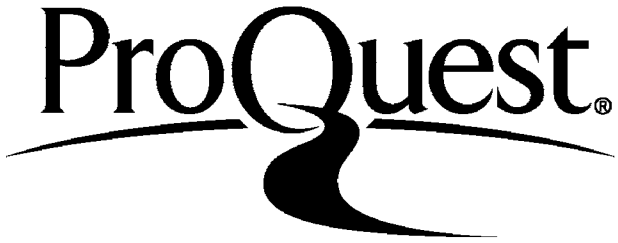
THESIS SUBMITTED FOR M.Sc. DEGREE

BY SUSAN CARTER, B.Sc.

SOME INVESTIGATIONS INTO THE SUCCESSION OF FUNGI ON RABBIT DUNG



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ABSTRACT

The experimental work has been divided into two sections. The first deals with succession. The most important groups of fungi appear to be the Phycomycetes and the Ascomycetes. The appearance of certain species when grown on dung agar has been investigated, and the effect of cellulose ascertained.

The second section is concerned with those factors affecting succession and is subdivided into three parts. Nutrient supply is dealt with in the first part. It has been demonstrated that the decline of the phycomycete population cannot be attributed to the depletion of nutrients. In the second part the importance of staling-products has been investigated.

The third part deals with inhibitory substances. No interaction has been detected between the species used in the experiments. However experimentation has shown that dung-inhabiting bacteria produce a diffusible substance which, in culture, retards the growth of the Phycomycetes to such an extent as to explain their disappearance from the pellets during the early stages of succession. This substance has no effect on the Ascomycetes used in the experiments.

CONTENTS

INTRODUCTION

It has long been known that a definite succession of fungi occurs on various substrates. However, very little experimental work was done to investigate the causes of such successions until Cook (1924) published the results of an investigation into the succession of fungi on decaying fruits and vegetables. From his results he concluded "that (the first) fungus, [which,] having exhausted the food supply, disintegrates and leaves favourable conditions for a second fungus".

As recently as 1953 Siu and Reese (1953) stated that the position is not clearly understood with regard to what they term primary and secondary invaders in wood-rotting and cellulose-destroying fungi. They mention briefly the fact that perithecial and pycnidial forms occur after earlier appearing Hyphomycetes in the decomposition of cellulose.

Massee and Salmon (1901) stated that theirs was the first specific work dealing with British coprophilous fungi. They were concerned with the morphology, but noted that the fungi appeared in a definite order - "first, Phycomycetes, usually heralded by Pilaira and Pilobolus, followed by species of Mucor, accompanied by their parasites Chaetocladium, Thamnidium, Piptocephalis, Syncephalis, etc.; next appear various members of the Hyphomycetes, probably in many instances representing the conidial condition of the ascigerous Fungi which are usually the last in the sequence to appear".

As has been stated above, previous work on fungal successions has dealt primarily with recording the occurrence of these successions and identification of the fungi concerned. The purpose of the present investigations has been to determine the possible factor or factors causing the succession of fungi on rabbit dung.

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SECTION 1. EXPERIMENTAL METHODS

Pellets used for the experiments were collected from a piece of waste-land west of Hailsham, Sussex, known as Milton Hide (O.S. 183, 556-7, 108-9 on National Grid) where a small colony of rabbits survived the outbreak of myxomatosis. They were collected in as fresh a condition as possible and packed into small closed tins the day before any experiment requiring fresh pellets was set up. It was probable therefore, that when used, such pellets were not more than 24 hours old. A large collection of fresh pellets was also made at the beginning of the investigations for use in making up dung-agar. It was stored in a large closed tin in the deep-freeze thus preventing growth of any sort without affecting the constitution of the dung. This stock lasted throughout all the experiments. By making a single collection the composition of the dung-agar for any two experiments carried out at different times was the same.

Dung-agar was prepared in all experiments according to the following method: 2.5 gms. of air-dried pellets were ground with a pestle and mortar with 100 ccs. distilled water. The suspension was filtered and 50 ccs. of the filtrate were made up to 200 ccs. with distilled water. Agar was then added at the rate of 20 gms. per litre, and the medium autoclaved.

For experiments employing liquid culture methods the formula used was that of Cook's No. 11 medium:

Glucose	20	gms.
Peptone	10	"
Potassium phosphate	0.25	"
Magnesium sulphate	0.25	"
Water	1000	ccs.

For isolation of the Phycomycetes and Fungi Imperfecti fresh pellets were incubated at room temperature (18°C - 20°C) in petri-dishes for a few days by which time sporing colonies had appeared.

With the aid of a binocular microscope groups of spores were transfered with a sterile needle to plates of dung agar.

It was found that the Ascomycetes could be isolated most satisfactorily by placing fragments of pellets, which were about two weeks old, on dung agar and subculturing from the resulting mycelial growth.

All the cultures were incubated at 24°C. Contaminated cultures were cleared by repeated subculturing.

A stock collection of the fungi isolated was maintained, and subculturing carried out every four months. The Phycomycetes were grown on oatmeal, the Ascomycetes on maltose or peptone, and the Fungi Imperfecti on Sabouraud media. The collection was kept at 20°C.

The bacteria used in certain of the experiments were isolated from the dung as follows. Some pieces of pellets about three weeks old were shaken up in a 2% sterile saline solution, and drops of this streaked on a plate of nutrient agar with a sterile loop. The plate was incubated at 24°C. The resulting bacterial growth was subcultured until free from fungal contaminants, nutrient agar being used as the medium. No attempt was made to separate or identify the various species.

Permanent slides were made of most of the fungi which appeared during the succession. The staining method used was as follows.

1. 2-3 mins. in cotton-blue in lacto-phenol.
2. Wash in 50% alcohol.
3. Take through 75% to absolute alcohol.
4. Wash in xylol.
5. Mount in Canada balsam.

Drawings were also made and are presented in this thesis.

Identifications were made from fresh material mounted in water. The genera were identified from Clements and Shears' standard work "Genera of the Fungi". From this specific determinations were made by consulting various books and monographs containing keys and/or illustrations (Refs. 1, 2, 3, 4, 8, 10, 11, 12, 14, 16, 17, 18). Of

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these Rabenhorsts' "Kryptogamen Flora" proved the most useful for identifying the Phycomycetes and Ascomycetes, and the illustrations in the "Flora Agaricina Danica" by Lange for final identification of the Basidiomycetes.

The most interesting species was finally identified as Pilobolus umbonatus. This was described for the first time in 1934 by Buller in his "Researches on Fungi". He stated that its habitat was usually sheep dung, but he had found it on horse manure. He gave its distribution as Canada and the eastern United States. There is no doubt that my specimen is the same species, and as far as can be ascertained this is the first time it has been recorded outside the North American continent.

A list of the identified fungi is given in the following table.

A rhizomorphous growth, which sometimes appeared on the pellets during the second to third week of the succession, was also grown in pure culture, but remained unidentified since no fruiting-bodies were produced.

PHYCOMYCETES

Chaetocladium brefeldii van Tiegh. & Le Monn.
Kickxella albastrina Coemans
* Mucor hiemalis Wehm.
* Pilaira anomala (Ces.) Schroet
Pilobolus crystallinus Tode ex van Tiegh.
" kleinii van Tiegh.
Pilobolus umbonatus Bull.
* Piptocephalis freeseniana de Bary.
Syncephalastrum sp.
Syncephalis cordata van Tiegh. & Le Monn.
" sphaerica van Tiegh.

ASCOMYCETES

Ascobolus immersus Pers. ex Fries
" leveillei Boud.
" stercorarius (Bull.) Schroet
" viridulus Phill. & Plow.
Ascophanus carneus (Pers. ex Fries) Boud.
" microsporus (Crouan) Phill.
Chaetomium globosum Kunze ex Fries
" spirale Zopf
* Lilliputia rufula (Bark. & Br.) Hughes
Patella coprinaria (Cooke) Seaver
Saccobolus violascens Boud.
Pleurage curvula (De Bary) Kuntze
Sordaria decipiens Wint.
" discospora (Auersw.) Nissl.
Pleurage fimicola (Corda) Kuntze
* Sordaria macrospora Auersw.

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Sordaria minuta *Fuckel*
* " myriaspora *(Crouan) Sacc.*
* Sporormia intermedia *Auersw.*
Sphaeronaemella fimicola *Marchal*

BASIDIOMYCETES

Coprinus ephemerus *Fries*
" fimetarius *(L.) Fries*
Psilocybe coprophila *(Bull. ex Fr.) Kaisner*

FUNGI IMPERFECTI

Actiniceps thwaitesii *Bark & Br.*
Arthrobotrys superba *Corda*
* Aspergillus candida *Link ex Fr.*
* Trichothecium roseum *(Pers.) Link ex Fr.*
Cladosporium herbarum *(Pers.) Link ex Fr.*
Didymostilbe sp.
* Fusarium merismoides *Corda*
* Gliocladium penicillioides *Corda*
* Isaria cretacea *van Beyma*
Oedocephalum lineatum *Bakshy*
* Penicillium expansum *Link emend Thom.*
Rhopalomyces elegans *Corda*
* Sepedonium chrysospermum *(Bull.) Fr.*
* Stysanus medius *Sacc.*
Tilachlidium sp.

