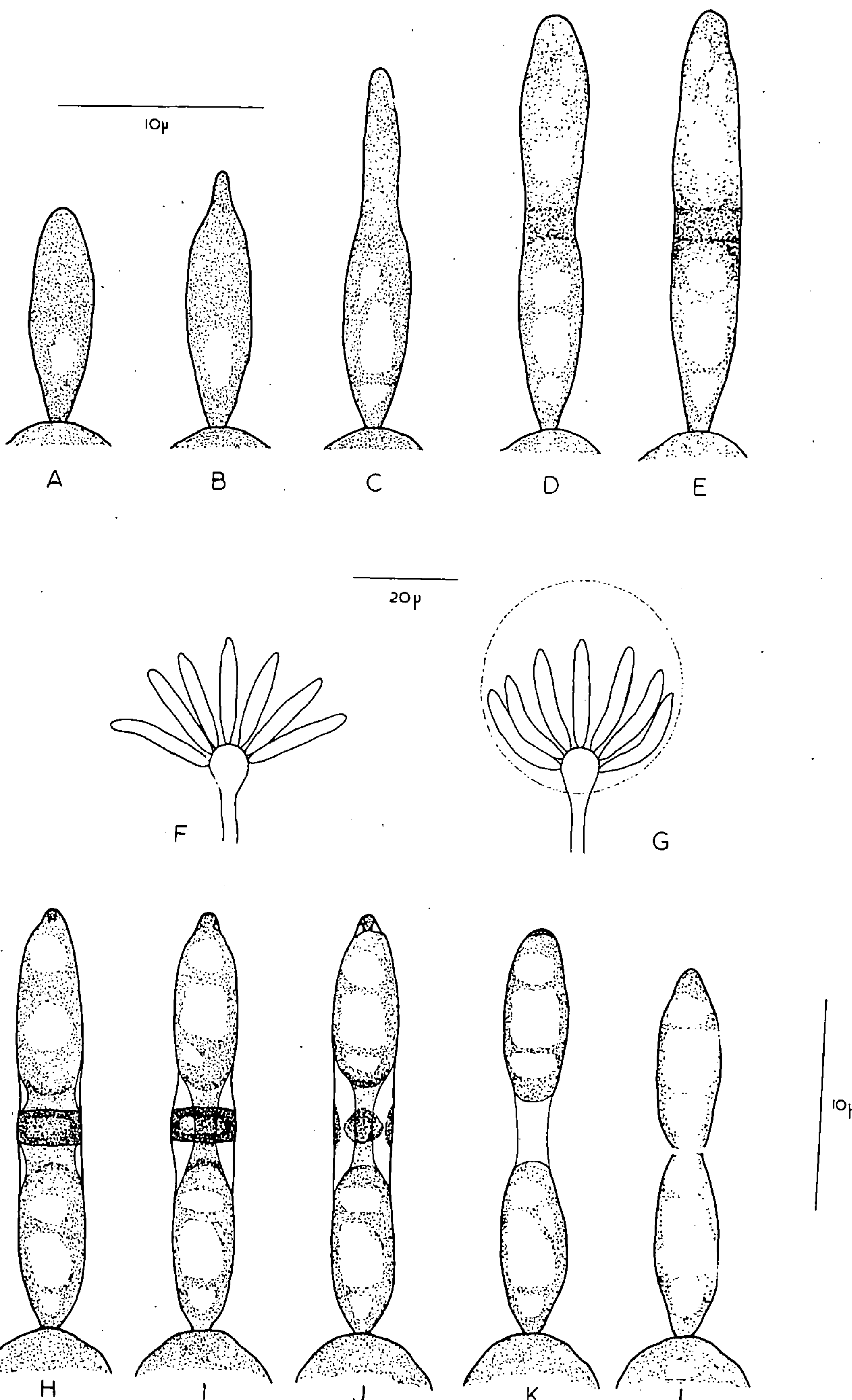
A - E. S.3 Later stage in merosporangial development.

F. S.3 Initial alignment of merosporangia.

G. S.3 Alignment of merosporangia after the formation of the spore droplet.

H - L S.3 Formation of spores within the merosporangium.

FIG. 10.



layer gradually disappeared and the two spores, at first separated from each other by a distance of 2 - 3 μ, approached each other until almost in contact.

Full details of spore formation were not obtained because of the onset of spore-droplet formation and the speed of subsequent events. Thus, rupture or lysis of the merosporangial wall at the junction of the two spores could not be determined; the fate of the distal separation layers at the merosporangial tip could not be described and the presence of a separation layer between the proximal spore and the sporophore head was not observed. Spore droplet formation certainly involved speedy terminal processes, which resulted in the dissociation of spores and their ready release, on disturbance, from the sporophore head. The dehiscence of spores left sporophore heads marked with very small tubercles, representing proximal portions of merosporangia not involved in spore formation (Fig. 14 D).

The spores thus formed are true sporangiospores, bearing remains of original merosporangial walls closely applied to their external surfaces.

Occasionally, some sporophores produced larger merosporangia, approximately 30 μ long. These formed three spores each, indistinguishable from the spores formed in normal merosporangia (Fig. 11 D.E.).

The nuclear behaviour leading to spore formation was followed by examination of successive preparations stained with basic fuchsin. (Figs. 12 A - I and 13 B).

Soon after the formation of the merosporangial initials, one nucleus moved from the actively dividing concentration of nuclei in the head of the sporophore, into each initial. When a merosporangium reached

Fig. 11

A. S.3 Mature spore.

B.C. S.3 Spore droplets. B, encompassing head and spores. Spores have been released. C. surrounding also part of sporophore stalk. Spores not yet released.

D.E. S.3 Large merosporangia (D), which form three spores each (E)

F-H. S.3 Distorted forms induced by continual subculturing on sugar-rich media.

FIG. 11.

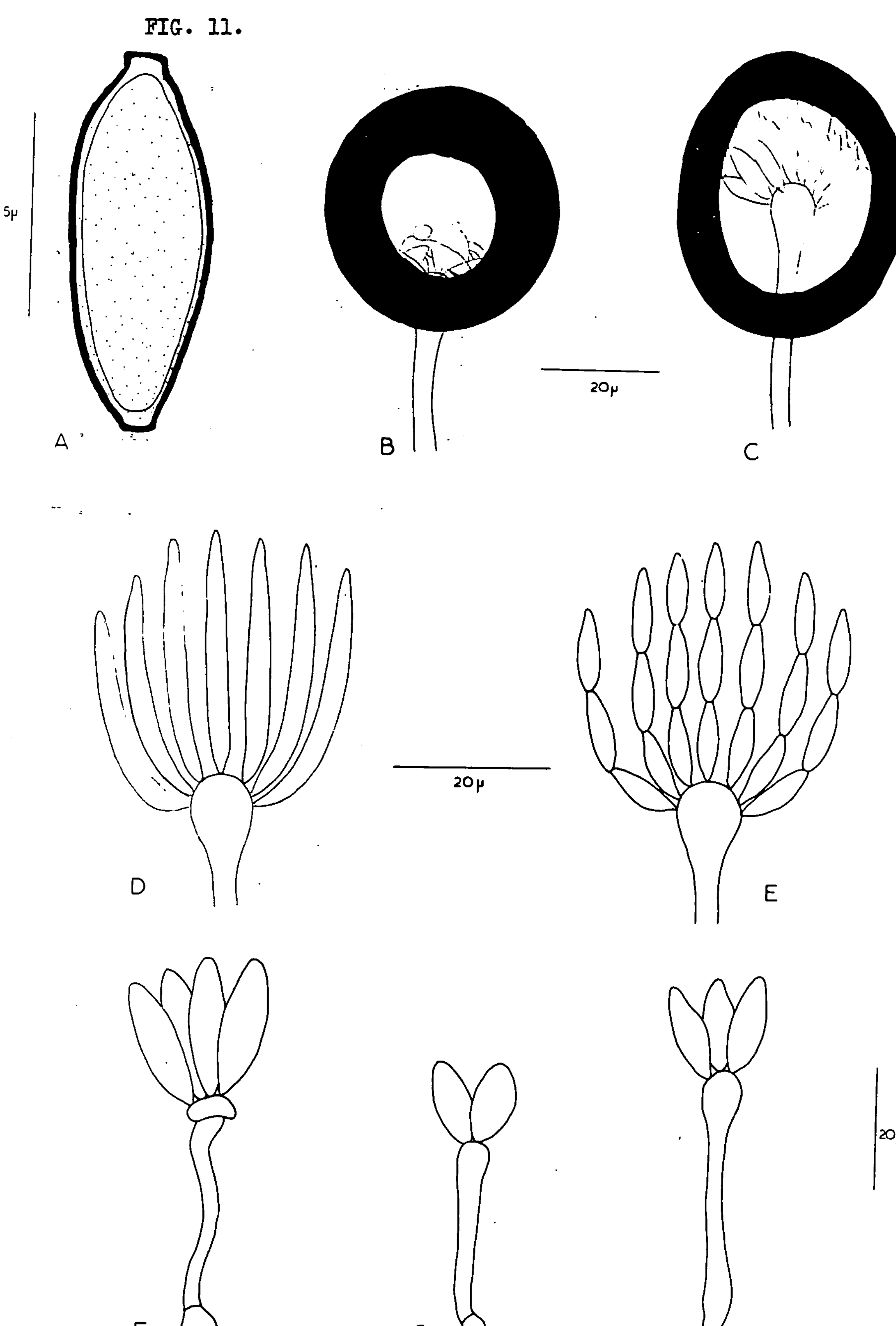


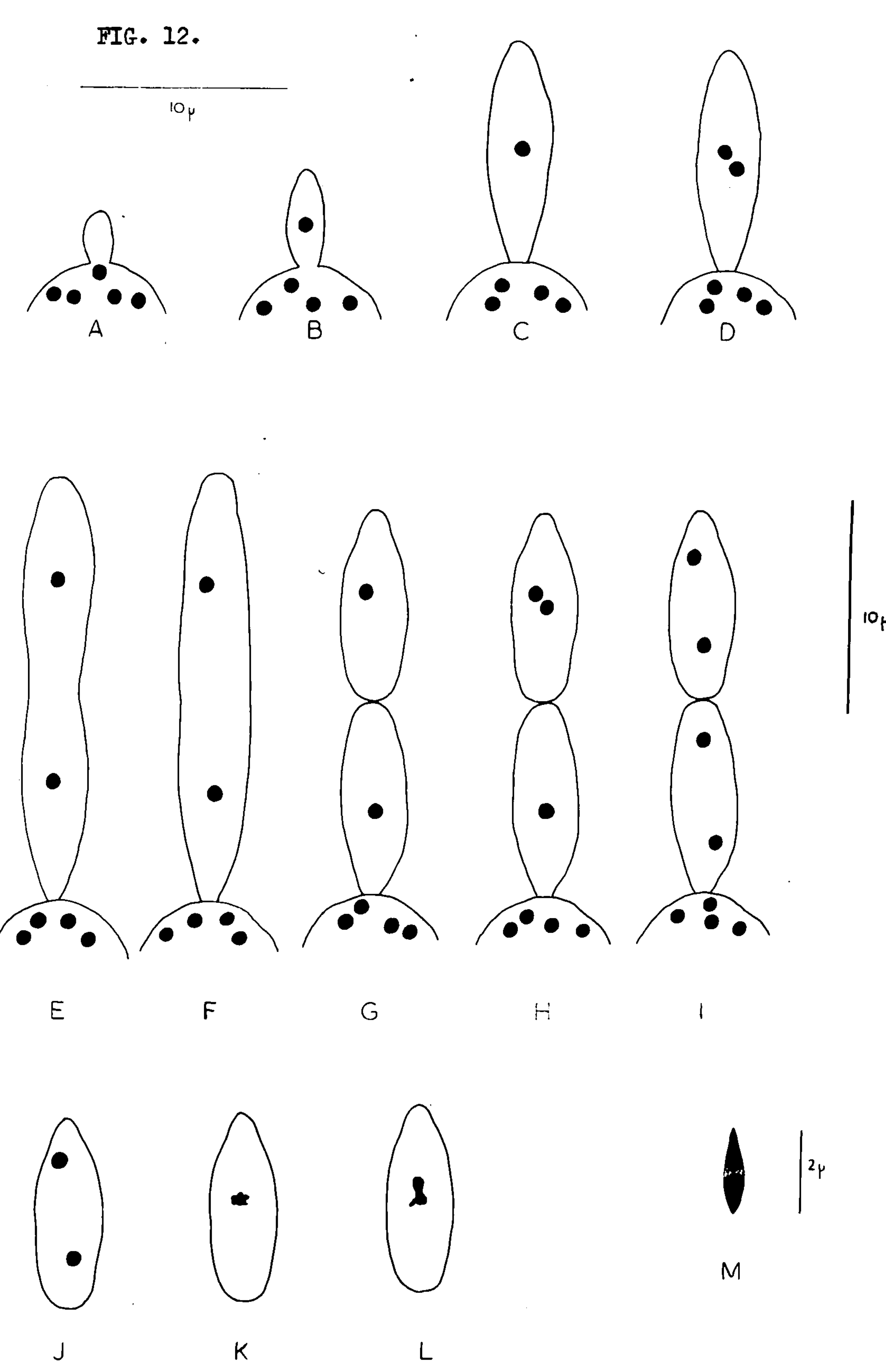
Fig. 12

A - I. S.3 Nuclear behaviour leading to spore formation.

J. S.3 Binucleate mature spore.

K - M. S.3 Nuclei, showing stages in division.

FIG. 12.



approximately 10 µ in length, this nucleus divided into two. The two nuclei then migrated with time, to opposite ends of the now fully elongated merosporangium. The merosporangium then formed its two uninucleate spores, at which stage, the spores were sometimes detached. A second nuclear division followed, so that each spore contained two antipodal nuclei (Fig. 12 J). The nuclei in the mature spores were spherical, definite in outline and 0.7 - 1.0 µ in diameter. The dividing nuclei in the merosporangia and sporophore were not so definite in outline, being variably regular, irregular, oval and spherical. (Fig. 12 K. L.) Some dividing nuclei took on an extreme eliptical form, 2.0 µ by less than 0.5 µ, with the concentration of nuclear material at the opposite ends (Fig. 12 M). Distinct chromosomes could not be demonstrated, but discrete chromatin granules and movements of them were observed during nuclear division.

Zygospores were not observed under any of the various cultural conditions adopted. However, as only one specimen of S.3 was available, the possibility arose of it being a heterothallic species.

(b) Discussion of taxonomic position of Syncephalis 3.

There are five species of Syncephalis which regularly form two spores per merosporangium. These are S. nana, S. tenuis, S. fusiger, S. wynneae, and Syncephalopsis bispora.