Those marked * were grown in pure culture and the species used in the experiments are available.

Section 2. INVESTIGATION OF SUCCESSION

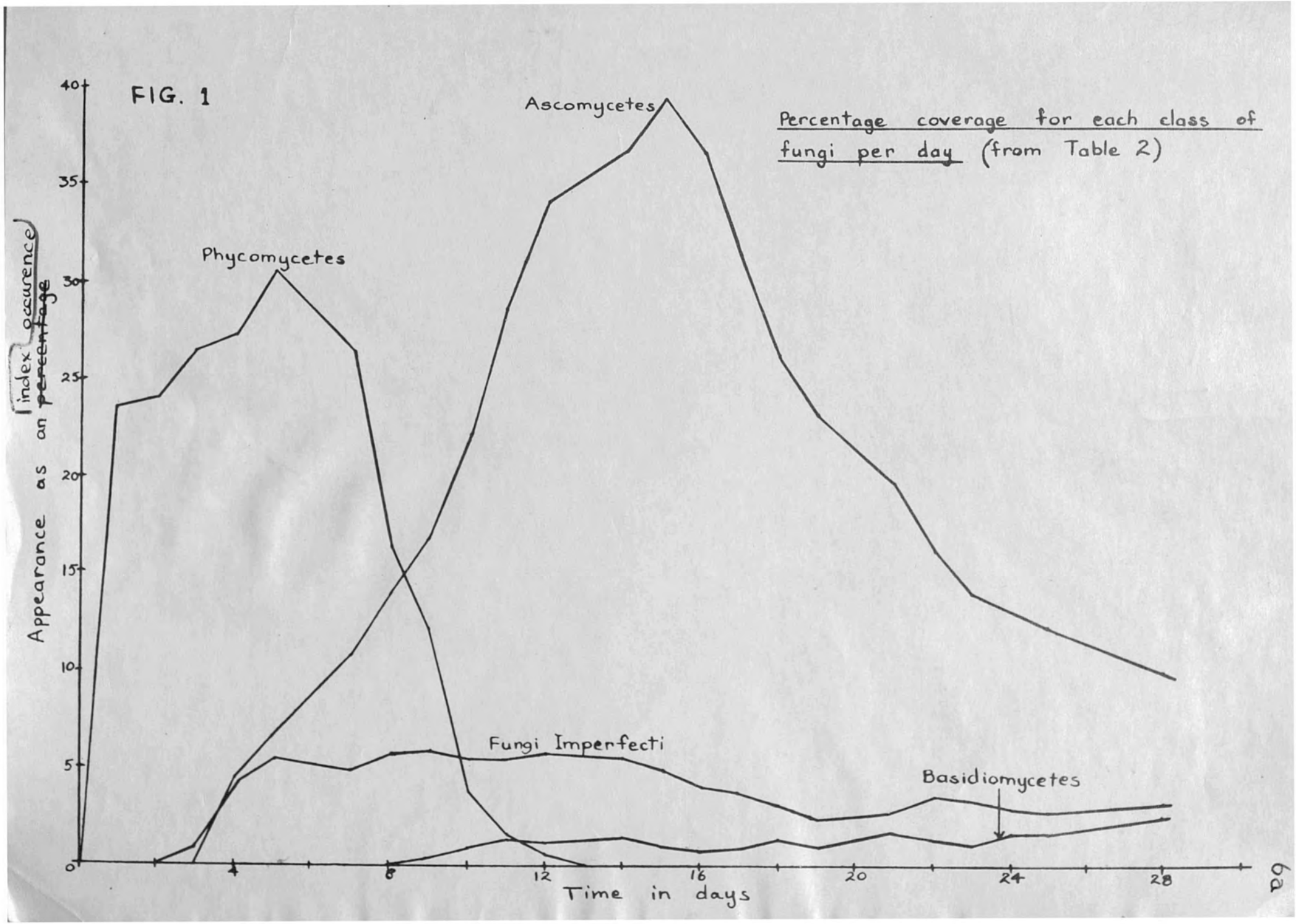
i. Sequence on pellets

In the second of their papers Massee and Salmon (1902) showed that the spores of most fungi appearing on rabbit dung actually passed through the animal by opening the intestine of a freshly killed rabbit under sterile conditions, and observing the sequence of appearance of any fungi on the intestinal contents. This was subsequently found to correspond with the succession on fresh pellets. Usually those spores which germinated were phycomycetes or ascomycetes, and bore a protective covering of mucilage. From this observation and the lack of growth of Hyphomycetes under such controlled conditions, they concluded that colonisation of pellets by this latter class of fungi resulted from dry, powdery, air-borne spores. This was confirmed during the present investigations by the fact that appearances on pellets of species of the Fungi Imperfecti were much less uniform than those of the Phycomycetes or Ascomycetes.

A clearer picture of the succession was needed as a start for these investigations, and the following experiment was devised in an attempt to provide results for a graphic recording.

Experiment 1. Fresh dung was collected, and 10 pellets placed in each of 5 petri-dishes. These had been lined with filter-paper and sterilised. The pellets were kept moist by the daily introduction of a few drops of sterilised distilled water on to the filter-paper. Each day the number of pellets upon which each species of fungus appeared was counted. In order to express results quantitatively, it was necessary to obtain a number representing maximum (i.e. 100%) colonisation of the pellets. This was achieved by summing over the whole range of species the greatest numbers of pellets upon which each species appeared during the succession. An index of occurrence for each class (i.e. Phycomycetes, Ascomycetes, Basidiomycetes, Fungi Imperfecti) was expressed as the ratio of the actual number of pellets occupied to this sum. A graph was drawn

FIG. 1


Percentage coverage for each class of fungi per day (from Table 2)
Ascomycetes
Phycomycetes
Fungi Imperfecti
Basidiomycetes
Appearance as an index occurence
Time in days
0
5
10
15
20
25
30
35
40
4
8
12
16
20
24
28

from this index ~~these percentage figures.~~ (Tables 1 & 2 in Appendix and Fig. 1.)

The graph shows that the two most important classes in the succession appeared to be the Phycomycetes and Ascomycetes. Colonisations by Basidiomycetes and Fungi Imperfecti were very slight. The Ascomycetes reached their maximum on the fifteenth day after which their decline was fairly rapid until the twenty-fourth day when it became more gradual. This was unlike the phycomycete decline which ended abruptly on the thirteenth day, its maximum having occurred on the fifth day. In fact the Ascomycetes persisted for as long as the pellets were kept under observation, which in some cases was for nearly eight weeks, while the Phycomycetes failed to reappear.

ii. Growth on dung-agar

Species of three of the classes of fungi (Basidiomycetes being excepted) were grown in pure culture on dung agar in the hope that comparisons could be made between their behaviour under these controlled conditions and the natural conditions prevailing when fresh pellets were incubated. The time of the appearance of fruiting-bodies after incubation began was considered to be the most satisfactory indication of whether conditions in the sterilised dung agar were similar to those in the pellets.

Experiment 2. Plates of dung-agar were inoculated in triplicate with the following fungi.

Phycomycetes:	Mucor hiemalis
	Pilaira anomala
Ascomycetes:	Sporormia intermedia
	Sordaria macrospora
	Lilliputia rufula
Fungi Imperfecti:	Penicillium expansum
	Cephalothecium roseum
	Stysanus medius

The third phycomycete to be isolated, Piptocephalis freeseniana, was

parasitic upon Mucor hiemalis, so could not be used as its growth rate would be controlled by that of the Mucor.

The plates were incubated at 24°C. Growth was measured each day by colony diameter, and the times of sporulation were noted. A graph was made from the average figures obtained from the three sets of plates. (See Tables 3 & 4 in Appendix and Fig. 2.)

Comparing this graph with Fig. 1 the growth of the phycomycetes should have fallen sharply after the fifth day. However this did not happen, the rates of both Mucor and Pilaira continuing steadily until checked by the mycelium reaching the edge of the plate (diameter c. 8.5 cms.). Sporangia were produced on the second day. Growths of the ascomycetes and fungi imperfecti were also steady, their fruiting-bodies being produced on the eighth and second days respectively.

The sporulation times correspond with those obtained from fungi appearing on fresh pellets in the cases of the phycomycetes and fungi imperfecti, and Sporormia intermedia (see Table 1) but are late for Sordaria and Lilliputia, which were expected to appear on the fourth or fifth day.

iii. Effect of cellulose

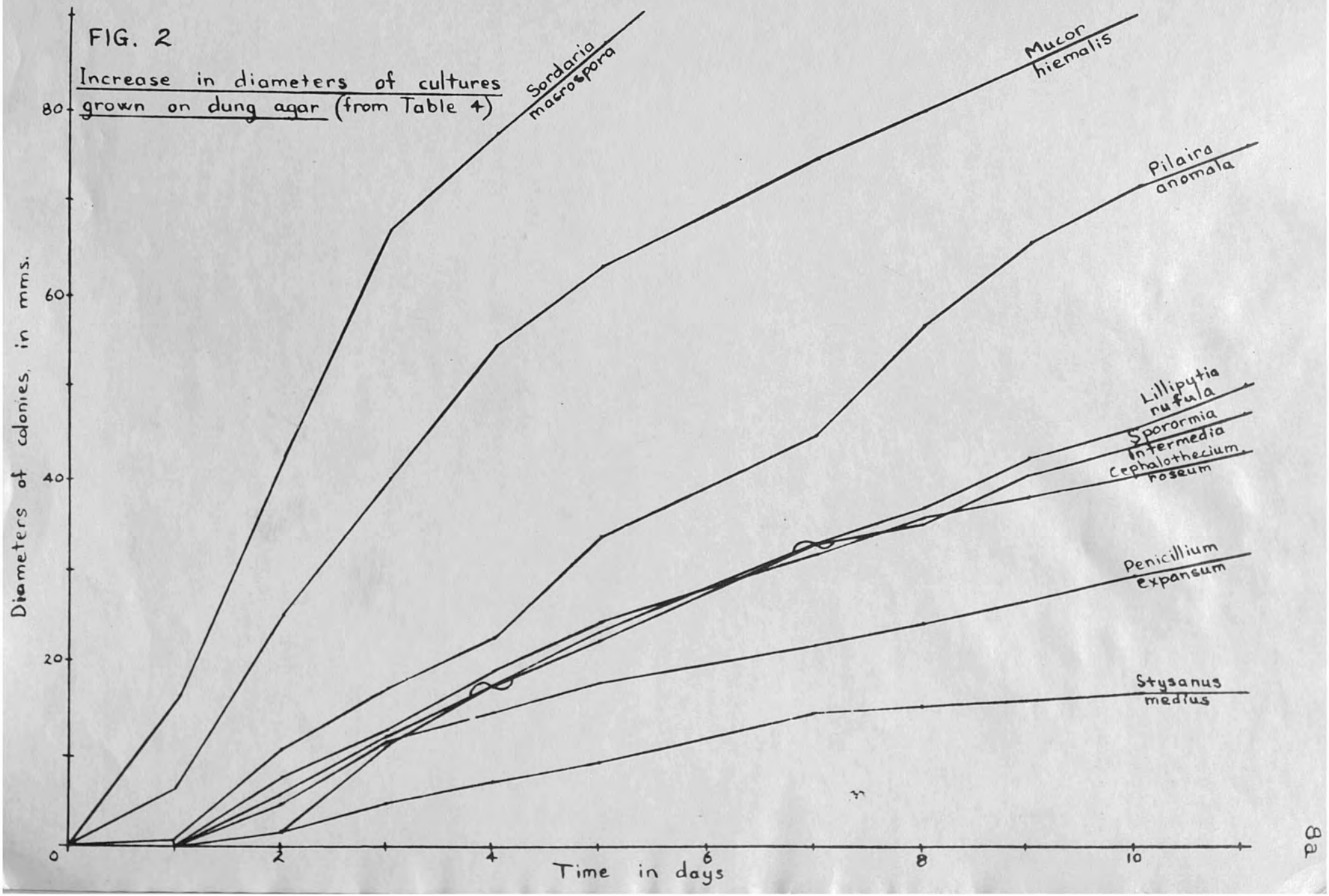
It has been shown by various workers that many ascomycetes can attack cellulose but that few phycomycetes can do so and never members of the Mucoraceae (e.g. Reese and Levinson, 1952; Siu and Reese, 1953). During Experiment 1 several ascomycetes spread from the pellets to the filter-paper, and produced fruiting-bodies. These species were:-

Chaetomium spirale
Ascophanus carneus
Saccobolus violascens
Sordaria minuta
Sordaria macrospora

However, when these were grown on pure cellulose (filter-paper) only

FIG. 2

Increase in diameters of cultures grown on dung agar (from Table 4)



Chaetomium and Ascophanus produced any growth and this was poor. This suggested that growth could not be supported by cellulose alone, but was possibly improved by its presence.

In Experiment 2 the dung-agar medium was devoid of a cellulose source, since the dung suspension was filtered. It was thus considered that this absence may have had some influence on the late appearance of the ascomycete fruiting-bodies on the dung-agar. An experiment was consequently carried out to investigate this possibility.

Experiment 3. The dung-agar solution was made up according to the standard method, and then divided into two portions. Plates poured with one of these portions were inoculated and used for controls. Filter-paper which had been cut into small pieces and mashed up in water was added to the other portion before sterilisation. The plates were inoculated with the following species.

Phycomycetes:	Mucor hiemalis
	Pilaira anomala
Ascomycetes:	Sordaria macrospora
	Lilliputia rufula
Fungi Imperfecti:	Cephalothecium roseum
	Penicillium expansum

The experiment was carried out in triplicate, and the averages, from the three sets of plates, of colony diameters were used to make a graph. The time of appearance of the fruiting-bodies was also noted. (See Tables 5-8 in Appendix and Fig. 3.)

The graph shows that there was no significant difference in the growth of any of the species when cellulose was present. The sporangia of the phycomycetes and conidia of the fungi imperfecti appeared on the second day, as in Experiments 1 and 2. The apothecia of Lilliputia rufula and the conidia of its asexual stage, Gliocladium penicillioides, appeared after five days in the presence of cellulose, compared with the time-lapse of eight days on the medium lacking cellulose. The perithecia of Sordaria macrospora appeared even earlier, on the fourth day compared with eight days in Experiment 2. This time-lapse corresponds to the time of appearance on the pellets (see

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FIG. 3

Increase in diameters of cultures grown on dung agar with & without cellulose (from Tables 6 & 8)

—— with cellulose

---- without "

Diameters of colonies, in mms.

0 20 40 60 80

Time in days

2 4 6 8 10 12

Sordaria macrospora

Mucor hiemalis

Pilaira anomala

Lilliputia rufula

Cephalotheclum roseum

Penicillium expansum

Table 1).

Thus it could be assumed that if the fungi were to be cultured on sterilised dung agar, conditions for growth would be similar to those present in fresh pellets at the beginning of the succession. Since the presence of cellulose served only to precipitate the formation of fruiting-bodies in the ascomycetes and did not affect growth in any other way it could be eliminated from the artificial medium. Furthermore, any introduced factors, which would influence growth on dung agar ~~in such a way as~~ to produce changes similar to those observed on the pellets, could be said to ~~apply to~~ operate in both media.

SECTION 3. FACTORS AFFECTING SUCCESSION

As has been previously mentioned the Phycomycetes disappear early in the succession. Before further experiments were carried out it was decided to discover whether they had been killed or whether they were still viable but remaining quiescent. Fragments of pellets which were in the late ascomycete stage of succession were placed on plates of dung agar and incubated at 24°C. Growth of phycomycetes always occurred, therefore viable spores or mycelial fragments must still have been present even when the phycomycetes seemed to have disappeared, thus showing that conditions prevailing in the pellets were preventing germination of the spores and growth of the fragments.

Experiments were then designed to try to establish the reason or reasons for the phycomycete decline. Three possible causes have been considered:- first, the exhaustion of the nutrient supply; second, the formation of staling-products by the phycomycetes preventing their further growth; and third, the production of some substance or substances by the ascomycetes or other micro-organisms having an inhibitory effect on the phycomycetes.

As a preliminary experiment pH values were taken throughout succession for an indication of whether any significant changes

occurred in the constitution of the pellets.

Experiment 4. Two or three pellets were taken each day during succession and broken up in a little distilled water. pH measurements were made using a BDH soil capillator set. These were discontinued after the twenty-third day, which was well past the time when all the phycomycetes had disappeared (see Table 9 in Appendix). The resulting figures indicate no significant change from the slightly alkaline state prevailing at the beginning of succession. The alkalinity would be due to the presence of ammonia.

i. Nutrient supply

Perhaps the exhaustion of suitable food materials would be the simplest explanation for the disappearance of the phycomycetes. This possibility was investigated by growing six of the fungi on an "exhausted" dung medium, and comparing growth with that obtained when they were grown on an agar medium made from fresh dung. "Exhausted" dung medium was made from pellets which had been collected fresh, had shown succession, and were now about four weeks old. It would therefore be expected, by refering to Fig. 1, that if exhaustion of suitable food materials was the explanation for their disappearance the phycomycetes would show slight or no growth, whereas the ascomycetes would be affected very little or not at all.