S. wynneae, S. fusiger and Syncephalopsis bispora have their merosporangia inserted on sterile sterigmata, while they are inserted directly on the head in S.3. S. tenuis, S. nana and S.3 possess spores of the same shape which appear to be formed in identical fashion. However, the sporophore of S. tenuis is tall, being 500 - 700 µ high,

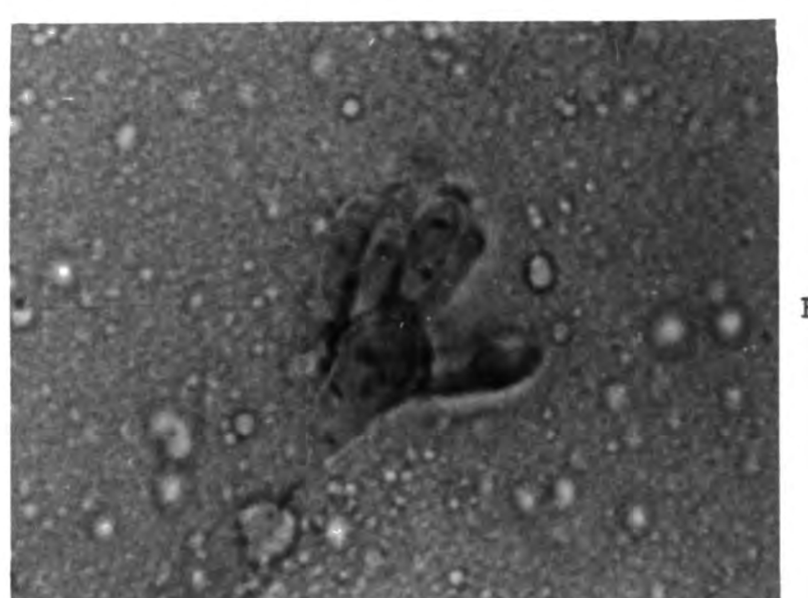
Fig. 13

A. S.3 Differentiation of spores, within the merosporangium. x 2,250

B. S.3 Young merosporangium at the bi-nucleate stage. x 1,600

FIG. 13.

A.



B.

whilst that of S.3 only 55 - 135 μ; the spores of the former are 20 - 27 μ long, whilst those of the latter only 6.5 - 13.0 μ.

S. nana closely resembles S.3 in general morphology of the sporophores, merosporangia and spores. The following table contains a comparison of these two species, which are also illustrated in Fig. 14.

Table 2. Summary of differences between S. nana and S. 3

Syncephalis nana	Syncephalis 3
Sporophore yellow	Sporophore white to colourless
Sporophore 100 μ in height	Sporophore 55 - 135 μ in height
8.0 μ wide at base. 2.0-3.0μ wide at neck	3.0-8.0 μ wide at base 2.0-4.5 μ wide at neck
Head 15.0 - 20 μ diameter	Head 5.0 - 11.0 μ diameter
30-40 merosporangia per head	15-20 merosporangia per head
Merosporangia 16.0-22.0 μ x 4.0 μ	Merosporangia 13.0-26.0 μ x 2.0-4.0 μ
Spores 10.0 - 12.0 μ x 4.0 μ clear honey coloured	Spores 6.5-13.0 μ x 2.0-4.0 μ colourless
"Wet spored"	"Wet spored"
When spores are detached, protuberances marking the position of the merosporangia are clearly visible on the head	When spores are detached, such protuberances only occur occasionally and then are only seen with difficulty
Zygospores not observed	Zygospores not observed
Parasitic on Absidia glauca on dried Cacao beans, Togoland, Africa	Parasitic on Absidia orchidis in manured soil nr. Kegworth, Leicestershire. Grid ref. SK 482294.

These differences may warrant the establishment of S.3 as a new species of Syncephalis.

(c) Ecology of Syncephalis

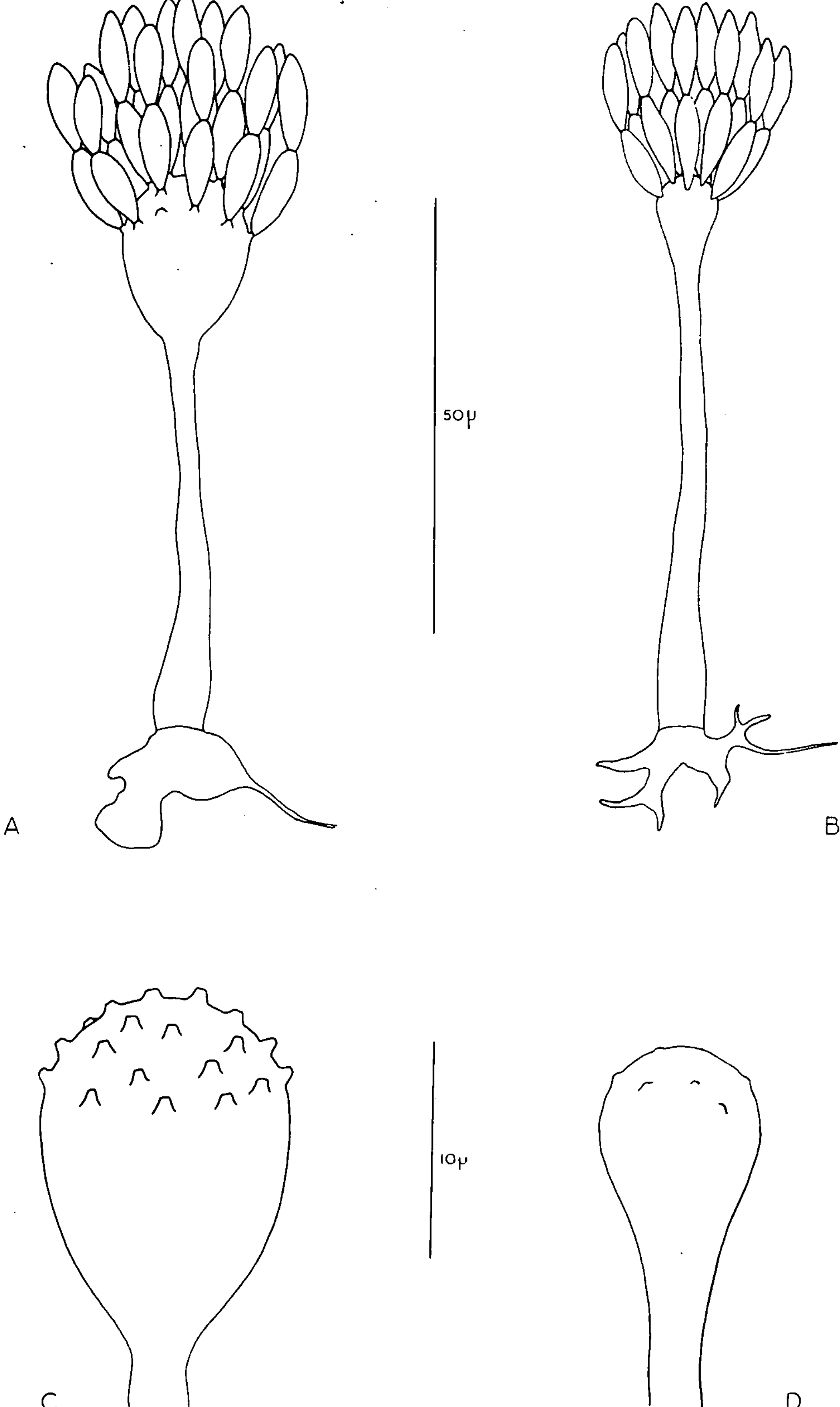
Additionally to the study of S.3, attempts were made to isolate

Fig. 14

A.C. *S. nana*

B.D. *S.3*

FIG. 14.



Syncephalis species from soils and dungs.

Many specimens of S. nodosa, S. cornu and S. depressa, together with a single specimen of S. ramosa were isolated from soils and dungs collected in Great Britain, Switzerland, Germany, Austria and France. S. sphaerica was identified, but attempts to bring it into culture were unsuccessful. (Table 3).

Table 3. Species of Syncephalis collected.

Jan 1960	Horse dung	Nottingham	S. nodosa
May 1960	Cow dung	Nottingham	S. nodosa
May 1960	Cow dung	Nottingham	S. nodosa
May 1960	Cow dung	Nottingham	S. nodosa
May 1960	Cow dung	Nottingham	S. nodosa
May 1960	Cow dung	Nottingham	S. nodosa
May 1960	Soil	Hamlin (Germany)	S. nodosa
Jul 1960	Soil	Oberammergau (Germany)	S. nodosa
Jul 1960	Soil	Reith bei Seefeld (Austria)	S. ramosa
Jan 1961	Dung	Basingfield (Notts.)	S. nodosa
Jan 1961	Dung	Edwalton (Notts.)	S. Nodosa
Jan 1961	Dung	Basingfield (Notts.)	S. nodosa
Jan 1961	Dung	Basingfield (Notts.)	S. nodosa
Feb 1961	Dung	Fylingthorpe (Yorks.)	S. nodosa
Feb 1961	Dung	Fylingthorpe (Yorks.)	S. nodosa
Feb 1961	Dung	Fylingthorpe (Yorks.)	S. nodosa
Apr 1961	Sheep dung	Edale (Derbys.)	S. nodosa
Jul 1961	Soil	Martigny (Switzerland)	S. cornu and S.(RAB.5)
Jul 1961	Soil	Martigny (Switzerland)	S. cornu
Aug 1961	Soil	Llanrwst (N.Wales)	S. nodosa
Aug 1961	Soil	Llanrwst (N.Wales)	S. nodosa
Aug 1961	Soil	Llanrwst (N.Wales)	S. nodosa
Aug 1961	Soil	Llanrwst (N.Wales)	S. nodosa

Table 3 cont.

Aug 1961	Soil	Llanrwst (N.Wales)	S. nodosa
Jul 1962	Soil	Chateau d'Oex (Switzerland)	S. nodosa
Jul 1962	Soil	Chateau d'Oex (Switzerland)	S. nodosa
Jul 1962	Soil	Chateau d'Oex (Switzerland)	S. nodosa
Jul 1962	Soil	Chateau d'Oex (Switzerland)	S. nodosa and S. depressa (R.A.B. 40)
Jul 1962	Soil	Chateau d'Oex (Switzerland)	S. nodosa and S. depressa
Jul 1962	Soil	Martigny (Switzerland)	S. (R.A.B.5)
Jul 1962	Soil	Martigny (Switzerland)	S. cornu
Jul 1962	Soil	Martigny (Switzerland)	S. depressa
Jul 1962	Soil	Martigny (Switzerland)	S. cornu
Jul 1962	Soil	Martigny (Switzerland)	S. cornu and S. sphaerica
Jul 1962	Soil	Martigny (Switzerland)	S. nodosa
Jul 1962	Soil	Martigny (Switzerland)	S.sphaerica
Jul 1962	Soil	Videmanette (Switzerland)	Syncephalis sp.
Jul 1962	Soil	Videmanette (Switzerland)	S. depressa
Jul 1962	Soil	Videmanette (Switzerland)	S. depressa
Jul 1962	Soil	Videmanette (Switzerland)	S. depressa
Jul 1962	Soil	Videmanette (Switzerland)	S. depressa
Jul 1962	Soil	Videmanette (Switzerland)	S. depressa
Jul 1962	Soil	Videmanette (Switzerland)	S. depressa
Jul 1962	Soil	Videmanette (Switzerland)	S. depressa

A number of other species were observed from time to time but were not identified or brought into culture. Some of these species could not be compared favourably with any previously described Syncephalis (eg. S. RAB.5), a factor which indicates that the genus contains many more species than at present recognised.

Syncephalis commonly appeared in association with species of Mucor, Absidia, Chaetocladium and Piptocephalis.

Three types of soil frequently yielded species of Syncephalis:-

(a) Sandy, well drained, rudimentary soils with little humus present, eg. screes, rock-wastes and mountain soils.

(b) Forest soils and litter.

(c) Alpine pastures.

Syncephalis appeared regularly when the dung of cows, sheep and horses was incubated.

They were found in samples obtained at all altitudes up to 2,525m (7,500 ft.) S. depressa was found in large numbers at 2,239 m. (6,720 ft.) in a thin grass covered soil.

(d) Other observations on Syncephalis

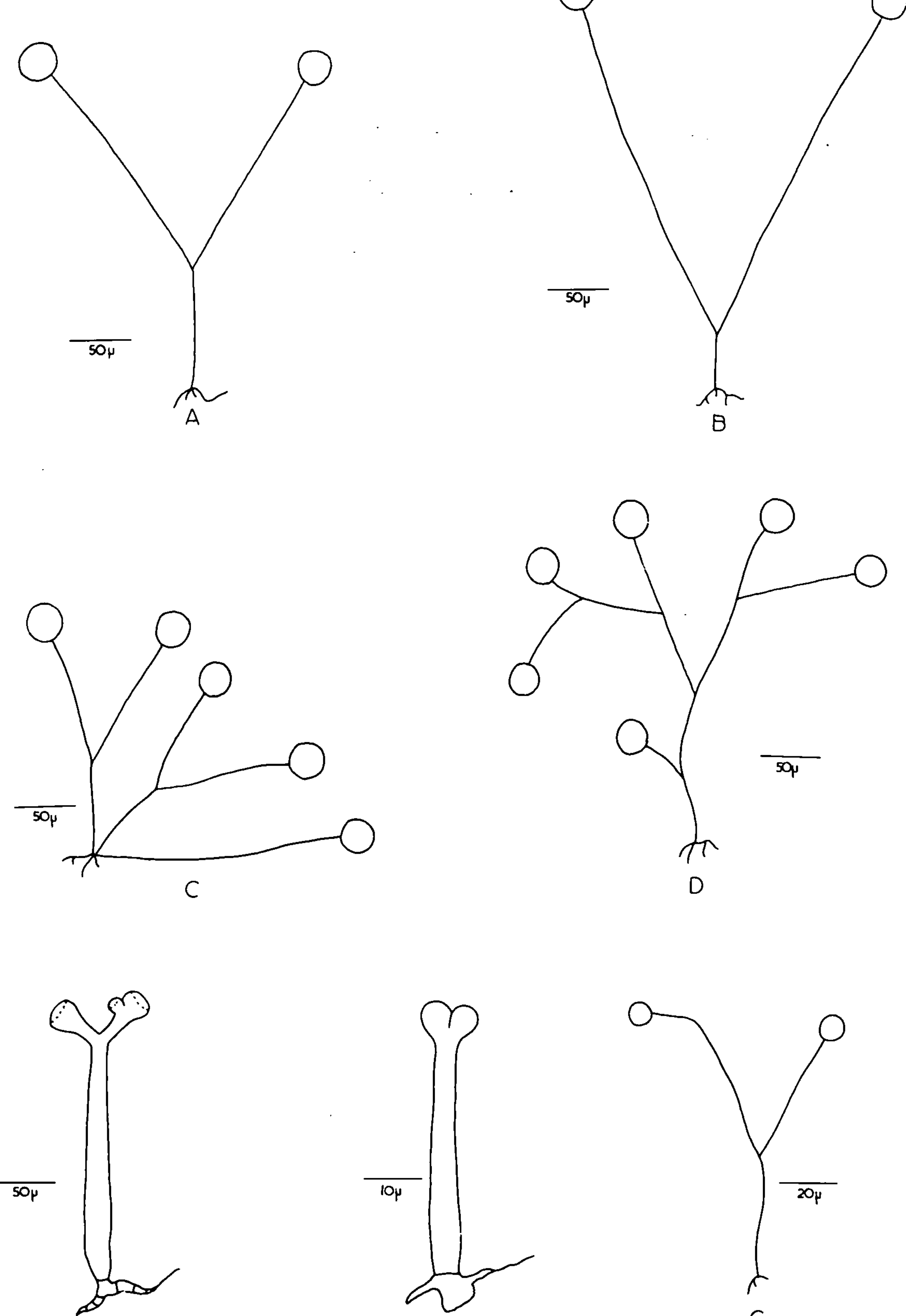
It is interesting to record that bifurcated sporophores occurred occasionally in S.3 (Fig. 15 F.G.) This is considered to be diagnostic of S. bifurcata, but certainly was recorded in several Syncephalis isolates, but particularly in S. depressa, where not only highly bifurcated sporophores but also bifurcated heads were regularly found (Fig. 15 A-E). In all bifurcated sporophores found, heads were normal and spores selected from such sporophores parasitised hosts, to give normal sporophores, with only the same regular percentage of bifurcated

Fig. 15

Bifurcated sporophores. (A-E, *S. depressa*, F.G. *S.3*)

(A-D, G, = diagrammatic representation)

FIG. 15.



ones. This indicated that there was no genetic involvement in bifurcation in S. depressa.