Experiment 5. An agar medium made from "exhausted" pellets and using the standard method was inoculated in petri-dishes with the following fungi:-

Phycomycetes:	Mucor hiemalis
	Pilaira anomala
Ascomycetes:	Sordaria macrospora
	Lilliputia rufula
Fungi Imperfecti:	Penicillium expansum
	Cephalothecium roseum

The experiment was carried out in triplicate. Growth was

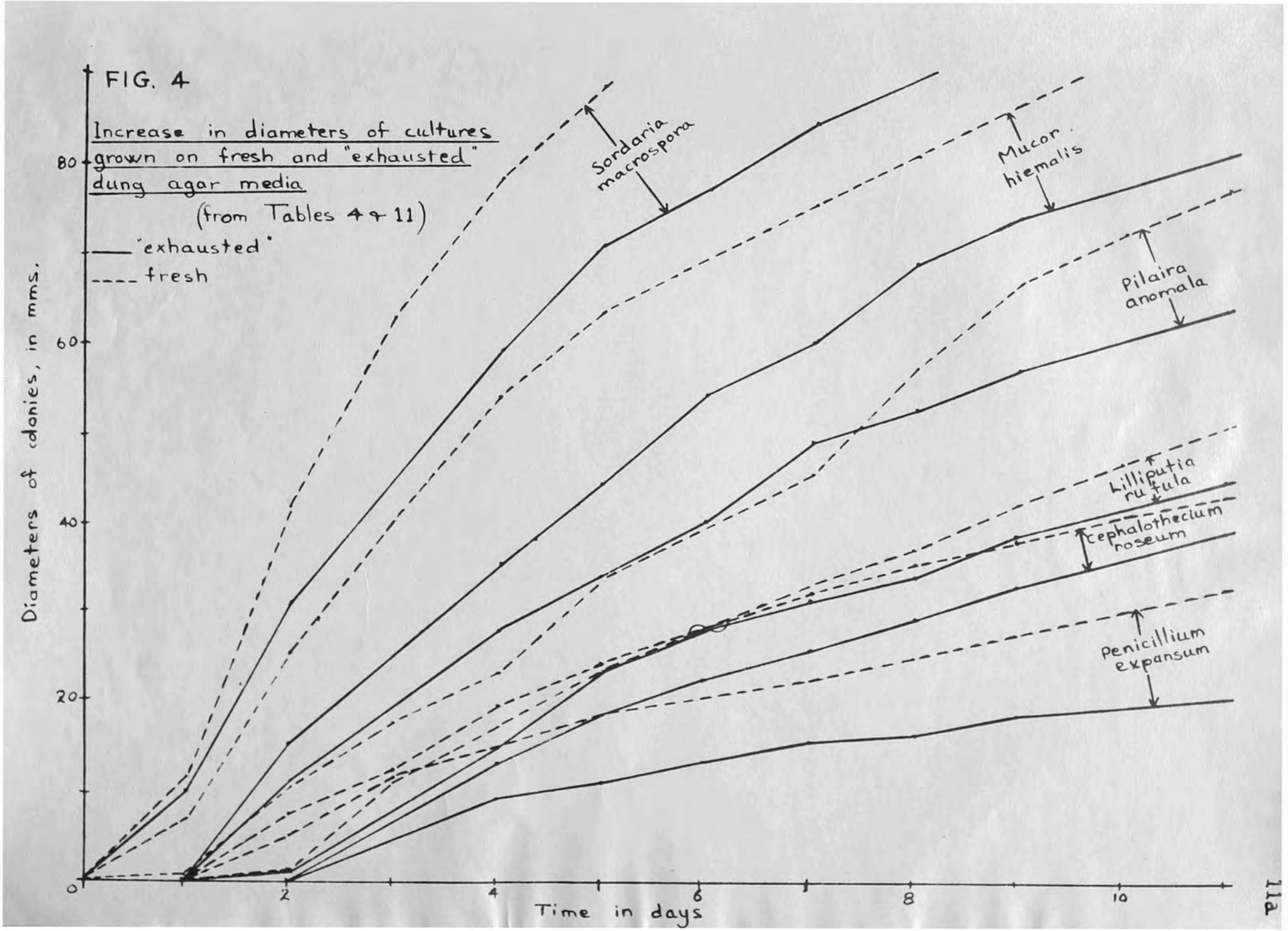


FIG. 4
Increase in diameters of cultures grown on fresh and "exhausted" dung agar media
(from Tables 4 & 11)
"exhausted"
fresh
Diameters of colonies, in mms.
0
20
40
60
80
2
4
6
8
10
Time in days
Sordaria macrospora
Mucor hiemalis
Pilaira anomala
Lilliputia rufula
Cephalotheciun roseum
Penicillium expansum

measured by increase in colony diameter, and the averages of the three sets of figures obtained. A graph was made comparing growth on the "exhausted" dung agar with that shown on dung agar made from fresh pellets. The figures used for this comparison were those resulting from Experiment 2. (Tables 3, 4, 10, 11 in Appendix and Fig. 4.)

The graph shows that growth of the fungi was slower on "exhausted" dung agar, except for Lilliputia rufula where it remained almost exactly the same. As growth of the phycomycetes was not significantly slower than that of the other species it could be concluded that they were not affected any more adversely by the lack of nutrients in the "exhausted" dung medium.

ii. Presence of staling products

The plates of the phycomycete and ascomycete species used in the last experiment were kept after measurements were terminated, to see how long the vigour of the cultures would last. The ascomycetes showed no signs of deterioration until they were about five weeks old. The phycomycete cultures however, remained vigorous for a further ten days only. But as they were then three weeks old this suggested that if staling-products were formed by them, they were not sufficient to account for the complete disappearance of the phycomycetes from the pellets after less than two weeks. The following experiment was carried out in an attempt to confirm this point.

Experiment 6. The experiment was duplicated and average measurements taken throughout, no significant discrepancies being observed between the two sets.

Eight 100 ml. conical flasks each containing 20 ccs. Cook's No. 11 medium were sterilised and divided into two groups of four flasks. One group was inoculated with Mucor hiemalis, the other with Pilaira anomala, and the flasks were incubated at 24°C for fourteen days. The fungal mats were then obtained by filtration, air-dried and weighed, and the weight of one mat for each species calculated. (See Table 12 in Appendix.)

Three groups of 2 flasks each for both fungi were then set up in the following manner.

The media were retained in two portions from the two former groups, each made up to 80 ccs. with distilled water, and 20 ccs. portions returned to the flasks. Two flasks from each set were replenished with the ingredients of the medium (as per 20 ccs.) thus making two groups.

The dried fungal mats from the two species were each ground to a fine powder, mixed thoroughly with 40 ccs. distilled water, and 20 ccs. portions filtered into 100 ml. flasks.

Finally the contents of each of the 12 flasks were sterilised by use of a Seitz-filter, so that any heat-labile substance possibly present in the media would not be destroyed. One flask from each pair was inoculated with Mucor, and the other with Pilaira. After incubation at 24°C for 14 days, the fungal mats were removed, air-dried and weighed. These weights were compared with those obtained during the first part of the experiment by means of histograms. (See Tables 13, 14 in Appendix and Figs. 5 and 6.)

The results show that no harmful staling-product was formed by either species during the two weeks of incubation. The fungal media promoted very little growth, and neither fungus grew any more satisfactorily on its own used medium. This could have suggested that nutrients necessary for the growth of the fungi were absent, or that a harmful substance was preventing growth. That the former explanation was the true one was shown, firstly, by some growth of both species on each other's used media (since neither would utilise the nutrients present in the same quantities); and secondly by vigorous growth of both species on used media which had further nutrients added.

iii. Inhibitory substances

To ascertain whether any substances were produced by one class of fungus which were inhibitory or stimulatory to those of another class, the fungi were inoculated in various combinations on plates of

FIG. 5

Dry weights of Mucor and Pilaira when grown on medium used by Mucor (from Table 13)