Absidia orchidis when grown alone on various media, soon took on a dark brown appearance as the sporangia matured. If parasitised by S.3, the culture remained a whitish-grey colour. This could easily be seen with the naked eye and microscopic examination showed that this was due to the suppression of sporangial formation in Absidia. Occasionally, where the parasite grew very vigorously, its mycelium could be seen as small white patches amongst the host mycelium.

Continual sub-culturing on sugar-rich media affected the diagnostic feature of S.3. The general size of the sporophore was reduced, both in length and breadth, in some cases the head was barely discernable from the rest of the sporophore or became distorted (Fig. 11 F - H). Occasionally, rhizoids failed to develop, the sporophore arising directly from the mycelium. The most noticeable alteration was in the form of the merosporangia, which did not develop beyond the "one-spored" stage previously mentioned and became relatively much thicker. The number of merosporangia was also reduced.

Section 2. Germination of spores and axenic growth of Syncephalis

(a) Introduction

Since van Tieghem (1875) generalised that all species of Syncephalis were facultative parasites on the evidence of saprophytic growth of S. cordata, S. nodosa and S. reflexa, no other workers have yet succeeded in growing any species of Syncephalis in the absence of a host. In some species, a small amount of mycelium may be formed and even a few atypical sporophores produced. (Benjamin 1959).

The series of experiments described in this section attempted to define the conditions for spore germination and the axenic growth of Syncephalis 3 in culture media. Spores of S. depressa (RAB 40) were also used concurrently in these experiments so that comparative conclusions could be made between the germination behaviour of spores of two contrasting species. Some authors have divided the genus into groups of species using as a criterion the type of merosporangial insertion on the sporophore head. This partition clearly separated S.3 and S. depressa. Further, with respect to other characters, such as the method of formation of the merosporangia, the arrangement of merosporangia on the sporophore heads, the complexity of the merosporangia and basal spores, the spore shape and the spore numbers in merosporangia, these two species clearly contrast with each other.

For the harvesting of Syncephalis spores, parasitic associations of each species on Absidia orchidis were grown on Dung Sucrose Agar at room temperature (18 - 23°C). Preliminary experiments had shown parasitic spores to be viable as soon as spore droplets were fully formed. Pure

inocula of 200 - 300 spores were gathered in sterile loops from such droplets and plated directly on test media prepared, at least, in triplicate. As a precaution during each harvesting, a control inoculum was used for a viability test, involving the successful parasitisation of, usually, Absidia.

(b) Swelling of spores on germination

Spores were placed on to the surface of Potato Carrot Agar. Small squares of the medium were immediately removed and the spores measured in length and width. The remaining spores were incubated and similarly measured after a period of time.

Results showed that spores of S.3 did not swell before germination, whilst those of S. depressa increased their volume by approximately 70%. With the latter, only swollen spores germinated, and further, the progress from a normal, to a swollen, to a germinated spore was rapid and intermediate conditions were difficult to detect at room temperature, but could be followed by decelerating this progression, at an incubation temperature of 6°C. A large vacuole developed simultaneously with the swelling of the spore.

(c) Degree and amount of spore germination

In the following experiments to determine conditions for spore germination, it was necessary to code the amount and degree of germination as follows:-

(i) Stages of germination

0 = no germination

1a = germination tube just emerging

1b = germination tube just emerging and once bifurcated (S.3 only)

2 = germination tube 2 - 4 times length of spore

3 = limited mycelium

Table 4. Stages in germination

TYPE	Stages in germination. 0	1a	1b	2	3
S.3				–	–
S. depressa			–		

The decision to exclude spore swelling as a germinative stage rested on two bases. The fact that spores of S.3 do not swell and those of S. depressa swell rapidly. Its exclusion made the comparative assessment of germination in these two species more readily appreciated.

(ii) Percentage germination

The percentage germination was estimated by random counts and recorded as follows:-

1a or 1b - 50-100% spores germinated to stage 1a or 1b

Some 1a or some 1b - 10-50% spores germinated to stage 1a or 1b

a few 1a or a few 1b - up to 10% spores germinated to stage 1a or 1b

0 - no germination

(iii) Germination time

The stages reached and the percentage of spores having attained such stages were recorded at fixed and/or appropriate intervals and entered in the following tables of results.

(d) pH and temperature ranges for spore germination

Prior to the use of established or formulated test media, optimum pH and temperature ranges for the germination of the spores of S.3 and S. depressa were determined.

For the pH range determination, Malt extract Agar and Milk Agar were selected as media because they were known to allow varying degrees of germination of the spores of both species. The media were prepared at double strength and mixed with equal quantities of autoclaved, double strength, Citric acid/di-Sodium hydrogen phosphate buffer solution* all at 50°C to produce, in each case, a series of media with varying pH. The final pH of each medium was determined, using narrow-range indicator papers.

For the temperature range determinations, Malt extract A, Dung Lactose A. and Potato Carrot A. were selected as another series of media, again permitting varying degrees of germination.

Test media were examined on each of seven days and results are presented in Figs. 16 and 17.

Such results indicated pH 6.0 to be a common optimum for the spore germination of both species. It is to be noted that S.3 spores took seven days to reach their maximum germinative stages, those of S.depressa

---

* See Appendix II for constituents and method of preparation.

FIG. 16. pH. range of spore germination.

S. TYPE 3.
(7 DAYS).
Malt extract A.
Milk A.
% stage Ib.
% stage Ia.
30
20
10
90
80
70
60
50
40
30
20
10
3·0
4·0
5·0
6·0
7·0
8·0
pH.

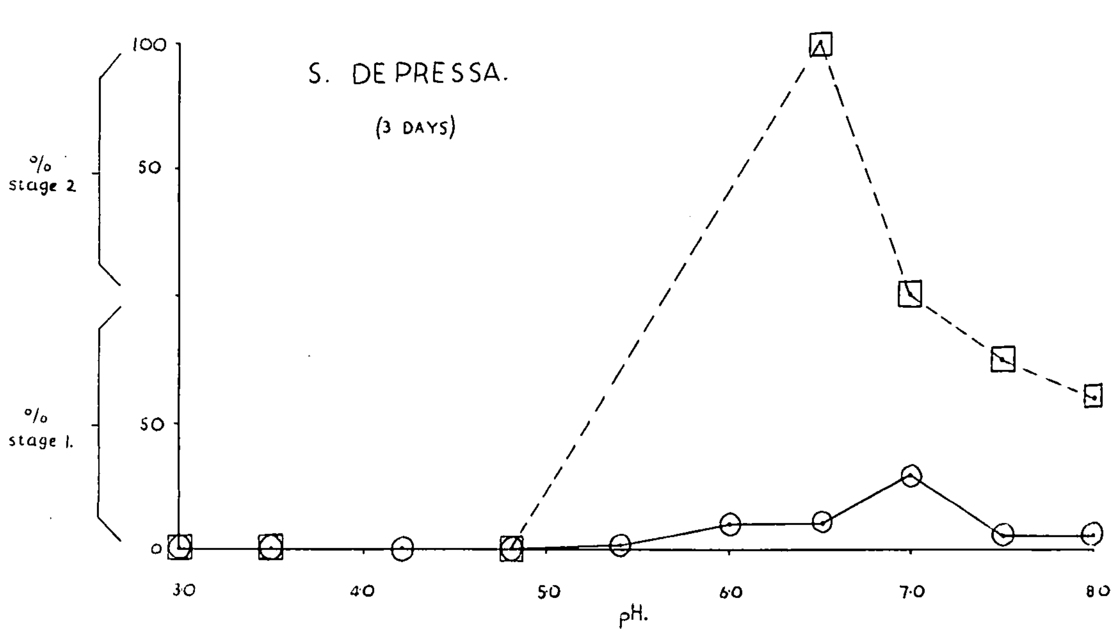
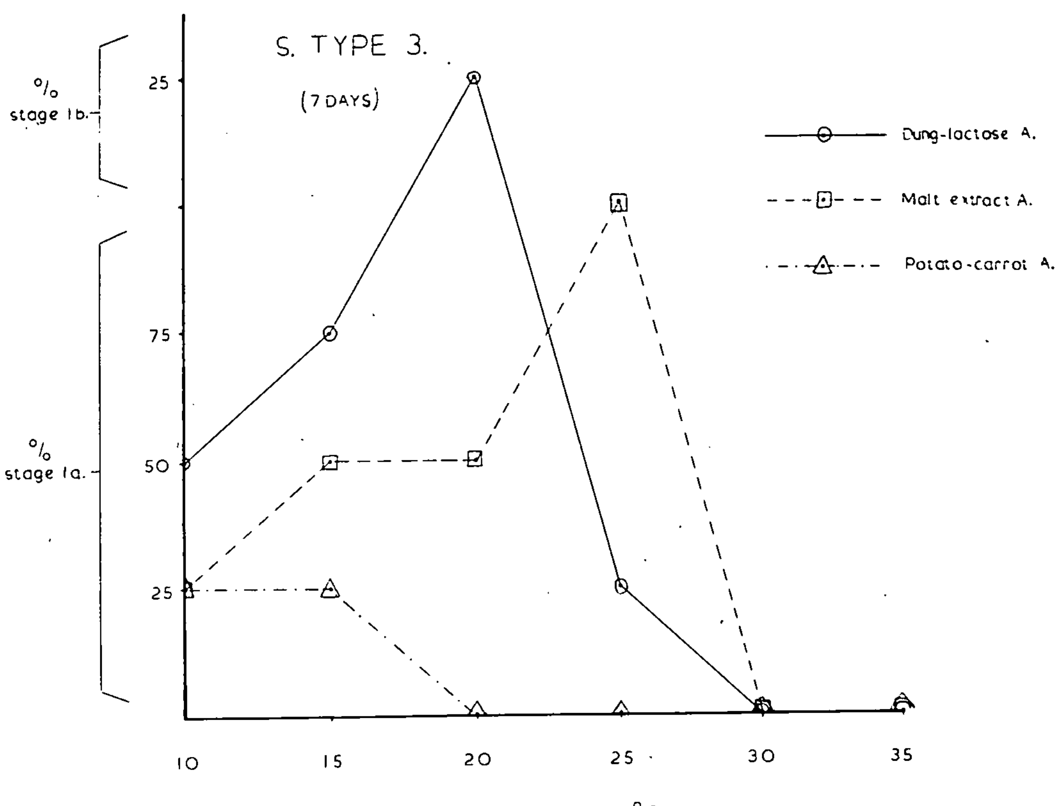
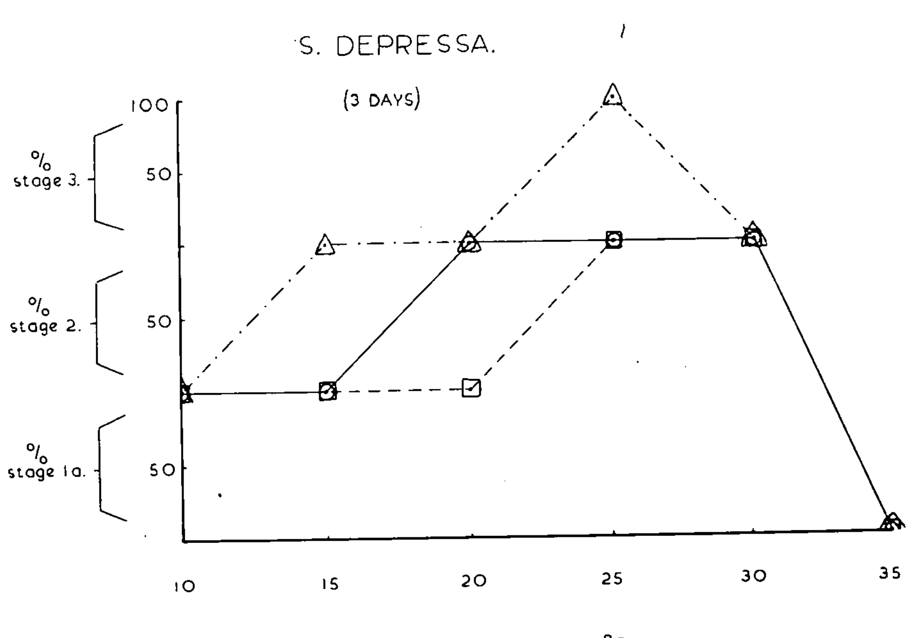


FIG. 17. Temperature range of spore germination.





only three days.

With respect to temperature, spores of S. depressa germinated readily on all three media between 10°c and 30°c. Dung Lactose A. and Malt extract A. permitted S.3 spores to germinate between 10°c and 25°c. Potato Carrot A. restricted both the degree and amount of germination to a limited range. This finding probably reflected its unsuitability as a medium.

The temperature of 22.5°c was selected as a common optimum for the spore germination of both species.

On the basis of the above, subsequent test media were adjusted to pH. 6.0 and incubations held at 22.5°c.

(e) Germination of spores on introductory test media.