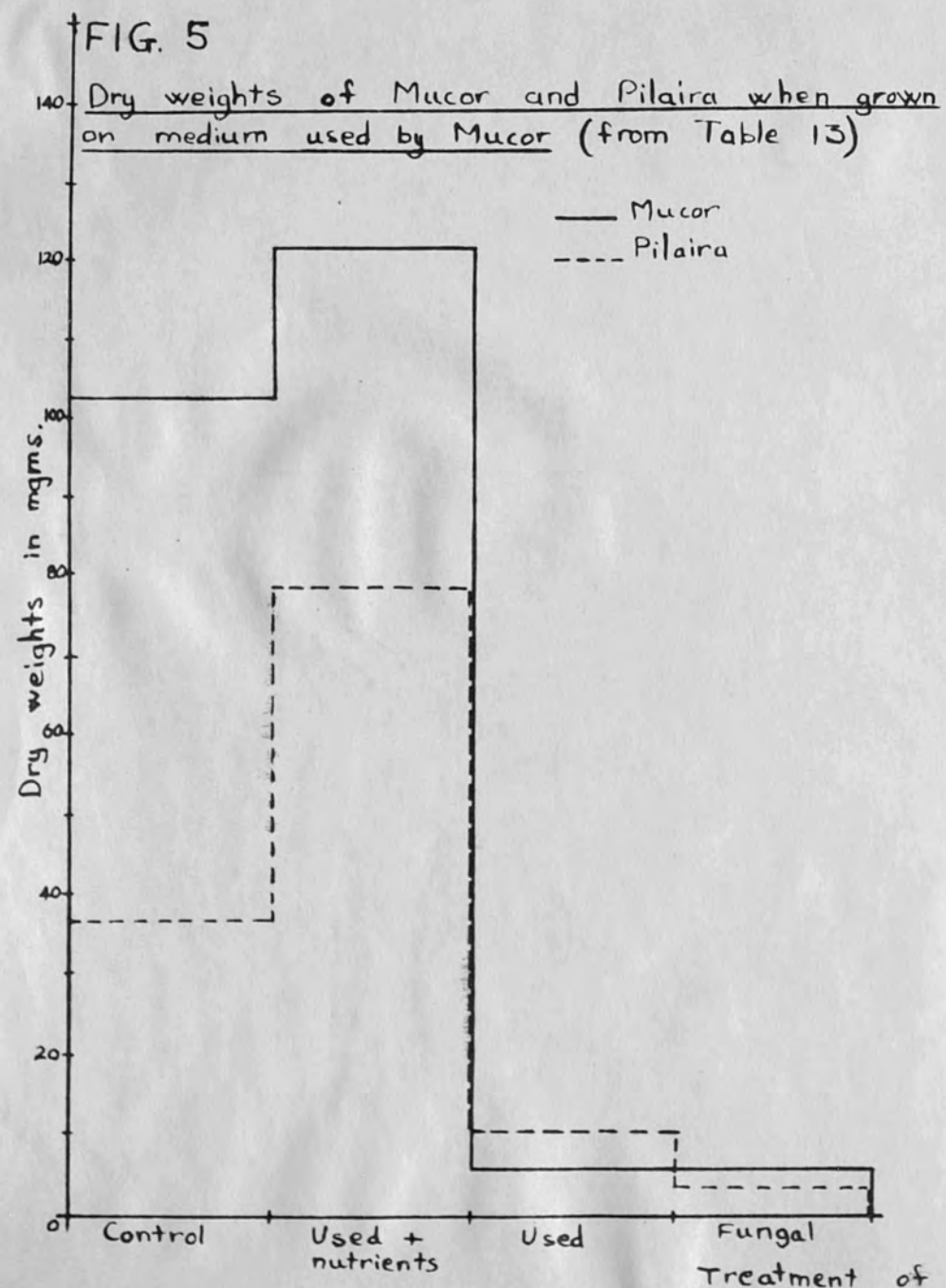
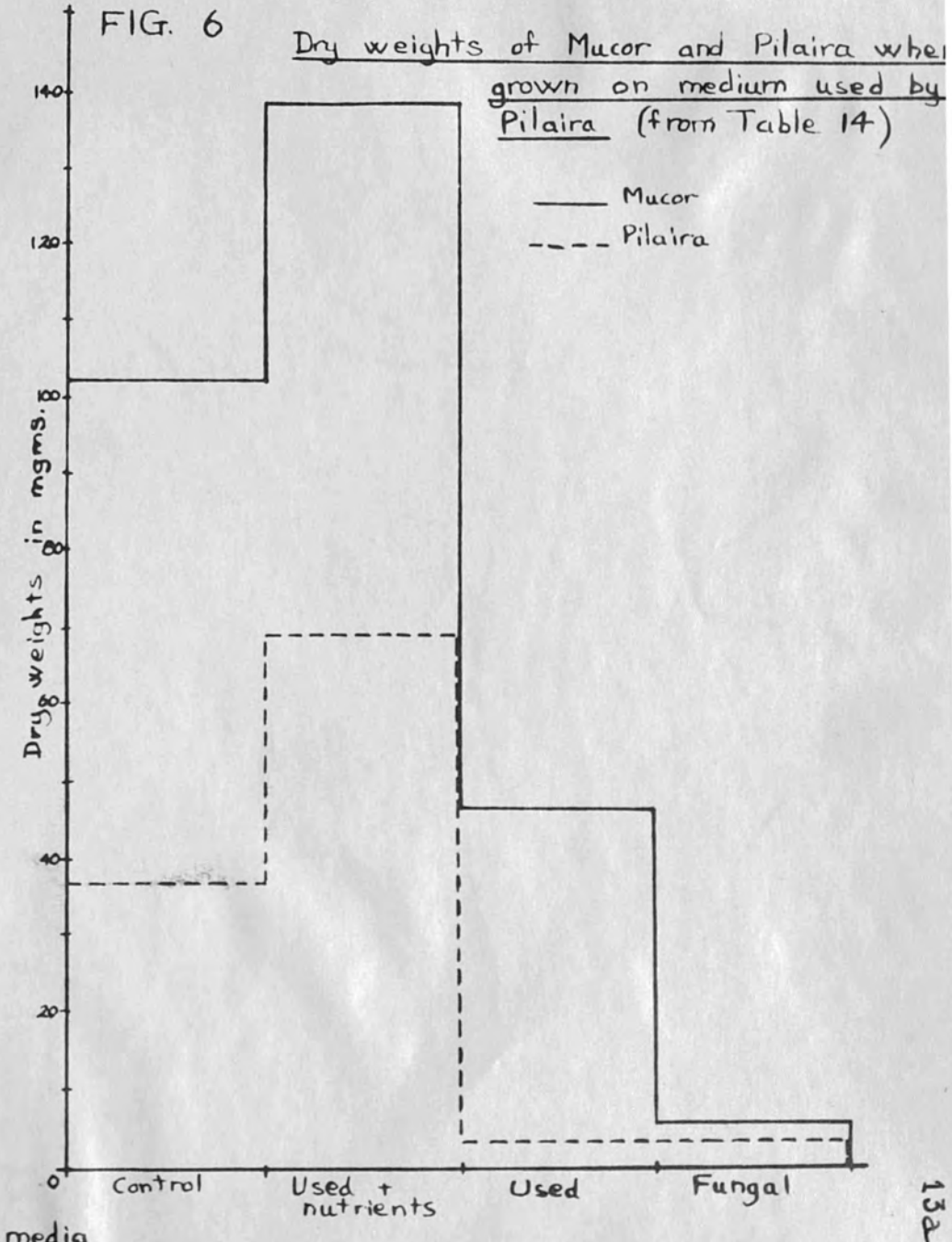


FIG. 6

Dry weights of Mucor and Pilaira when grown on medium used by Pilaira (from Table 14)



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dung agar.

Experiment 7. Duplicated plates of dung agar were inoculated in the following manner.

Plate 1. Pilaira anomala + Mucor hiemalis
" 2. Sordaria macrospora + Lilliputia hiemalis
" 3. Penicillium expansum + Cephalothecium roseum
" 4. Pilaira + Mucor + Sordaria + Lilliputia
" 5. Pilaira + Mucor + Penicillium + Cephalothecium
" 6. Sordaria + Lilliputia + Penicillium + Cephalothecium
" 7. Pilaira + Mucor + Sordaria + Lilliputia + Penicillium + Cephalothecium

Behaviour of the fungi on plates 1 to 3 was used as a control against their behaviour when grown in combination with fungi of another class. No measurements were taken, but observations were made of their growth and sporing activities.

The fungi all behaved as in the previous experiments when they were grown singly. In every case fruiting-bodies appeared at the same times - after two days for the phycomycetes and fungi imperfecti, and eight days for the ascomycetes (no cellulose present in the medium, see Experiment 3.) Colonies of the different species produced when grown in combination with those of a different class spread over the agar at the same rate as the controls, and on all the plates intermingled freely.

Cook, in his experiments investigating the succession of fungi on culture media (1924) found no substance produced by a primary invader of decaying fruits and vegetables which was inhibitory to a secondary invader. However, all his media were sterilised by heat, so he suggested that if a toxin was produced at any stage during his experiments it was subsequently destroyed by this treatment. In an effort to eliminate this possibility an experiment was devised using his method of growing the fungi in succession in liquid-culture but all sterilisation was made through a Seitz filter. (Experiment 9).

Dalton and Hurwitz (1948) stated that soil could be sterilised by steam but that changes result in its properties, especially in the organic matter. They found the fumes of volatile disinfectants were the most satisfactory for sterilisation, inducing a minimum change in the soil itself. Thus, in the following experiment where pellets were used as a medium, sterilisation was effected by propylene oxide vapour.

Experiment 8. For this experiment only phycomycetes and ascomycetes were used, these being the two most prominent classes in the succession. The experiment was duplicated.

Some fresh pellets were sterilised by propylene oxide vapour in the following way. The liquid was put in the bottom of a jar, above which the pellets rested on a platform of wire-mesh. The jar was left closed, (but not sealed) for ten days, which proved quite long enough to effect complete sterilisation. Four pellets were placed in each of twelve sterilised petri-dishes lined with filter-paper, which was kept moist by the daily introduction of a few drops of sterile distilled water. The pellets were inoculated as follows:

Plates	1 - 3	: Pilaira anomala
"	4 - 6	: Mucor hiemalis
"	7 - 9	: Sordaria macrospora
"	10 - 12	: Lilliputia rufula

Abundant mycelial growth resulted at once. The sporangia of the phycomycetes appeared on the second day, and the perithecia and apothecia of the ascomycetes on the fourth and fifth days respectively - these times would be expected with cellulose present in the medium. After three weeks when growth of all the species was still quite vigorous the pellets were sterilised again using propylene oxide vapour. The contents of each plate were then re-inoculated with the other three species used in the experiment, as shown in Table 15. Each fungus consequently colonised the pellets as it did after the first inoculation. Thus no substance was shown to be produced by any one species which could have had an inhibitory or stimulatory effect on the others.

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Table 15. Inoculation of pellets sterilised with propylene oxide vapour

Plates	First fungus	Second fungus
1	Pilaira anomala	Mucor hiemalis
2	"	Sordaria macrospora
3	"	Lilliputia rufula
4	Mucor hiemalis	Pilaira anomala
5	"	S. macrospora
6	"	L. rufula
7	Sordaria macrospora	P. anomala
8	"	M. hiemalis
9	"	L. rufula
10	Lilliputia rufula	P. anomala
11	"	M. hiemalis
12	"	S. macrospora

/17.

Experiment 9. As mentioned above this experiment was carried out employing Cook's method with the exception that sterilisation was effected by a Seitz-filter. It is also an extension of the method used in Experiment 6, (staling-products in liquid culture) and as such the results from that experiment are incorporated.

20 ccs. of the solution were sterilised (by autoclaving) in each of 32 100 ml. conical flasks, which were then divided into four groups of 8. Each group was inoculated with a single fungus, the species being the same as those used in the previous experiment:

Mucor hiemalis
Pilaira anomala
Sordaria macrospora
Lilliputia rufula

The cultures were incubated for 14 days at 24°C, by which time it was judged that maturity had been reached. The fungal mats were removed, air-dried and weighed, and the remaining media treated in different ways. As in Experiment 6, four flasks of each group were replenished with the original solids of the medium (as per 20 ccs.) and the volume brought up to 20 ccs. with distilled water. The other four flasks of each group had distilled water only added. Thus any lack of growth of fungi inoculated into these last four flasks due to loss of nutrients could be compared with those grown in the first four containing replenished media.

A third set of flasks was set up at this point. As before the dried fungal mats from the first inoculations were collected, and each species ground to a fine powder. This was mixed thoroughly with 80 ccs. distilled water and divided into 20 ccs. portions. Thus a third medium was made from the fungus itself.

Finally the contents of each flask were sterilised separately by Seitz-filtering. Thus if any inhibitory substance had been produced by the first fungus it would be present in an unaltered form to affect the second fungus.

There were now 48 flasks in four groups of twelve, each group in three sections of four flasks. The flasks in these sections were

/18.

FIG. 7

Dry weights of cultures when grown on medium used by Mucor (from Table 17)

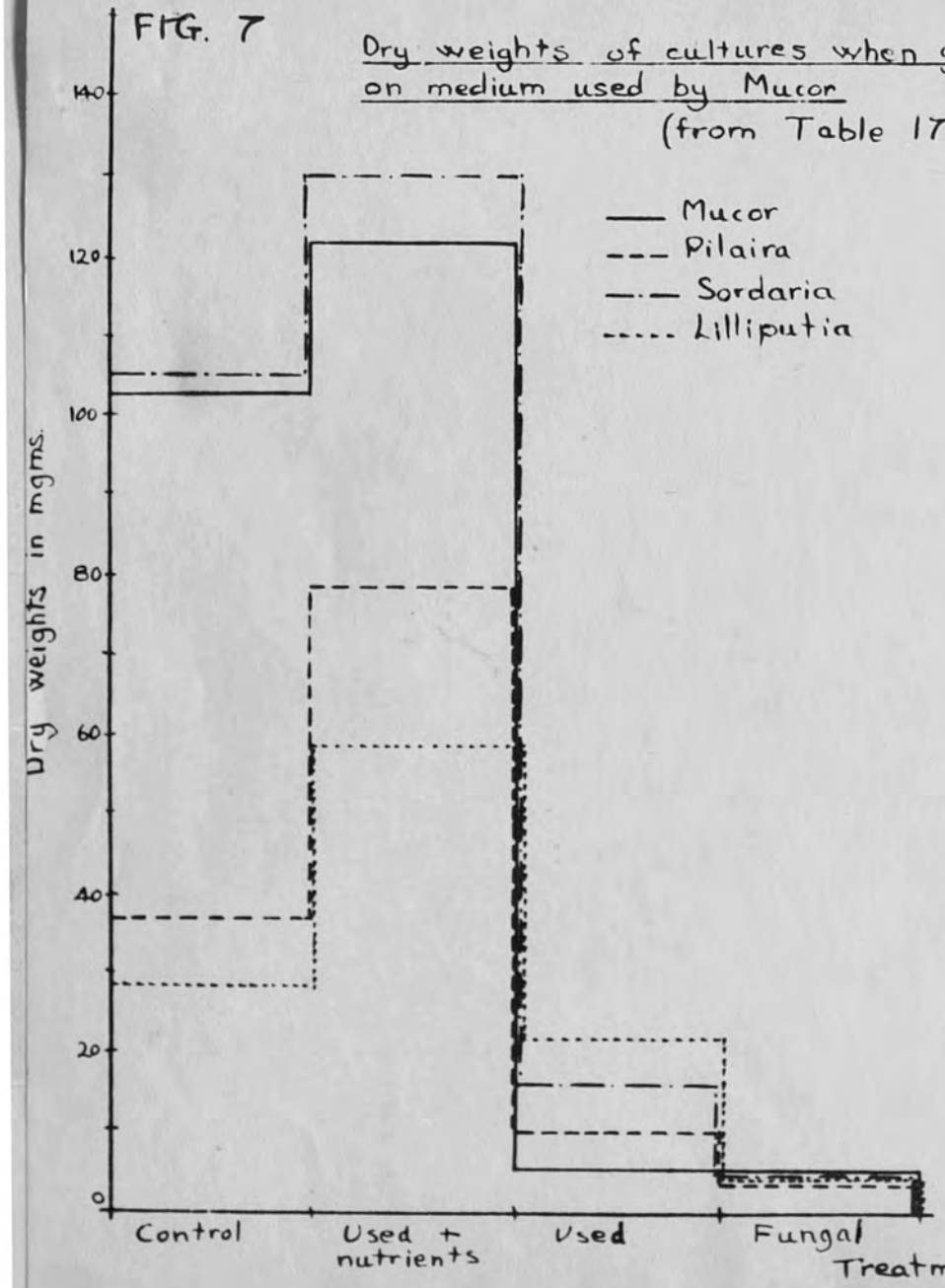


FIG. 8

Dry weights of cultures when grown on medium used by Pilaira (from Table 18)

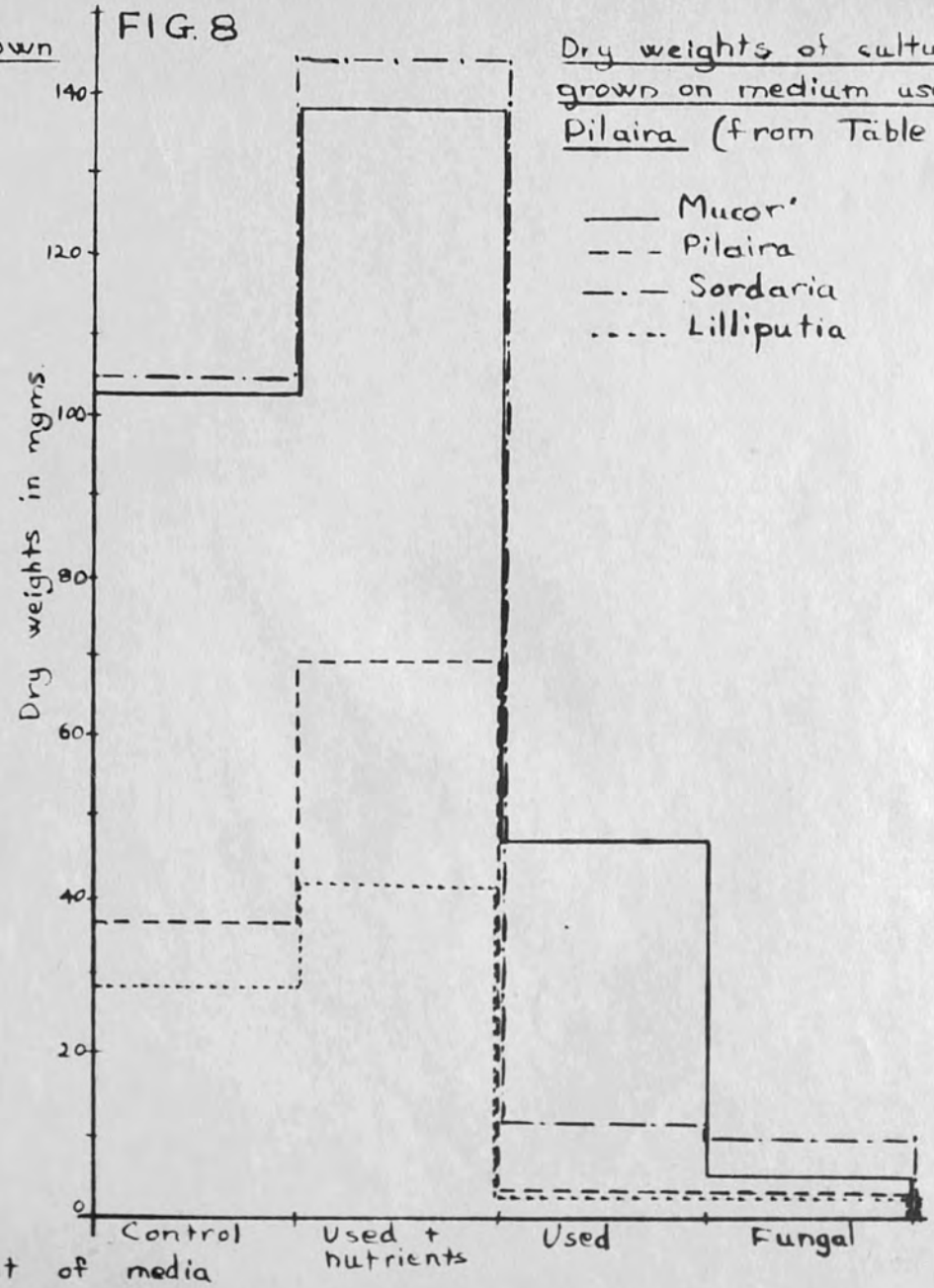


FIG. 9

Dry weights of cultures when grown on medium used by Sordaria (from Table 19)