Tables 5a.b. record results obtained for germination, using a range of arbitrarily selected mycological and bacteriological media, all adjusted to pH. 6.0 and incubated at 22.5°c. All culture plates were examined daily for seven days and plain Water Agar test plates were used as controls.

Table 5a. Germination of spores of S.3 on standard media.

Medium	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Water A.	0*	0	0	0	0	0	0
Dung Sucrose A.	1a	1b	1b	1b	1b	1b	1b
Dung Lactose A.	1a	some 1b	some 1b	1b	1b	1b	1b
Dung A.	1a	some 1b	1b	1b	1b	1b	1b
Polyporus extract A.	1a	some 1b	1b	1b	1b	1b	1b
Milk A.	1a	1a	1a	some 1b	some 1b	some 1b	1b
MacConkey A.	1a	1a	1a	some 1b	some 1b	some 1b	1b
Dextrose Peptone A.	1a	1a	1a	1a	some 1b	some 1b	some 1b
Corn steep liquor Lactose A.	1a	1a	1a	1a	1a	some 1b	some 1b
Mixed A.	some 1a	1a	some 1b	1b	1b	1b	1b
China Blue Lactose A.	some 1a	some 1a	some 1b	some 1b	some 1b	some 1b	some 1b
Polyporus extract Lactose A.	1a	1a	1a	1a	1a	1a	1a
Sabouraud Maltose A.	1a	1a	1a	1a	1a	1a	1a
Polyporus extract Sucrose A.	some 1a	some 1a	1a	1a	1a	1a	1a
Brilliant Green Bile A.	some 1a	1a	1a	1a	1a	1a	1a
Malt extract A.	0	0	some 1a	1a	1a	1a	1a
Potato Carrot A.	0	0	some 1a	some 1a	some 1a	some 1a	some 1a
Nutrient A.	0	0	0	some 1a	some 1a	some 1a	some 1a
Tomato juice A.	0	0	0	0	some 1a	some 1a	some 1a
Czapek Dox A.	0	0	0	0	some 1a	some 1a	some 1a
Corn meal Dextrose A.	0	0	0	0	D	0	0
Corn meal A.	0	0	0	0	0	0	0
Cotton seed meal A.	0	0	0	0	0	0	0
Spent grain A.	0	0	0	0	0	0	0

* For explanation of amount of germination see Table 4.

Table 5b. Germination of spores of S. depressa on standard media.

Medium	1 day	2 days	3 days	4 days	5 days	6 days	7 days
Water A.	2	2	2	3	3	3	3
Dung Sucrose A.	2	3	3	3	3	3	3
Dung Lactose A.	2	2	3	3	3	3	3
Dung A.	2	3	3	3	3	3	3
Polyporus extract A.	2	2	2	2	2	2	2
Milk A.	3	3	3	3	3	3	3
MacConkey A.	la	2	3	3	3	3	3
Dextrose Peptone A.	2	3	3	3	3	3	3
Corn steep liquor Lactose A.	la	3	3	3	3	3	3
Mixed A.	some 2	3	3	3	3	3	3
China Blue Lactose A.	some 2	3	3	3	3	3	3
Polyporus extract Lactose A.	2	2	2	2	2	2	2
Sabouraud Maltose A.	2	3	3	3	3	3	3
Polyporus extract Sucrose A.	some la	la	la	la	la	la	2
Brilliant Green Bile A.	2	2	2	2	2	2	2
Malt extract A.	la	la	some 2	2	2	3	3
Potato Carrot A.	la	la	2	2	3	3	3
Nutrient A.	2	2	2	3	3	3	3
Tomato juice A.	0	0	0	0	0	0	0
Czapek Dox A.	0	la	la	2	2	2	2
Corn meal Dextrose A.	some la	some la	la	la	la	2	2
Corn meal A.	la	la	la	la	la	la	la
Cotton seed meal A.	some la	la	la	la	la	la	la
Spent Grain A.	0	0	0	0	0	0	0

S.3 spores, it will be noted, did not germinate on Water Agar, but did germinate to the stage coded as 1b on certain other media. Contrarily, the spores of S. depressa germinated on Water Agar to an extent only equalled by that on some test media and not achieved by that on others.

With S.3, spore germination benefited from certain medial contents; with S. depressa, certain media accelerated the rate of germination, only, whilst others inhibited both rate and degree of germination.

The following media, Dung A, Dung Sucrose A, Dung Lactose A, Polyporus extract A, Polyporus extract Sucrose A, Polyporus extract Lactose A, Milk A, MacConkey A, Malt extract A, Mixed A, Dextrose Peptone A, Corn steep liquor Lactose A, China blue Lactose A, Sabouraud Maltose A and Brilliant green Bile A were found to allow germination of S.3 spores to at least stage 1a. These media could therefore contain some substances which stimulate germination. Of the above media however, Polyporus extract A, Polyporus extract Lactose A, Polyporus extract Sucrose A, and Brilliant green Bile A inhibited the spore germination of S. depressa (note however, the object of concentrating on S.3)

In the media stimulating S.3 spore germination, a number have common constituents, such as Lactose, which occurs in nine of the media; Peptone in eight, Bile salts in four; Polyporus extract, in three and Dung in three. It is interesting to note that Lactose, Bile salts and Dung are associated with animal metabolism and that Syncephalis is frequently found parasitising coprophiles.

To assess whether or not these constituents singly or in combination could enhance spore germination, a series of 31 media with a Dung A base,

Table 6. Germination of spores of S.3 and S. depressa on Lactose (Lact.)/Peptone (Pept.)/Bile-salts (Bile.)/Polyporus extract (Poly ex.)/Dung(Dung) media.

* For explanation of amount of germination, see table 4.

Medium	S.3.		S.depressa	
	2 days	5 days	2 days	5 days
Lact	0*	0	0	0
Pept	la	la	2	2
Bile	0	some la	0	0
Dung	la	la	3	3
Poly ex	la	la	2	2
Lact/Pept	la	la	2	2
Lact/Bile	la	la	0	0
Lact/Dung	0	la	0	0
Lact/Poly ex	la	la	la	3
Pept/Bile	la	la	2	2
Pept/Dung	la	la	3	3
Pept/Poly ex	la	la	2	2
Bile/Dung	la	la	la	la
Bile/Poly ex	la	la	la	la
Dung/Poly ex	la	la	3	3

Medium	S. 3		S.depressa	
	2 days	5 days	2 days	5 days
Lact/Pept/Bile	la	la	2	2
Lact/Pept/Dung	la	la	la	2
Lact/Pept/Poly ex	la	la	2	3
Lact/Bile/Dung	la	la	la	la
Lact/Bile/Poly ex	la	la	la	la
Lact/Dung/Poly ex	la	la	la	2
Pept/Bile/Dung	la	la	la	la
Pept/Bile/Poly ex	la	la	la	la
Pept/Dung/Poly ex	la	la	2	3
Bile/Dung/Poly ex	la	la	la	la
Lact/Pept/Bile/Dung	la	la	la	la
Lact/Pept Bile/Poly ex	la	la	la	la
Lact/Pept/Dung/Poly ex	la	la	la	la
Lact/Bile/Dung/Poly ex	la	la	la	la
Pept/Bile/Dung/Poly ex	la	la	la	la
Lact/Pept/Bile/Dung/Poly ex	la	la	la	la

containing the first four of the above constituents singly and in combination were formulated. The concentrations of such constituents in all these media were:- Lactose 1%, Bile salts 1%, Peptone 1% and Polyporus extract 2%.

Results in Table 6 show that, apart from Lactose A, each medium allowed germination of S.3 spores to 1a only, thus providing no enhancement to the axenic development of this fungus.

All but six of the media, viz. Dung A, Lactose-Polyporus extract A, Peptone-Dung A, Dung-Polyporus extract A, Lactose-Peptone-Polyporus extract A and Peptone-Dung-Polyporus extract A, showed marked inhibition of germination of spores of S. depressa.

Again it is thus emphasised that the spores of these two species of Syncephalis differ in their germinative requirements.

(f) Germination of spores on defined media

Defined, synthetic media were prepared by adding various Carbon and Nitrogen sources to a standard basal medium. The basal medium* consisted of a dilute solution in distilled water of the mineral salts, Potassium di hydrogen phosphate, Magnesium Sulphate and Calcium Chloride and trace-elements supplied as Ferrous Sulphate, Cupric Sulphate, Zinc Sulphate, Manganese Sulphate, Cobalt Chloride and Sodium Molybdate, made into a 2% agar medium to which were added compounds under test. Media were prepared with and without the addition of a solution* of the following growth factors:- Thiamin, Riboflavin, Nicotinic acid, Pyridoxal, Biotin, p-Amino Benzoic acid, Pantothenic acid, Folic acid, Vitamin B_{12},

---

* See appendix II for method of preparation.

Inositol, Adenine, Guanine, Hypoxanthine, Xanthine, Uric acid, Uracil and Cytosine. A plain basal agar medium was included in each experiment as a control.

(i) Media with single Nitrogen sources.

Test compounds were incorporated into the basal medium at 0.1% concentration. Test media with inorganic Nitrogen sources were supplemented with 0.1% Lactose. The pH. values of all media were checked after autoclaving, and in no case was there evidence of changes, indicating serious thermo-lability of medial contents.

Results are shown in Table 7.

Table 7. Germination of spores of S.3 and S. depressa on a basal medium of KH_2PO_4/$MgSO_4$/$CaCl_2$/Trace elements, to which the following N. compounds were incorporated.

* For explanation of amount of germination, see table 4.

Medium	S. 3.				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Plain Agar	0*	0	0	0	2	3	2	3
Sodium Nitrite	0	0	0	0	0	0	0	0
Sodium Nitrate	0	0	0	0	2	3	2	3
di-Ammonium Hydrogen Phosphate	0	0	0	0	2	3	2	3
Glycine	0	0	0	0	1a	2	1a	2
L α Alanine	0	0	0	0	2	3	2	3
L Valine	0	0	0	0	2	3	2	3
L Leucine	0	0	0	0	2	3	2	3
L iso Leucine	some 1a	some 1b	0	1a	2	3	2	3
L Proline	0	0	0	0	2	3	2	3
L Hydroxy-proline	0	0	some 1a	some 1a	2	3	2	3
L Serine	0	0	0	0	1a	1a	1a	1a
L Threonine	0	0	0	0	1a	2	1a	2
L Cystine	0	0	0	0	3	3	3	3
L Methionine	0	0	0	some 1a	3	3	2	3
L Aspartic acid	0	0	0	0	1a	1a	1a	1a
L Glutamic acid	0	some 1a	0	some 1a	1a	1a	1a	1a

Table 7 contd.

Medium	S. 3.				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
L Lysine mono hydrochloride	0	0	0	0	3	3	3	3
L Arginine	a few 1a	1a	some 1a	some 1b	3	3	some 3	some 3
L Histidine	some 1b	some 1b	0	some 1a	3	3	2	2
L β Phenyl α alanine	0	0	0	0	2	3	2	2
L Tyrosine	a few 1b	some 1b	some 1b	some 1b	2	3	2	3
L Tryptophane	0	0	0	0	1a	2	1a	1a
L Asparagine	some 1b	some 1b	0	0	1a	1a	1a	1a
L Glutamine	0	0	a few 1a	a few 1a	3	3	3	3
Urea	some 1a	1a	some 1a	1a	2	3	3	3

Such results show that S.3 spores germinated to stage 1a in three days on media containing iso-Leucine, Histidine, Tyrosine, Asparagine and Urea as test compounds.

Some inhibition of the germination of spores of S. depressa on Glycine, Threonine, Histidine and Phenyl-Alanine media and strong inhibition on Serine, Aspartic acid, Glutamic acid, Tryptophane and Asparagine media occurred.

With neither fungus did the addition of the vitamin supplement affect the amount and degree of germination. This was a general finding throughout experiments detailed in this section, and repetition of this fact is avoided.

(ii) Media with mixed Nitrogen sources

Twenty-six media were prepared containing all possible combinations of the most satisfactory single Nitrogen sources for S.3 spore germination, viz. Iso-Leucine, Histidine, Tyrosine, Asparagine and Urea. The total concentration of the Nitrogen sources in each of these media was 0.1%. Results are shown in Table 8.

Table 8. Germination of spores of S. 3 and S. depressa on a basal medium of $KH_2PO_4/MgSO_4/CaCl_2$ + trace elements, to which all possible combinations of iso-Leucine, Histidine, Tyrosine, Asparagine and Urea were incorporated. Total N. conc. = 0.1%

* for explanation of amount of germination, see Table 4.

Medium	S. 3				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
iso-Leucine/Histidine	some* la	some la	0	0	3	3	3	3
iso-Leucine/Tyrosine	some la	some la	0	0	3	3	3	3
iso-Leucine/Asparagine	0	0	0	0	3	3	3	3
iso-Leucine/Urea	some lb	some lb	0	0	3	3	3	3
Histidine/Tyrosine	0	0	0	0	some 3	3	3	3
Histidine/Asparagine	some la	some la	0	0	some la	some 3	2	3
Histidine/Urea	0	0	0	0	3	3	3	3
Tyrosine/Asparagine	0	0	0	0	2	3	2	2
Tyrosine/Urea	some la	some la	some la	some la	3	3	3	3
Asparagine/Urea	some la	la	0	some la	3	3	3	3
Iso-Leucine/Histidine/Tyrosine	0	0	0	0	3	3	3	3
iso-Leucine/Histidine/Asparagine	0	0	0	0	3	3	3	3
iso-Leucine/Histidine/Urea	some la	some la	some la	la	3	3	3	3
iso-Leucine/Tyrosine/Asparagine	0	0	0	0	3	3	3	3
iso-Leucine/Tyrosine/Urea	0	0	0	0	some 3	3	3	3

Table 8 contd.

Medium	S. 3				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
iso-Leucine/Asparagine/ Urea	0	0	0	0	3	3	3	3
Histidine/Tyrosine/ Asparagine	0	0	0	0	a few 3	a few 3	2	3
Histidine/Tyrosine/ Urea	0	0	some la	some la	3	3	3	3
Histidine/Asparagine/ Urea	0	0	0	0	2	3	2	3
Tyrosine/Asparagine/ Urea	0	0	0	0	2	3	2	3
iso-Leucine/Histidine/ Tyrosine/Asparagine	0	0	0	0	3	3	3	3
iso-Leucine/Histidine/ Tyrosine/Urea	some la	la	la	some lb	3	3	3	3
iso-Leucine/Histidine/ Asparagine/Urea	some la	some la	some la	some la	3	3	3	3
iso-Leucine/Tyrosine/ Asparagine/Urea	la	la	la	la	3	3	3	3
Histidine/Tyrosine/ Asparagine/Urea	some la	some la	some la	some la	2	3	2	3
iso-Leucine/Tyrosine/ Histidine/Asparagine/ Urea	la	la	la	la	3	3	3	3

It can be seen from Table 8 that iso-Leucine/Urea, Asparagine/Urea, iso-Leucine/Histidine/Tyrosine/Urea, iso-Leucine/Tyrosine/Asparagine/Urea, and iso-Leucine/Histidine/Tyrosine/Asparagine/Urea media gave germination of S.3 spores to stage 1a or better. Antagonistic effects may explain the fact that some media did not allow germination to at least stage some 1b, as most of the compounds did singly (Table 7).

All media allowed germination of *S.depressa* spores to stage 3. The inhibiting effects of Asparagine were counteracted by the synergistic effects of additional Nitrogen sources.

(iii) Media with Carbon and Nitrogen sources.

Prior to using test media with Carbon and Nitrogen sources, a preliminary trial was carried out adding Sucrose or Lactose singly to a basal medium containing separately, Tyrosine, Urea and Tyrosine/Urea as Nitrogen sources. These latter were the simplest of those which had provided the highest consistent degree of germination of S.3 spores. The object of this trial was to select the best of the Nitrogen sources for continued work. The total concentration of the Nitrogen compounds was 0.1% and that of the Carbon compounds also 0.1%. The result of the germinative studies are given in Table 9.

Table 9. Germination of spores of S.3 and S. depressa on a basal medium of $KH_2 PO_4/MgSO_4/CaCl_2$ + trace elements, to which Nitrogen +/- Carbon containing compounds were added. Total conc. of each = 0.1%

* for explanation of amount of germination, see table 4.

	S. type 3.				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
Medium	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Urea/Sucrose	la*	la	la	la	2	3	2	3
Urea/Lactose	some lb	some lb	some lb	some lb	3	3	2	3
Urea	la	la	some la	some la	2	3	2	3
Tyrosine/Sucrose	a few la	some la	some la	some la	2	3	2	3
Tyrosine/Lactose	some lb	some lb	some la	some la	3	3	3	3
Tyrosine	some lb	some lb	a few la	a few la	2	3	2	3
Tyrosine/Urea/Sucrose	some la	some la	a few la	la	2	3	2	3
Tyrosine/Urea/Lactose	some la	some la	some la	la	2	3	2	3
Tyrosine/Urea	la	la	some la	some la	2	3	2	3

Urea was the best choice of Nitrogen source, giving consistently good germination of S.3 spores. Urea has the advantage of being a simple compound, but decomposes on heating. However, the minimal autoclaving gave no change in pH. and its use was continued for further studies.

It had been observed in preliminary experiments, that a high concentration of Urea tended to suppress spore germination. Consequently, the spores of S.3 and S. depressa were tested on media containing Urea in concentrations between 0.01% and 1.0%, the results being given in Table 10.

Table 10. Germination of spores of S.3 and S.depressa on a basal medium of $KH_2PO_4/MgSO_4/CaCl_2$ + trace elements, to which varying concentrations of Urea were incorporated.

* for explanation of amount of germination see table 4.

	S. 3.				S. depressa.			
	- vits.		+ vits.		- vits.		+ vits.	
Medium	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
0.01% Urea	0	0	0	0	2	3	1a	2
0.05% Urea	some 1a	1a	0	some 1a	2	3	1a	2
0.10% Urea	some 1a	1a	some 1a	some 1a	2	3	2	3
0.50% Urea	some 1a	some 1a	some 1a	some 1a	2	3	2	3
1.00% Urea	0	0	0	0	2	3	2	3

Table 10 shows that 0.1% Urea is a suitable concentration for the spores of both S.3 and S depressa.

Therefore, test media containing 0.1% Urea and various Carbon sources were prepared and results for spore germination are given in Table 11. Some oils were also used as Carbon sources, likely to exhibit permeability differences from the other Carbon sources used, with respect to spore walls and membranes. Such oils were emulsified with 0.1% autoclaved Gum Tragacanth prior to addition to the test media and an additional Gum Tragacanth/Urea Agar was included as a control medium.

Results in Table 11 show that all media except Clove oil/Urea allowed germination of the spores of S.3 to at least stage some la. Pyruvic acid/Urea, Lactic acid/Urea and Acetic acid/Urea media allowed germination to a degree above that allowed by Urea medium alone. Several media also allowed a slightly, but not significantly so, higher degree of germination than the control medium. Addition of the vitamin supplement improved development on such media as Glucose/Urea, Cellobiose/Urea and Inulin/Urea.

Germination of S. depressa spores was not stimulated by any of the media, whilst several of them inhibited germination.

The stimulations of germination of S.3 spores produced by Pyruvic acid/Urea, Lactic acid/Urea and Acetic acid/Urea media over and above that by Urea alone were re-examined by obtaining the results entered in Table 12, the possibility of the influence of the Na^+ ions used in pH. adjustment being also considered.

Table 11. Germination of spores of S.3 and S. depressa on a basal medium of KH_2PO_4/$MgSO_4$/$CaCl_2$/trace-elements/0.1% Urea to which was incorporated separately 0.1% of the following Carbon containing compounds.

* For explanation of amount of germination, see table 4.

Medium	S. 3.				S. depressa.			
	- vits.		+ vits.		- vits		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Plain Agar (+ Urea)	some la	some la	some la	some la	2	3	2	3
D Xylose	some la	some la	some la	some la	2	3	2	3
L Arabinose	some la	some la	la	la	3	3	3	3
D Ribose	some la	some la	some la	some la	2	2	2	3
D Glucose	0	0	some la	la	2	3	2	3
D Fructose	some la	some la	la	la	3	3	2	3
D Galactose	some la	some la	some la	some la	2	3	3	3
D Mannose	some la	some la	some la	some la	2	3	2	3
Sucrose	some la	some la	some la	some la	2	3	2	3
Maltose	la	la	la	la	2	3	2	3
D Lactose	some la	some la	some la	some la	3	3	la	la
D Cellobiose	0	0	some la	la	2	3	2	3
Soluble Starch	some la	some la	some la	some la	2	3	2	3
Cellulose	some la	some la	some la	some la	2	3	2	3
Glycogen	some la	some la	some la	some la	2	2	2	2

Table 11 cont.

Medium	S.3				S. depressa.			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Inulin	0	0	some la	some la	2	2	2	3
Chitin	some la	some la	some la	la	2	3	2	3
Dextrin	some la	some la	la	la	2	3	2	3
L Tartaric acid	la	la	some la	some la	3	3	3	3
L Malic acid	some la	la	la	la	3	3	2	3
Citric acid	some la	la	some la	some la	3	3	3	3
DL Lactic acid	some lb	some lb	some lb	some lb	3	3	2	3
Pyruvic acid	some lb	some lb	some lb	lb	2	2	la	la
Acetic acid	la	la	some la	la	2	3	3	3
Stearic acid	some la	some la	some la	some la	2	2	2	2
Glycerol	some la	some la	some la	some la	la	la	la	2
D. Mannitol	some la	some la	some la	some la	3	3	3	3
D. Sorbitol	some la	some la	la	la	la	2	la	la
L Ascorbic acid	some la	some la	some la	some la	la	2	la	2
Olive Oil	some la	some la	some la	some la	0	0	0	0
Cedar-wood oil	some la	some la	some la	some la	la	2	la	2
Clove Oil	0	0	0	0	0	0	0	0
Gum Tragacanth	some la	some la	some la	some la	2	3	2	3

Table 12. Germination of spores of S.3 and S. depressa on a basal medium of $KH_2PO_4/MgSO_4/CaCl_2$/trace elements to which acids (0.1%) and/or Urea (0.1%) and/or Sodium ions were added.

* for explanation of amount of germination, see table 4.

Medium	S. 3				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Pyruvic acid + Na^+ ions	1b*	1b	some 1b	1b	2	2	2	2
Lactic acid + Na^+ ions	some 1b	some 1b	some 1b	some 1b	3	3	3	3
Acetic acid + Na^+ ions	some 1b	some 1b	some 1b	some 1b	2	3	3	3
Pyruvic acid + Urea + Na^+ ions	some 1b	1b	some 1b	1b	2	2	2	2
Lactic acid + Urea + Na^+ ions	some 1b	some 1b	some 1b	some 1b	2	2	2	2
Acetic acid + Urea + Na^+ ions	1a	1a	some 1b	some 1b	2	3	3	3
Urea + Na^+ ions	some 1a	some 1a	some 1a	1a	3	3	2	3
Na^+ ions	0	0	0	0	2	2	2	2
Urea	some 1a	some 1a	some 1a	some 1a	3	3	3	3

It can be seen that stimulation of the germination of S.3 spores could be ascribed to single acid anions, particularly Pyruvate.

Because of the above finding, that stimulation was ascribable to the Carbon Sources, the stimulating effects indicated in Table 12 were checked by formulating a series of test media with 0.1% of selected Carbon sources only. The results are given in Table 13.

Table 13. Germination of spores of S. 3 and S. depressa on a basal medium of $KH_2PO_4/MgSO_4/CaCl_2$/trace elements to which the following Carbon containing compounds (0.1%) were added.

* for explanation of amount of germination, see table 4

Medium	S. 3.				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Plain Agar	0*	0	0	0	2	3	3	3
D Xylose	0	0	0	0	3	3	3	3
L Arabinose	0	0	0	0	3	3	3	3
D Ribose	0	0	0	0	3	3	3	3
D Glucose	0	0	0	0	3	3	3	3
D Fructose	a few la	a few la	0	0	3	3	3	3
D Galactose	0	0	0	0	3	3	3	3
D Mannose	0	0	0	0	3	3	3	3
Sucrose	0	0	0	0	3	3	3	3
Maltose	0	0	0	0	3	3	3	3
D Lactose	0	0	0	0	3	3	3	3
D Cellobiose	0	0	0	0	3	3	3	3
Soluble Starch	0	0	0	0	2	2	2	2
Glycogen	0	0	0	0	3	3	3	3
Inulin	0	0	0	0	3	3	3	3
Chitin	0	0	0	0	3	3	3	3

Table 13 contd.

Medium	S. 3				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
L Tartaric acid	0	0	0	0	2	3	3	3
L Malic acid	0	0	0	0	2	3	2	3
Citric acid	0	0	0	0	3	3	3	3
Glycerol	0	0	0	0	2	3	3	3
D. Sorbitol	0	0	0	0	3	3	3	3
D. Mannitol	0	0	0	0	2	3	3	3
L Ascorbic acid	some la	some la	0	some la	la	la	la	la
Pyruvic acid	lb	lb	some lb	lb	2	2	2	2
Lactic acid	some lb	some lb	some lb	some lb	3	3	3	3
Acetic acid	some lb	some lb	some lb	some lb	2	3	3	3

Pyruvate, Lactate and Acetate were amply confirmed as being the only single Carbon sources stimulating the germination of S.3 spores, none of the others being successful without Urea.

Tables 11, 12 and 13 showed again that Pyruvate strongly inhibited the germination of the spores of S. depressa. Many of the inhibitions of the spore germination of this species shown by the Urea/Carbon source media in Table 11, did not appear when the Carbon source was used alone, with the exception of Ascorbate, which inhibited with or without Urea. This fungus may be sensitive to compounds which may arise during the preparation and autoclaving of media.

Because of their involvement in metabolic pathways, seven media were prepared containing all possible combinations of Pyruvate, Lactate and Acetate. The total concentration of the Carbon sources in all cases was 0.1%. Results are shown in Table 14.

Table 14. Germination of spores of S.3 and S. depressa on a basal medium of $KH_2PO_4/MgSO_4/CaCl_2$/trace elements to which all possible combinations of Pyruvate, Lactate and Acetate were added (total C. conc. = 0.1%)

* for explanation of amount of germination, see table 4.

Medium	S. 3				S. depressa			
	- vits.		+ vits.		- vits.		+ vits.	
	3 days	7 days	3 days	7 days	3 days	7 days	3 days	7 days
Pyruvate	1b*	1b	some 1b	1b	2	2	2	2
Lactate	some 1b	some 1b	some 1b	some 1b	3	3	3	3
Acetate	some 1b	some 1b	some 1b	some 1b	2	3	3	3
Pyruvate/Lactate	some 1b	1b	some 1b	1b	2	2	2	2
Pyruvate/Acetate	some 1b	some 1b	some 1b	some 1b	2	2	2	2
Lactate/Acetate	1a	some 1b	some 1b	some 1b	2	2	2	2
Pyruvate/Lactate/Acetate	some 1b	1b	some 1b	some 1b	2	2	2	2

These results show that no further stage in S.3 spore germination could be induced on such test media, the maximum germination of stage 1b being observed on the Pyruvate medium alone.

Except for the Lactate and Acetate media; S. depressa spore germination was inhibited by all other media.

(v) Summary of substances stimulating or inhibiting the germination of spores of S.3 and S. depressa

From the foregoing experiments, it can be seen that a number of test compounds stimulated the germination of S.3 spores to at least stage some 1a. These, and their effects on S. depressa are summarised in Table 15 which has the following key.

Key.

Stimulation	=	+
Good stimulation	=	++
Inhibition	=	-
Strong inhibition	=	- -
No effect	=	0

This key compares the superiority or inferiority of media, containing test compounds as matrices for spore germination, with Water Agar. Spores of S.3 do not germinate on Water A, so (0), (-) or (--) cannot validly be used, because no germination on a particular medium might be due to:- (a) no stimulation (o) or (b) inhibition (-) or (- -).

Spores of S. depressa germinate to stage 3 on Water Agar. This is the highest degree of germination observed on any medium. Therefore, no test substances caused stimulation. Only inhibiting (-) (- -) or neutral (o) substances can be shown and (+) or (++) effects are inapplicable.

Table 15. Summary of substances causing stimulation or inhibition of germination of spores of S.3 and S. depressa.

* for explanation of amount of germination, see Table 4.

	S. 3.		S. depressa.	
	stage	stim/inhib.	Stage	stim/inhib
Water	0*		3	
Dung	1b	++	3	0
Polyporus extract	1a	+	2	-
Bile salts	some 1a	+	0	- -
Peptone	1a	+	2	-
Glycine	0		2	-
L iso-leucine	some 1b	++	3	0
L hydroxyproline	some 1a	+	3	0
L serine	0		1a	- -
L threonine	0		2	-
L methionine	some 1a	+	3	0
L aspartate	0		1a	- -
L glutamate	some 1a	+	1a	- -
L arginine	some 1b	++	some 3	0
L histidine	some 1b	++	3	0
L tyrosine	some 1b	++	3	0
L tryptophane	0		2	-
L asparagine	some 1b	++	1a	- -

	S. 3		S. depressa	
	stage	stim/inhib.	Stage	stim/inhib.
L glutamine	a few lb	+	3	0
Urea	la	+	3	0
Fructose	a few la	+	3	0
Soluble Starch	0		2	-
L ascorbate	some la	+	la	- -
Pyruvate	lb	++	2	-
D.L.Lactate	some lb	++	3	0
Acetate	some lb	++	3	0

	stage	stim/inhib. over Urea	stim/inhib. over Water	Stage	stim/inhib.
$NaNO_3$/Lactose	0			0	- -
Urea/Xylose	some la	0	+	3	0
" /L arabinose	la	0	+	3	0
" /D ribose	some la	0	+	3	0
"/ D glucose	some la	0	+	3	0
"/ D fructose	la	0	+	3	0
"/ D galactose	some la	0	+	3	0
"/ D mannose	some la	0	+	3	0
"/ Sucrose	some la	0	+	3	0
"/ Maltose	la	0	+	3	0

Table 15 contd.