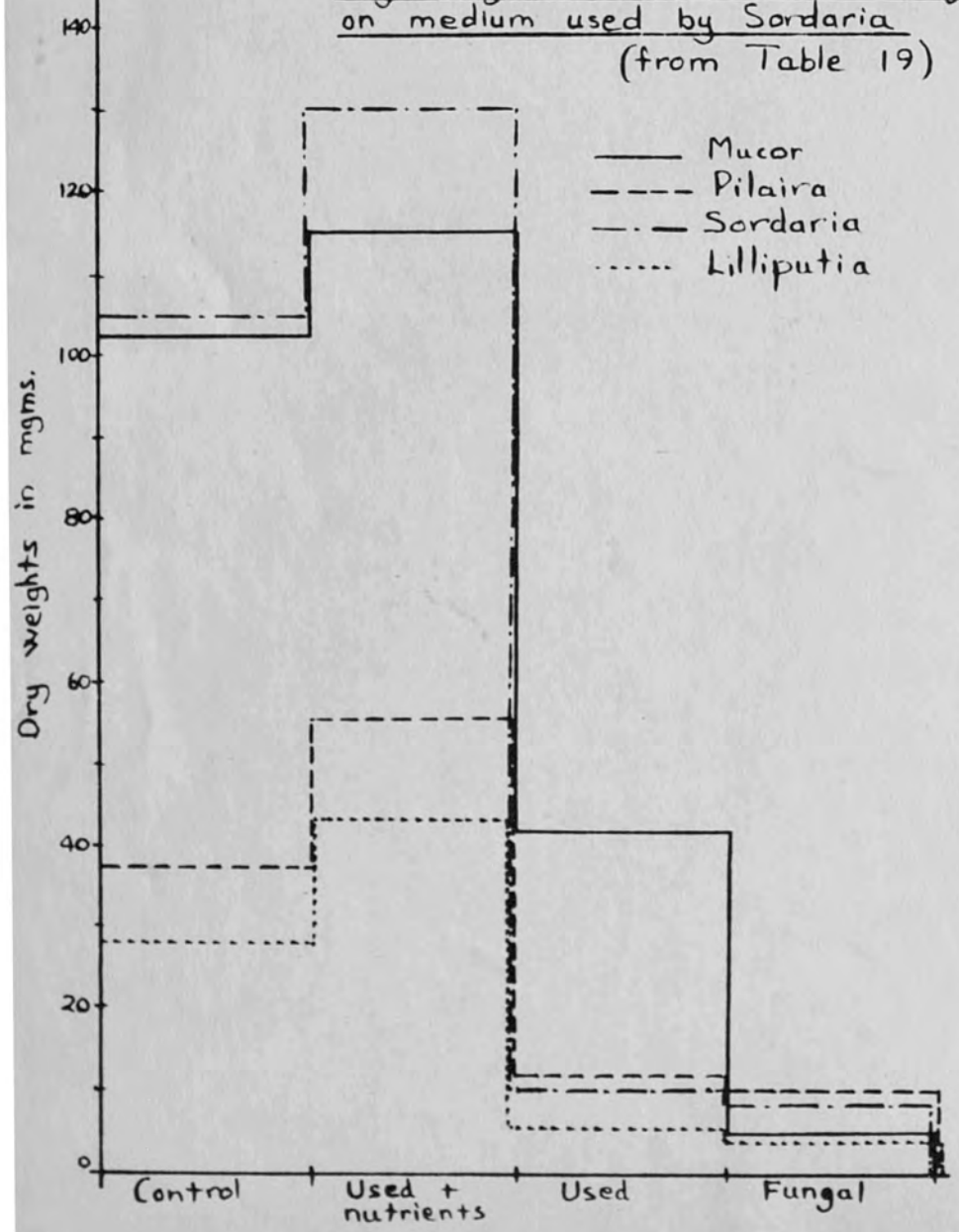
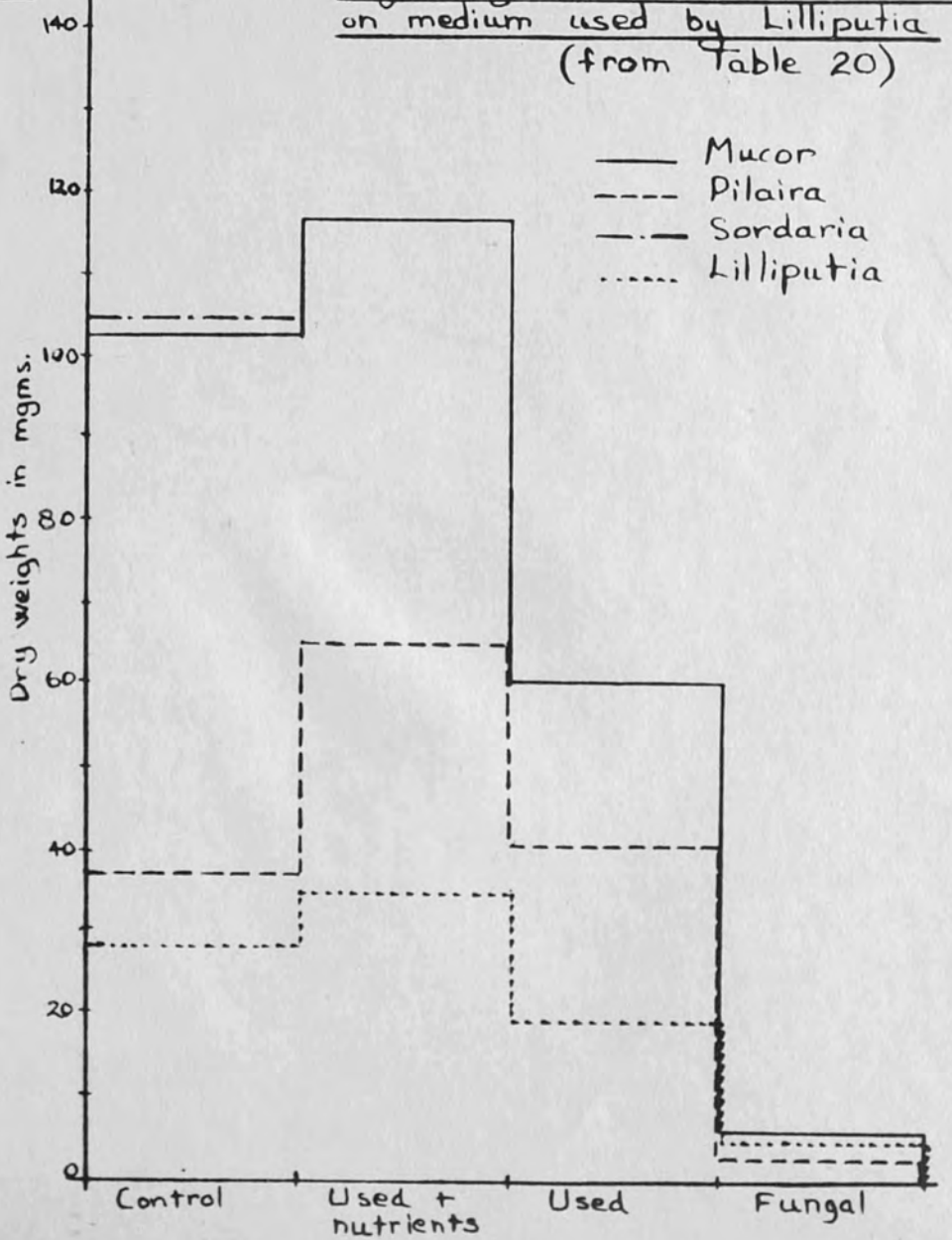


FIG. 10

Dry weights of cultures when grown on medium used by Lilliputia (from Table 20)



inoculated separately with the four fungi. After 14 days of incubation at 24°C the fungal mats were removed, air-dried and weighed. The results are set out in Tables 16, 17-20 in the Appendix, and Figs. 7-10, the latter being in the form of histograms.