	S. 3			S. depressa	
	stage	stim/inhib. Over Urea	stim/inhib. over Water	stage	stim/inhib.
Urea/D lactose	some la	0	+	3	0
" /D cellobiose	some la	0	+	3	0
" /Soluble starch	some la	0	+	3	0
" /Cellulose	some la	0	+	3	0
" /Glycogen	some la	0	+	2	-
" /Inulin	some la	0	+	3	0
" /Chitin	la	0	+	3	0
" /Dextrin	la	0	+	3	0
" /L tartrate	la	0	+	3	0
" /L malate	la	0	+	3	0
" /L citrate	la	0	+	3	0
" /D.L. Lactate	some lb	+	++	3	0
" /Pyruvate	lb	+	++	2	-
"/ Stearate	some la	0	+	2	-
" /Glycerol	some la	0	+	2	-
" /Mannitol	some la	0	+	3	0
" /Sorbitol	la	0	+	2	-
" /L ascorbate	some la	0	+	2	-
" /Olive oil	some la	0	+	0	- -
" /Cedar-wood oil	some la	0	+	2	-
" /Clove oil	0	-		0	- -
" /Acetate	la	0	+	3	0

DUBLIN UNIVERSITY SCIENCE 20 OCT 1966 SECTION LIBRARY

From this summary emerged the following points.

(i) The germination of S.3 spores was stimulated most by single substances such as L iso-Leucine, L Arginine, L Histidine, L Tyrosine, L Asparagine, Lactate, Acetate and Pyruvate. Of these, the latter consistently gave the greatest amount and degree of germination.

(ii) Media with combinations of Carbon and Nitrogen sources never equalled the stimulatory effects of Pyruvate.

(iii) The germination of *S. depressa* spores was never enhanced but frequently inhibited by test substances and inexplicably, even by some which stimulated that of S.3 spores.

(iv) The inclusion or not of a vitamin supplement in the test media in no way affected results.

(g) Effect of host secretions and extracts on spore germination

During the study of the development of S.3, parasitisation was established only by spores in contact, or almost in contact, with host hyphae. This fact, coupled with the utter failure to promote the axenic development of parasite spore germination, suggests a dependency on materials provided by the host.

For this reason, it was decided to incorporate in selected media, secretions and extracts from *Absidia orchidis*, a fungus proven to be a ready host. The latter was grown at 22.5°c on 100 mls. liquid Corn steep liquor contained in 250 ml. flasks, for eight days, after which the mycelial mat was removed and the remaining liquor filtered and then Seitz sterilised. The mycelial mat was then washed in distilled water, ground with Aluminium Oxide or ultrasonically disintegrated in distilled

water. In both cases these extracts were Seitz sterilised and made up to 15 mls. volume.

Plates of test media were prepared with central bore holes, 1 cm. in diameter. Into these bore holes were then introduced the secretions or extracts in 1 ml. amounts. Inoculations of parasite spores were made to surround these bore holes.

Table 16. Effect of host secretions or extractions on the germination of spores of S. 3 and S. depressa.

* for explanation of amount of germination, see table 4.

		S.3		S.depressa	
		3 days	7 days	3 days	7 days
Dung Lactose A.	Medium alone	1b*	1b	2	3
	Medium + liquor	1b	1b	3	3
	Medium + Al_2O_3 ground extr.	some 1b	1b	3	3
	Medium + Ultrasonic extr.	some 1b	1b	3	3
Malt Extract A.	Medium alone	1a	1a	2	3
	Medium + liquor	1a	1a	2	3
	Medium + Al_2O_3 ground extr.	1a	1a	2	3
	Medium + Ultrasonic extr.	1a	1a	3	3
Potato Carrot A.	Medium alone	0	some 1a	3	3
	Medium + liquor	0	some 1a	3	3
	Medium + Al_2O_3 ground extr.	0	some 1a	3	3
	Medium + Ultrasonic extr.	0	some 1a	3	3

Results given in Table 16 showed no influences on axenic development of parasite spores. Thermo-lability, quick lability or indiffusibility of stimulatory host substances are possible explanations for this failure. The latter two are particularly relevant to the observation that only parasite spores in close proximity attack the host.

Parasite spores separated from live host only by cellophane, might therefore be expected to show responses of a stimulatory nature. Consequently a small piece of cellophane, previously boiled in distilled water to remove plasticizers, was made into a small bag (eventually 2 cm x 2 cm x 0.5 cm) using Araldite (C.I.B.A.) as an adhesive. The bag was sterilised in boiling water and placed in a petri-dish on top of damp filter paper. The bag was partly filled with molten Dung Sucrose A. by infection with a hypodermic syringe, making sure that small pockets of air remained inside the bag. When the medium was set, a suspension of <u>Absidia orchidis</u> spores in sterile distilled water was injected into the bag. The bag was then inverted, placed horizontally on to the filter paper and its upper, outer surface inoculated with parasite spores. As a control, a similar bag was prepared, but omitting the <u>Absidia</u> spores.

Table 17. Germination of spores of S.3 after seven days, separated from an active culture of Absidia orchidis, by cellophane.

* for explanation of amount of germination, see table 4.

Medium	Dung Sucrose A. with Absidia orchidis	Dung Sucrose A. alone
	Some 1b*	Some 1b

Results showed that if there was a stimulating substance diffusible from the host, either it could not permeate the cellophane membrane or became inactive before influencing Syncephalis spores.

(h) Summary

All experiments to stimulate the axenic development of parasite spores failed. The only salient features which emerged were the definite differences in the responses of the two parasites tested. These are given in Table 18.

Table 18. Differences in physiology of spore germination between S.3 and S. depressa.

	S. 3	S. depressa
1.	Do not swell before germination	Swell before germination
2.	Temp. range 10 - 25°c. Optimum 20 - 25°c.	Temp.range 10 - 30° c. Optimum 15 - 30° c.
3.	Do not germinate on Water A.	Germinate on Water A.
4.	Germinate to stage 1b only (after 2 days on best medium)	Germinate to stage 3 (after 1 day on best medium)
5.	Stimulated by Pyruvate, Ascorbate, Asparagine and Glutamate	Inhibited by Pyruvate, Ascorbate, Asparagine and Glutamate

Part IV

Part IV Discussion

Host ranges for several species of Piptocephalis have been investigated and except for P. xenophila, this genus is a mucorine parasite. Most species of Syncephalis have also been reported as mucorine parasites. However, the reports of S. glabra on Boletus sp., S. ubatubensis on an entomogenous fungus, S. tenuis on moss, S. wynneae on Wynnea macrotis query the status of Syncephalis as a mucorine parasite. They can, however, be explained readily by the frequent observation made of Syncephalis on a wide variety of plants and plant debris, but only when these are already parasitised or being disintegrated by mucorine fungi. Thus the association of forest litter, Mortierella and a parasitic Syncephalis species is of frequent occurrence. Leadbeater informs the writer of finding Piptocephalis lepidula on Coprinus, but which also bore a saprophytic Mucor.

Whilst host range experiments on S.3 and S. depressa only confirmed the genus as a mucorine parasite, a categoric statement on its status cannot be made. The laboratory establishment of parasitic associations of Syncephalis with its hosts can be readily achieved with certain Syncephalis species, but with other species is most difficult. Leadbeater et al. readily admit, even with their experience in establishing parasitic associations of Piptocephalis, that they have been unable to obtain or maintain certain species of Syncephalis in pure culture. Further, Dobbs (1942) with his interesting P.xenophila and Mercer (1959) show how a general rule can be broken.

Studies on the axenic development of S.3 and S. depressa have

revealed little, except to emphasise the improbability of the statements of van Tieghem and de Bary that here is a genus capable of saprophytic growth. Supplementary results are however of interest. Thus, it is interesting that the responses of S.3 and S. depressa to axenic culture are so different. The germination of the spores of the latter require only water to germinate to stage 3 and the introduction of metabolites in no way enhances and can even inhibit, this maximum degree of germination. It thus follows, that the growth metabolism of the spore involves stored materials only and none are beneficially incorporated from axenic media provided. The maximum germination, under axenic conditions, of the spores of S.3 to the stage 1b, is very limited and is only attained when certain metabolites are provided, notably Pyruvate and certain Amino-acids.

Nothing was found to confirm the work of Berry and Barnett (1957), Shigo (1960b), Shigo, Anderson and Barnett (1961) on Piptocephalis sp. that C:N ratios affected, or Mn, Fe, Zn and Ca elements were necessary in the media, for the axenic growth of Syncephalis.

The spores of S.3 and S. depressa are metabolically different and it is interesting that they possess different methods of spore formation.

The apparent confinement of parasitism of Syncephalis to the Mucorales suggests the need for some aspect or aspects of metabolic twinning between host and parasite. The presence of necessary growth factors for increased germination of the two fungi could not be shown either by the use of host secretions or host extracts as additives to test media.

It is not pertinent to justify the inability to demonstrate growth factors, by invoking the lability of such possible compounds. One must recall the behaviour of S.3 spores in the presence of susceptible hosts. Parasitisation is only achieved by spores in contact or angled so that their short 1 - 2 μ germ tubes will contact host hyphae. Spores lying in contact with, but parallel to, host hyphae have been often observed unable to achieve parasitisation although possessing germ tubes. One can now invoke, if one wishes, a growth factor secreted by the host but of low diffusibility. It is unlikely, however, that a compound capable of diffusing through a cell wall would possess such a low diffusibility as to be unable to travel 2 μ in agar.

Contact with the host wall by the spores own germinative capacity is indicated as essential. The cell wall of the host, with its cellulose, chitin and associated enzymes necessary for its construction, is unlikely to alter the metabolic pathways of the parasite spore. Host wall enzymes very probably change the elasticity of the proximal germ tube wall, leading to local turgor preceding the act of penetration. Once inside, the parasite must utilise a metabolic step or pathway for which it is deficient.

The parasites under test, showed no toxic effects upon their hosts, but certainly suppressed their formation of sporangia. It is rather surprising that the two neighbours Piptocephalis and Syncephalis attack their hosts in such different ways. Piptocephalis has its local haustorial systems whilst Syncephalis has its systemic invasion of the host. Internal parasite hyphae of Syncephalis can emerge from and

repenetrate the host. No such records have been made for the haustorial rhizoids of Piptocephalis. Does this indicate that Syncephalis is immune to a host defence reaction to which Piptocephalis is susceptible? It is interesting that Leadbeater and Mercer (1957) once observed a restricted systemic Syncephalis-like attack by a Piptocephalis isolate in their possession, an occasion perhaps when the host was unable to produce normal reaction to parasitisation.

Another interesting point which emerges, is the inability of the systemic hyphae of Syncephalis to penetrate internal host septa. The resistance of internal septa to penetration may rely on a chemical composition different from that possessed by hyphal walls. Penetration of walls by parasites has been explained as a mechanical and/or enzymatic process. If one assumes no, or unimportant, differences in the composition between the hyphal walls and internal septa, why is there no penetration of the latter? If the act of penetration is mechanical, only the inability to penetrate internal septa can be readily explained by the failure of the systemic parasitic hyphae to acquire, in competition with ambient host protoplasm, sufficient turgor for penetrative power. If one invokes force and enzymatic activity for penetration, one must postulate the suppression of either turgor or enzymatic activity, or degrees of both.

Again, concerning the properties of cell walls, the spores of Syncephalis on germination produce two terminal germ tubes. The end walls are free of merosporangial remains and must be more elastic than the side walls. Indeed, in S.3, the side walls may be considered inelastic since spore swelling is never observed. It is peculiar that

in Piptocephalis the reverse is true. Terminal germ tubes are constantly absent, spores become highly swollen and are characterised by peripheral germ tubes. Here presumably, the end walls are rigid and the side walls, with merosporangial remains, elastic.

A detailed investigation of the spore formation in both genera, involving the chemistry of cell walls and, since the process is beyond the resolution of the light microscope, involving also electron-microscope studies, might prove interesting. This would be of additional interest, since, as illustrated by S.3 and S. depressa, spores formed by different processes, possess different germinative capacities. Those formed in S.3 fashion always offer greater difficulties for their culture maintenance.

Again, the role of spore droplets is intriguing. In those examined, viz. S. nana, S. ramosa, S. nodosa, S. depressa, S. cornu and S.3, spores are eventually free within spore droplets. At such a stage, spores are easily detached from the plant onto a solid object. Beyond this stage and especially on air exposure, the spore droplet dries and the spores are cemented to the head. The spores can only be displaced by "strong-arm" methods and then only as groups or even a single group. Traditionally, spore droplet formation is considered to aid the dispersal of individual spores. At the wet stage, this is true, but in the dry state, only "pollinia" of spores are released. This latter could have a biological advantage in providing infection thresholds to achieve parasitisation. Leadbeater and Hood (1966) have recently been interested in this problem within the genus Piptocephalis. They have found spore droplets to contain as many as fourteen Amino-acids.

Cemented spore "pollinia" could therefore use, to a greater extent than single spores, such contained Amino-acids and possibly other factors for germination. Could different methods of spore formation in *Syncephalis* determine different Amino-acid and growth factor pools in spore droplets and thus explain the different germinative abilities as illustrated by *S.3* and *S. depressa*?

The isolation of *Syncephalis* species from various substrates, underlines that the genus is not, as is indicated by its frequent occurrence on dungs, coprophilous. It can occur on any substrate occupied by suitable hosts. Experimentally, it has been shown that *S.3* spores survive in dry soil for 16 months. Resistance to extreme conditions is better indicated by the isolation of *Syncephalis* species in the Swiss Alps at heights of 6,720 ft and 7,500 ft. At the latter station, the daytime temperature exceeds 0°c only for a few weeks during each year. Indeed, *S. depressa* is so readily found at these stations, that it could be called an alpine.

Isolations of *Syncephalis* on its host from various substrates, permits one to say that if *Syncephalis* has a predilection for a particular mucorine host, then that host must be *Absidia*.

Ecological studies also emphasised that in addition to the 36 species of *Syncephalis* already erected, there are many distinctive undescribed species. One special occasion involved the inspection of litter samples collected from Swithland Wood in Leicestershire, in which there were no less than five new, distinct species, which refused all attempts to be isolated and subcultured. The most remarkable of these

possessed sporophores 20 μ only in height, each bearing only one, unispored merosporangium, greater in length than the sporophore.

Comparison of S.3 with erected species shows it to be a quite distinctive isolate warranting specific rank.

Thus, this work has shown that the genus *Syncephalis* is still not well known and points to many areas for future investigation.

APPENDIX I.

Host range of Syncephalis 3. and Syncephalis depressa.

	S.3.	S. depressa
I MYXOMYCOTINA		
1. ACRASIALES		
Dictyostelium mucoroides	-	-
II EUMYCOTINA		
1. PHYCOMYCETES		
a. Mucorales		
Absidia glaucus	-	-
Absidia orchidis	+	+
Actinomucor elegans	+	+
Azygozygum chlamydosporum	-	-
Chaetostylum fresenii	-	+
Circinella minor	-	-
Circinella nigra	-	-
Circinella tenella	-	-
Coemansia sp.	-	-
Cokeromyces poitrasi	+	+
Cunninghamella bainieri	+	+
Haplosporangium bisporale	-	-

	S. 3.	S. depressa.
Martensiomyces pterosporus	-	-
Mucor hiemalis (-)	+	+
Mucor hiemalis (+)	+	+
Mucor sp. (R.A.B. 36)	+	-
Mucor sp. (R.A.B. 28)	+	-
Mortierella longicollis	-	-
Mortierella ramanniana	-	+
Mortierella isabellina	-	+
Mortierella vinacea	-	+
Mortierella alpina	+	+
Mortierella spinosa	+	+
Mortierella reticulata	-	-
Mortierella polycephala	-	-
Mycotypha microspora	+	-
Parasitella simplex	+	+
Phascolomyces articulosus	-	-
Radiomyces sp.	+	+
Rhizopus migricans	-	+
Rhizopus oryzae	+	+
Syncephalastrum racemosum	+	-
Thamnidium elegans	-	-
Zygorhynchus exponens	-	+

	S. 3.	S. depressa.
2. ASCOMYCETES		
a. Eurotiales		
Gymnoascus reesii	-	-
b. Hypocreales		
Melanospora zamiae	-	-
c. Sphaeriales		
Sordaria fimicola (white spored)	-	-
Sordaria fimicola (black spored)	-	-
d. Pezizales		
Ascodesmis porcina	-	-
Ascobolus sp.	-	-

	S.3.	S. depressa.
3. BASIDIOMYCETES		
a. Agaricales		
Corticium sp.	-	-
b. Sclerodermatales or Nidulariales		
Sphaerobolus sp.	-	-
4. FUNGI IMPERFECTI		
a. Moniliales		
Arthrobotrys oligospora	-	-
Arthrobotrys robusta	-	-
Arthrobotrys sp.	-	-
Aspergillus flavus	-	-
Alternaria sp.	-	-
Botryosporium sp.	-	-
Cephalosporium acremonium	-	-
Curvularia sp.	-	-
Cladosporium sp.	-	-
Neurospora crassa	-	-
Hormodendrum sp.	-	-

	S.3.	S. Depressa.
Penicillium waksmani	-	-
Penicillium clavariformis	-	-
Stemphylium sp.	-	-
Stachybotrys sp.	-	-
Trichoderma viride	-	-
Verticillium sp.	-	-

APPENDIX II.

(a) Staining solutions

(i) Nuclear staining solutions

Schaudinn's Solution.

Saturated solution of Mercuric chloride	200mls.
90% Ethyl alcohol	100mls.
Glacial Acetic acid	6mls.

Basic Fuchsin.

Basic Fuchsin	0.4gr.
0.01N HCI	100mls.

Heated with stirring, to 80°C. Cooled. Filtered.

(ii) General Cytoplasmic Stain, and Mountant.

Cotton-Blue in Lactophenol.

Cotton blue	0.25gr.		
Lactophenol	99.75gr.	(Lactic acid conc.	10gr.
		Phenol cryst.	10gr.
		Glycerine	10gr.
		Water	10ml.)

(b) Cultural formulae

(i) Preparation of Mineral Salt and Trace Element Basal Medium

Mineral Salts.

KH_2PO_4	1.5gr.
$MgSO_4$ $7H_2O$	1.5gr.
$CaCl_2$ $6H_2O$	0.25gr.
Agar	25gr.
Glass distilled water	1 litre

Mineral salts dissolved in the water. pH. adjusted to approx. 6.

Steamed to dissolve the agar. 5mls. of Trace element solution added.

Trace Elements

$FeSO_4$ $5H_2O$	0.003gr.
$CuSO_4$ $5H_2O$	0.001gr.
$ZnSO_4$ $7H_2O$	0.002gr.
$MnSO_4$ $4H_2O$	0.001gr.
$CoCl_2$ $6H_2O$	0.001gr.
Na Molybdate	0.001gr.
H_2SO_4 (conc.)	0.01mls.
Glass distilled water	100mls.

The solution is distributed and autoclaved.

Test compounds are added to this basal medium before autoclaving, unless they are heat sensitive, when they are Seitz sterilised and added to the cooling medium.

The final pH. of the medium is always tested with narrow range indicator papers.

(ii) Vitamin Growth Factors.

Thiamin	2mg.	(100µg. in 100mls.)
Riboflavin	2mg.	(100µg. ")
Nicotinic acid	2mg.	(100µg. ")
Pyridoxal	4mg.	(200µg. ")
Biotin	10µg.	(0.5µg. ")
p-Amino benzoic acid	200µg.	(10µg. ")
Pantothenic acid	2mg.	(100µg. ")
Folic acid	1mg.	(50µg. ")
B_{12}	0.08µg.	(0.004µg. ")
Inositol	200mg.	(10mg. ")
Adenine	10mg.	(500µg. ")
Guanine	10mg.	(500µg. ")
Hypoxanthine	10mg.	(500µg. ")
Xanthine	10mg.	(500µg. ")
Uric acid	10mg.	(500µg. ")
Uracil	10mg.	(500µg. ")
Cytosine	10mg.	(500µg. ")
Glass distilled water	100mls.	

The above vitamins, purines and pyrimidines dissolved in the and Seitz sterilised. 5ml. portions added to 100mls. of the cooling culture media, to give the final concentration in brackets.

(iii) Buffer solutions

di Sodium Hydrogen Phosphate - Citric acid Buffer solutions - * (double strength)

0.4Molar Na_2HPO_4 with 0.2Molar Citric acid.

pH.	Phosphate	Citric	pH.	Phosphate	Citric
2.2	0.40mls.	19.60mls.	5.2	10.72mls.	9.28mls.
2.4	1.24	18.76	5.4	11.15	8.85
2.6	2.18	17.82	5.6.	11.60	8.40
2.8	3.17	16.83	5.8	12.09	7.91
3.0	4.11	15.89	6.0	12.63	7.37
3.2	4.94	15.06	6.2	13.22	6.78
3.4	5.70	14.30	6.4	13.85	6.15
3.6	6.44	13.56	6.6.	14.55	5.45
3.8	7.10	12.90	6.8	15.45	4.55
4.0	7.71	12.29	7.0	16.47	3.53
4.2	8.28	11.72	7.2	17.39	2.61
4.4.	8.82	11.18	7.4	18.17	1.83
4.6	9.35	10.65	7.6	18.73	1.27
4.8	9.86	10.14	7.8	19.15	0.85
5.0	10.30	9.70	8.0	19.45	0.55

This double strength buffer solution is added to an equal amount of double strength culture medium after autoclaving. The final pH. is checked with narrow range indicator papers.

* adapted from Dawson et al. (1959)

(iv) Culture Media *

Brilliant Green Bile (2%) Agar (Oxoid C.M.31 + Agar)

Peptone (Oxoid L 37)	10gr.	
Lactose	10gr	
Ox Bile (purified)	20gr.	
Brilliant Green	0.0133gr.	
Agar	15gr.	
Water	1 litre	pH. 7.4 approx.

China Blue Lactose Agar (Oxoid C.M. 209)

Peptone (Oxoid L 37)	5gr.	
"Lab-Lemco" Beef extract	3gr.	
Lactose	10gr.	
Sodium chloride	5gr.	
China Blue	0.03gr.	
Agar	12gr.	
Water	1 litre.	pH. 6.7. approx.

Czapek Dox /. Agar Modified. (Oxoid C.M. 97)

Sodium nitrate	2gr.	
Potassium chloride	0.5gr.	
Magnesium glycerophosphate	0.5gr.	
Ferrous sulphate	0.01gr.	
Potassium sulphate	0.35gr.	
Sucrose	30gr.	
Agar	12gr.	
Water	1 litre.	pH. 6.8. approx.

Dextrose Peptone Agar. (Oxoid C.M. 13)

Peptone (Oxoid L 37)	20gr.	
Dextrose	10gr.	
Sodium chloride	5gr.	
Agar	15gr.	
Water	1 litre.	pH. 7.2. approx.

---

* pH. as indicated, except where adjusted to other values as stated in the text.

MacConkey Agar. (Oxoid C.M.7)

Peptone (Oxoid L 37)	20gr.	
Lactose	10gr.	
Bile salts (Oxoid L 55)	5gr.	
Sodium chloride	5gr.	
Neutral Red	0.075gr.	
Agar	12gr.	
Water	1 litre.	pH. 7.4. approx.

Malt Extract Agar. (Oxoid C.M. 59)

Malt extract (Oxoid L 39)	30gr.	
Mycological Peptone (Oxoid L 40)	5gr.	
Agar	15gr.	
Water	1 litre	pH. 5.4. approx.

Milk Agar. (Oxoid C.M. 21)

Yeast extract (Oxoid L 20)	3gr.	
Peptone (Oxoid L 37)	5gr.	
Milk solids (equivalent to 10mls. fresh milk)	1gr.	
Agar	15gr.	
Water	1 litre.	pH. 7.2. approx.

Nutrient Agar. (Oxoid C.M. 3)

"Lab-Lemco" Beef extract	1gr.	
Yeast extract (Oxoid L 20)	2gr.	
Peptone (Oxoid L 37)	5gr.	
Sodium chloride	5gr.	
Agar	15gr.	
Water	1 litre.	pH. 7.4. approx.

Potato Dextrose Agar. (Oxoid C.M. 139)

Potato extract (Oxoid L 101)	4gr.	
Dextrose	20gr.	
Agar	15gr.	
Water	1 litre.	pH. 5.6. approx.

Potato Dextrose Agar. (Alternative)

Potato (Fresh, macerated, filtered)	4gr.	
Dextrose	20gr.	
Agar	15gr.	pH. 5.0. approx.
Water	1 litre.	

Potato Dextrose Streptomycin Agar.

As Potato Dextrose Agar with 0.2gr. Streptomycin sulphate per litre added when the medium is cool but still molten.

Potato Carrot Agar.

Fresh Potato	20gr.	
Fresh Carrot	20gr.	

Macerated in an M.S.E. "Atomix". Boiled in water for ½hour. Strained to remove any large lumps.

Agar	15gr.	
Tap water	1 litre.	pH. 5.5. - 6.0.

Sabouraud Maltose Agar (Oxoid C.M. 41a.)

Mycological Peptone (Oxoid L 40).	10gr.	
Maltose	40gr.	
Agar	15gr.	
Water	1 litre.	pH. 5.2. approx.

Tomato Juice Agar. (Oxoid C.M. 113)

Tomato juice (solids from 400mls.)	20gr.	
Peptone (Oxoid L 37)	10gr.	
Peptonized Milk (Oxoid L 32)	10gr.	
Agar	12gr.	
Water	1 litre	pH. 6.1. approx.

Corn Meal Agar (Oxoid C.M.103)

Corn Meal extract	2gr.	
Agar	15gr.	
Water	1 litre.	pH. 6.0 approx.

Corn Meal Dextrose Agar. (Oxoid C.M. 105)

Corn Meal extract	2gr.	
Dextrose	10gr.	
Agar	15gr.	
Water	1 litre.	pH. 6.0 approx

Mixed Agar.

Equal quantities of Yeast extract A., Malt extract A., Tryptone Soya A., MacConkey A., Sabouraud Maltose A., Czapek-Dox A., Corn meal A., Milk A., Potato Dextrose A., and Nutrient A.

pH. 6.4. approx.

Corn-steep liquor Agar.

Corn-steep liquor	7.5gr.	
Sucrose	10gr.	
Magnesium sulphate 7 H_2O	0.25gr.	
Potassium di-Hydrogen Orthophosphate	4 gr.	
di-Ammonium Hydrogen Orthophosphate	2gr.	
Agar	20gr.	
Tap water	1 litre.	pH. 5.9 approx.

Corn-steep liquor Lactose Agar.

As for Corn-steep liquor Agar, but with 10gr./litre of Lactose substituted for Sucrose.

pH. 5.7 approx.

Cotton Seed Meal Agar.

Cotton seed meal (10gr. boiled in water. Filtered)

Sucrose	10gr.	
di-Ammonium Hydrogen Orthophosphate	2.5gr.	
Agar	20gr.	
Water	1 litre.	pH. 7.0 approx.

Dung Agar

Cow dung (1,000gr. soaked in tap water for three days. Filtered. Liquid diluted until a pale straw colour)

Agar 20gr./litre

pH. 6.7 approx.

Dung Sucrose Agar.

Dung agar with 10gr. sucrose per litre pH. 6.4 approx.

Dung Lactose Agar.

Dung agar with 10gr. Lactose per litre pH. 6.4 approx.

Polyporus extract Agar.

Polyporus squamosus fruiting body (20gr. macerated in an M.S.E. "Atomix". Filtered)

Agar 20gr.
Water 1 litre pH. 6.0 approx.

Polyporus extract Lactose Agar.

Polyporus extract agar with 10gr. Lactose per litre.
pH. 6.0 approx.

Polyporus extract Sucrose Agar.

Polyporus extract agar with 10gr. Sucrose per litre
pH. 6.0 approx.

Spent Grain Agar.

Spent Grain (10gr. boiled in water. Filtered.)
Agar 15gr.
Water 1 litre. pH. 5.5 approx.

Tap Water Agar.

Tap water 1 litre
Agar 15gr. pH. 7.0 approx.

BIBLIOGRAPHY.

ARNAUD (1952). Mycologie concrète. Genera. Bull. soc. Mycol. France, 68, 181-223.

AYRES, T.T. (1933). Growth of Dispira cornuta in artificial culture. Mycologia, 25, 333 - 341.

AYRES, T.T. (1941). The distribution and association of Gonatorhodiella with Nectria coccinea in the U.S.A. Mycologia, 33, 178-187.

BAINIER, G. (1882). Etude sur les Mucorinées. Thèse, F. Pinchon et A. Cotillon. Imp. Paris.

BAINIER, G. (1883). Observations sur les Mucorinées. Ann. Sci. Nat. Bot. 6, (15), 70-104.

BARNETT, H.L. (1958). A new Calcarisporium parasitic on other fungi. Mycologia, 50, 497-500

BARNETT, H.L. and LILLY, V.G. (1962). A destructive mycoparasite, Gliocladium roseum. Mycologia, 54, 72-77.

BENJAMIN, R.K. (1959). The merosporangiferous Mucorales. Aliso, 4, 321-433.

BENJAMIN, R.K. (1961). Addenda to The merosporangiferous Mucorales. Aliso, 5, 11-19.

BERLESE, A.N. and DE TONI, J.B. (1888). Phycomyceteae, in Saccardo, Sylloge Fungorum, 7, 225-233.

BERRY, C.R. and BARNETT, H.L. (1957). Modes of parasitism and host range of Piptocephalis virginiana. Mycologia, 49, 374-386

BERRY, C.R. (1959) Factors affecting the parasitism of Piptocephalis virginiana on other Mucorales. Mycologia, 51, 824-832.

BESSEY, E.A. (1961) Morphology and Taxonomy of Fungi. Hafner Publishing Co. New York, 1st ed. 1950.

BLYTH, W. (1949). Studies on Gonatorhodiella highii. A. L. Smith Trans. Proc. Bot. Soc. Edinburgh, 35, (Pt. 11), 157-159

BOEDIJN, K.B. (1959). Notes on the Mucorales of Indonesia. Sydowia, 12, 321-362.

BOOSALIS, M.G. (1956). The effects of soil temperature and green manure amendment of unsterilised soil on the parasitism of Rhizoctinia solani by Penicillium and Trichoderma spp. Phytopath. 46, 473-478

BREFELD, O. (1872). Mucor mucedo, Chaetocladium jonesii, Piptocephalis Freseniana. Bot. Unters. Schimmelp. 1, 1-64.

BULLER, A.H.R. (1934). The biology and taxonomy of Pilobolus. Researches on the Fungi, Vol. VI.

BUTLER, E.E. (1957). Rhizoctinia solani as a parasite of fungi. Mycologia 49, 354-374

CHRISTENBERRY, G.A. (1940). A taxonomic study of the Mucorales in the South Eastern United States. Journ. Elisha Mitchell Sci.Soc. 56, 333-366

COEMANS, E. (1862). Notice sur un champignon nouveau: Kickxella alabastrina Cms. Bull. Soc. Roy. Bot. Belgique, 1, 155-159.

COEMANS, E. (1863). Quelques hyphomycètes nouveaux. 1. Mortierella Polycephala et Martensella pectinata. Bull. Soc. Roy. Bot. Belgique, 2, sér. 15, 536-540

DADE, H. A. (1937). New Gold Coast Fungi. I. Trans. Brit. mycol. Soc. 21, 16-28.

DADE, H.A. (1960). Laboratory methods in use in the culture collection, C.M.I. Herb. I.M.I. Handbook. Commonwealth Mycological Institute.

DAWSON, R.M.C., ELLIOT, D.C., ELLIOT, W.H. and JONES, K.M. (eds.) (1959). Data for Biochemical Research. Clarendon Press, Oxford.

DE BARY, A. (1865). Zur Kenntnis der Mucorineen. Abl. seckenb. naturf. Ges. 5, 345-375.

DE BARY, A. (1887). Comparative morphology and biology of the Fungi, Mycetozoa and Bacteria. Oxford, Clarendon Press.

DE LAMATER, E.E. (1948). Basic fuchsin as a nuclear stain for fungi. Mycologia, 40, 423-429.

DOBBS, C.G. (1942). A mucorine parasite on Penicillia. Nature, Lond. 150, 319

DOBBS, C.G. and ENGLISH, M.P. (1954). Piptocephalis xenophila sp. nov. parasitic on non-mucorine hosts. Trans. Brit. mycol. Soc. 37, 375-389.

DRECHSLER, C. (1938). Two hyphomycetes parasitic on oospores of root-rotting oomycetes. Phytopath. 28, 81-103

DRECHSLER, C. (1961). Production of large solitary sporangiospores in some species of the Mucorales. Mycologia, 53, 439-442.

DUGGAR, B.M. (1909). Fungous diseases of plants. Ginn and Co.

ELLIS, J.J. (1963). A study of Rhopalomyces elegans in pure culture. Mycologia, 55, 183-198.

ELLIS, J.J. and HESSELTINE, C.W. (1962). Rhopalomyces and Spinellus in pure culture and the parasitism of Rhopalomyces on nematode eggs. Nature, Lond. 193, 699.

EMBREE, R. W. (1963a). The status of Gliocephalis. Mycologia, 55, 127-128.

EMBREE, R.W. (1963b). Observations on Mortierella vesiculosa. Trans. Brit. mycol. Soc. 46, 560-564.

FISCHER, A. (1892). Rabenhorsts Kryptogamen-Flora, 4, 294-308.

FITZPATRICK, H.M. (1930). The lower fungi. Phycomycetes. McGraw-Hill, N. Y.

FOSTER, J. W. (1949). Chemical Activities of Fungi. Academic Press Inc. New York. (Reprinted 1952).

GAUMANN, E. (1926). Vergleichende Morphologie der Pilze. Gustav Fischer, Jena.

GINAI, M.A. (1936). Further contributions to our knowledge of Indian Coprophilous Fungi. J. Indian Bot. Soc. 15, 269-284

GODFREY, R. M. (1957). Fungal parasites II. Studies on British species of Endogone. Trans. Brit. mycol. Soc. 40, 136-144

HESSELTINE, C.W. (1955). Genera of the Mucorales with notes on their synonymy. Mycologia, 47, 344-362.

INDOH, H. (1962). Studies on the Japanese Mucorales. I. On the genus Syncephalis. Sc. Rep. T.K.D. Sect. B. Vol. II. No.160.

INGOLD, C.T. and ZOBERI, M.H. (1963). The asexual apparatus of Mucorales in relation to spore liberation. Trans. Brit. mycol. Soc. 46, 115-134.

KRZEMIENIEWSKA, H. and BADURA, L. (1954). A contribution to the knowledge of the micro-organisms from the litter and soil of the beech wood. Acta, Soc. Bot. Poloniae, 23, 727-781.

LEADBEATER, G. and MERCER, C.K. (1957). Piptocephalis virginiana sp. nov. Trans. Brit. mycol. Soc. 40, 461-471

LEADBEATER, G. and RICHARDSON, M. (1963). Spinellus in pure culture. Nature, Lond. 198, 1015.

LEADBEATER, G. and RICHARDSON, M. (1965). Private communication.

LEADBEATER, G. and HOOD. (1966). Private communication.

LEGER, M. (1896). Recherches sur la structure des Mucorinées. E. Druinard, Libraire-Editeur, Poitiers.

LENDNER, A. (1908). Les Mucorinées de la Suisse. Beitrage zur Kryptogamen-Flora der Schweiz, 3, 1-180.

LINDER, D.H. (1943). The genera Kickxella, Martensella and Coemansia, (Phycomycetes). Farlowia, 1, 49-77.

MALENCON, M.G. (1942). Etudes de parasitisme mycopathologique. Revue de Mycologie, 7, 27-52.

MANGIN, L. (1899). Observations sur la membrance des Mucorinées. Journ. Bot. Paris. 13, 209-216, 277,287, 307-316, 371-378.

MARCHAL, E. (1891). Champignons Coprophiles de Belgique VI. Mucorinées et Sphaeropsidées nouvelles. Bull. de la Société Royale de la Botanique de Belgique, 30, 134-146.

MARTIN, G.W. (1940). Outline of the Fungi. Univ. of Iowa studies in Natural History, 18, (1), 1-40. Univ. of Iowa, Iowa City.

MATRUCHOT, M.L. (1899). Notes Mycologique. 1. Gliocephalis hyalina. Bull. Soc. Mycol. France, 15, 254-262.

MEHROTRA, B.S. and PRASAD, R. (1964). Syncephalis depressa from India. Mycologia, 56, 905-908.

MERCER, C.K. (1959). Studies on Piptocephalis: Ph.D. Thesis, Univ. of London

MIGULA, W. (1910). Pilze, in Thomes Kryptogamen-Flora von Deutchland, Deutch-Osterreich und der Schweiz, Band III, Teil. 1, 58-342. Verlag Friedrich von Zezschwitz, Gera.

MOLLER, A. (1901). Phycomyceten und Ascomyceten. Botanische Mittheilungen aus den Tropen, 9, 1-319.

MOREAU, F. (1913). Recherches sur la reproduction des Mucorinées et de quelques autres Thallophytes. Le Botaniste, 13, 1-136.

MORINI, F. (1902). Contributo allo studio del gen. Syncephalis. Mem. R. Acad. Sci. Inst. Bolognia, Ser.5, 9, 229-236.

NAUMOV, N.A. (1924). Les bases morphologiques de la systematique dans la famille des Mucoracées. Bull Soc. Mycol. France, 40, 86-92.

NAUMOV, N.A. (1935). Opredelitel Makorovych (Mucorales). Bot. Inst. Acad. Sci. U.S.S.R. (2nd rev. ed.) Moscow and Leningrad.

NAUMOV, N.A. (1939). Clés des Mucorinées (Mucorales). Enclyclopédie Mycologique, 9, 1-137. Paul Lechevalier, Paris.

OU, S.H. (1940). Phycomycetes of China. Sinensia, 11, 35-57.

OUDEMANS, C.A.J.A. (1897). Revision de Champignons (Tant supérieurs qu'inférieurs trouvés jusqu'à ce jour) dans le Pays-Bas, 2, 52-56.

POVAH, A.H.W. (1917). A critical study of certain species of Mucor. Bull. Torrey Botan. Club, 44, 241-259, 287-313.

RACIBORSKI, M. (1909). Parasitische und epiphytische Pilze Java's. Bull. Internat. l' Acad. Sci. Cracovie, Classe Sci. Maths. et Nat. 346-394.

RAMAKRISHNAN, K. (1955). Some aspects of soil fungal ecology. Proc. Indian Acad. Sci. B, 41, 110-116.

RICKER, P.L. (1906). Journ. Mycol. 12, 61.

SCHROETER, J. (1886). Pilze, in Die Pilze Schlesiens. F. Cohn's Kryptogamen-Flora von Schlesien, 3, 198-217. (Vol. printed 1889).

SCHROETER, J. (1893). Mucorineae. Die naturlichen Pflanzenfamilien, 1, 119-134. Engler & Prantl. 1897.

SHIGO, A.L. (1958). Fungi isolated from Oak-wilt trees and their effects on Ceratocystis fagacearum. Mycologia, 50, 757-769.

SHIGO, A.L. (1960a). The parasitism of Gonatobotryum fuscum on a species of Ceratocystis. Mycologia, 52, 584-598.

SHIGO, A.L. (1960b). Mycoparasitism of Gonatobotryum fuscum and Piptocephalis xenophila. Trans. N.Y. Acad. Sci. 365-375.

SHIGO, A.L., ANDERSON, C.D. and BARNETT, H.L. (1961). Effects of concentration of host nutrient on the parasitism of Piptocephalis xenophila and Piptocephalis virginiana. Phytopath. 51, 616-620.

SLIFKIN, M. (1963). The parasitism of Olpidiopsis on members of the Saprolegniaceae, I, Effects of pH. and host nutrition. Mycologia, 55, 172-182.

THAXTER, R. (1891). On certain new or peculiar North American Hyphomycetes. Bot. Gaz. 16, 14-26.

THAXTER, R. (1897). New or peculiar Zygomycetes. 2, Syncephalastrum and Syncephalis. Bot. Gaz. 24, 1-15.

VAN TIEGHEM, P. and LE MONNIER, G. (1873). Recherches sur les *Mucorinées*. Ann. Sci. Nat. Bot. Sér. 5, 17, 261-399.

VAN TIEGHEM, P. (1875). Nouvelle recherches sur les *Mucorinées*. Ann. Sci. Nat. Bot. Sér. 6, 1, 5-175.

VAN TIEGHEM, P. (1876). Troisième mémoire sur les *Mucorinées*. Ann. Sci. Nat. Bot. Sér. 6, 4, 312-398.

VIEGAS, A.P. and TEIXIERA, A.R. (1943). Alguns Fungos do Brasil (Phycomycetos). Bragantia, 3, 223-269.

VON HOHNEL, F. (1902). Fragmente zur Mykologie (I. Mitheilung). Sitzungsberichte Der Kaiserlichen Akademie der Wissenschaften. Mathematisch-Naturwissenschaftliche classe, CXI band, X Heft.

VUILLEMIN, P. (1886). La membrane des Zygospores de *Mucorinées*. Bull. Soc. Bot. France, Tome 33, Sér. 2, Tome 8.

VUILLEMIN, P. (1902). Les *Cephalidées*. Bull. mensuel séances Soc. Sci. Nancy, Sér. 3, 3, 21-83.

VUILLEMIN, P. (1903). Le *Syncephalis adunca* sp. nov. et la série des *Cornutae*. Ann. Mycol. 1, 420-427.

VUILLEMIN, P. (1904). Le *Spinalia radians* g. et sp. nov. et la série des *Dispirées*. Bull. Soc. Mycol. France, 20, 26-33.

VUILLEMIN, P. (1922). Une nouvelle espèce de *Syncephalastrum*; affinités de ce genre. C.R. Acad. Sci. 174, 986-988.

WARCUP, J.H. (1951). Effect of partial sterilisation by steam or formalin on the fungus flora of an old forest nursery soil. Trans. Brit. mycol. Soc. 34, 519-532.

WARREN, J.R. (1948). An undescribed species of Papulospora parasitic on Rhizoctinia solani Kuhn. Mycologia, 40, 391-401.

WATSON, P. (1964). Spore germination in Spinellus macrocarpus. Trans. Brit. mycol. Soc. 47, 239-245.

WATSON, P. (1965). Nutrition of Spinellus macrocarpus. Trans. Brit. mycol. Soc. 48, 73-80.

WEINDLING, R. (1932). Trichoderma lignorum as a parasite of other soil fungi. Phytopath. 22, 837-845.

WHALLEY, J.W. and BARNETT, H.L. (1963). Parasitism and nutrition of Gonatobotrys simplex. Mycologia, 55, 199-210.

ZOPF, W. (1888). Zur Kenntniss der Infections-Krankheiten niedener Thiere und Pflanzen. Nova Acta Acad. Caes. L.-C. Nat. Cur. 52, 313-376.

ZYCHA, H. (1935). Mucorinae, in Kryptogamen-Flora der Mark Brandenburg. 6a, 1-264. Gebruder Borntraeger, Leipzig.

DURHAM UNIVERSITY SCIENCE 20 OCT 1966 SECTION LIBRARY