The experiment was repeated and comparable results obtained. However, it was not until after several trial experiments had first been made that all contamination was finally eliminated. It was subsequently found that the only sure way was to filter the solution for each flask separately, and to take great care in transfering the sterilised solution from the filter-flask to the sterilised 100 ml. conical flask.

All the histograms follow the same pattern. In each case, when the used medium was replenished with the original quantities of solid nutrients, weights were increased, in comparison with the decreased weights from unreplenished media. If some inhibitory or stimulatory substance had been produced it would have been present in both these media, and would have affected the cultures in the same way. It could not be argued that such a substance would possibly have no effect until the available nutrients were used up, as it was shown that there was no shortage of food materials in the pellets even after four weeks. (See Experiment 5). It would not, therefore, have the deleterious effect on the phycomycetes evident on the pellets after only one week. Apart from this, at least one histogram would be expected to show a difference from the rest.

Grown on a used medium, Mucor hiemalis showed an increase in weight when this medium was replenished with the original nutrients, and a decrease when it was unreplenished. The increase was slightly more on the medium used by Pilaira anomala. Sordaria macrospora also showed a greater increase in this case, whereas Pilaira increased more when grown on a Mucor medium. On its own unreplenished medium Mucor made very poor growth, as did Pilaira and Sordaria when grown on their own media. Lilliputia rufula did better in comparison, most probably because, owing to its slower growth, a greater proportion of the original nutrients still remained. This probability is strengthened by the fact that the other three species also grew better

on this medium as compared to their growth on the other unreplenished media. Growing on a used Pilaira or Sordaria medium, Mucor showed a smaller decrease in weight than the other species. This suggests that some ingredient of the medium unused by them was readily utilised by Mucor. None of the species made any appreciable growth when grown on a fungal medium.

The results of these last two experiments indicate that no substance was produced by any of the fungi which could have accounted for the sudden disappearance of the phycomycetes from the pellets.

When isolations were made from the pellets bacteria were usually present as contaminants. Further, pellets which were five or six weeks old were sometimes completely covered with bacteria, provided they were kept under moist conditions. It was therefore considered possible that the bacteria, or some substance(s) produced by them, could be harmful to either or both the phycomycetes and ascomycetes.

Experiment 10. As a preliminary experiment to an investigation of this possibility some pellets which were four weeks old were divided into three groups and treated as follows:

Group 1. Pellets unsterilised
" 2. Pellets sterilised by heat (autoclaving)
" 3. Pellets sterilised by propylene oxide vapour.

The pellets were placed in sterilised petri-dishes lined with filter-paper as before, and inoculated in each group with the fungi used in the latest experiments:

Mucor hiemalis
Pilaira anomala
Sordaria macrospora
Lilliputia rufula

No growth was produced by the phycomycetes on pellets in Group 1, and very little on those in Group 3, but abundant growth occurred in Group 2. The ascomycetes however had colonised the pellets of all groups completely after five or six days. The extent

of this colonisation was judged by the appearance of young perithecia and apothecia, and the spread of hyphae over the surface of the pellets. (This was not noticeable under natural conditions, as the mycelium was buried under the surface of the dung.)

These results show that some substance which was present in the dung, at least after four weeks, was preventing the phycomycetes from growing. It has been shown in previous experiments that this was not produced by any of the fungi. It was also a heat-labile substance, as growth of the phycomycetes was not inhibited on the heat-sterilised pellets.

Experiment 11. Some dung agar was made up according to the standard method. Before pouring into petri-dishes however, an inoculum of the dung-inhabiting bacteria was introduced into the warm agar. Duplicated plates were inoculated separately with the four fungi used in the previous experiment. Colony diameters were measured, and a graph drawn from the results comparing the growth with that obtained in Experiment 2, when the fungi were grown on untreated dung agar. (See Tables 4 and 21 in Appendix and Fig. 11.)

It is clear from the graph that growth of the phycomycetes was prevented by the fourth day, when bacteria were present in the medium, whereas that of the ascomycetes was unaffected.

Since some inhibitory substance was shown to be present in four-week-old pellets after sterilisation by propylene oxide vapour (see Experiment 10) it seemed fairly certain that the bacteria must have produced a diffusible inhibitor, and not have actively destroyed the fungal mycelium. The following experiment was performed in an attempt to provide confirmation for this possibility.

Experiment 12. Duplicated plates of dung agar were inoculated on one side with one of the four fungi, and on the other with bacteria. Two radii of the fungal colonies were measured: i) the radius growing towards the bacterial inoculum; and ii) the radius at right-angles to i). This latter was unaffected, it was found, by any inhibitory substance produced by the bacteria, and was therefore considered to show normal development. A third measurement taken

FIG. 11

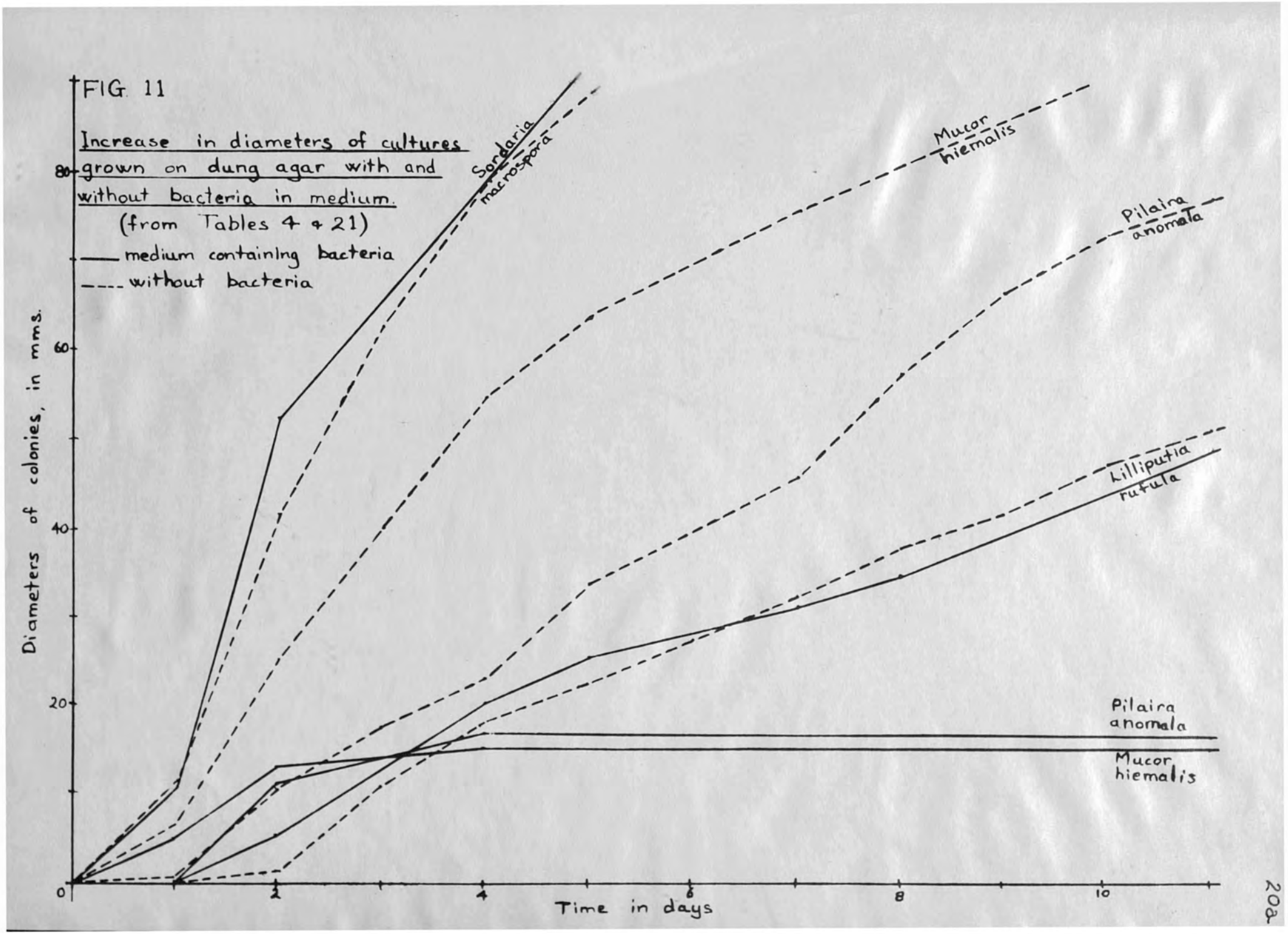

Increase in diameters of cultures grown on dung agar with and without bacteria in medium.
(from Tables 4 & 21)
medium containing bacteria
without bacteria
Sordaria macrospora
Mucor hiemalis
Pilaira anomala
Lilliputia rufula
Pilaira anomala
Mucor hiemalis
Diameters of colonies, in mms.
0
20
40
60
80
2
4
6
8
10
Time in days

FIG. 12.

Development of a Mucor hiemalis colony when growing with a bacterial colony on dung agar

(from Table 22)

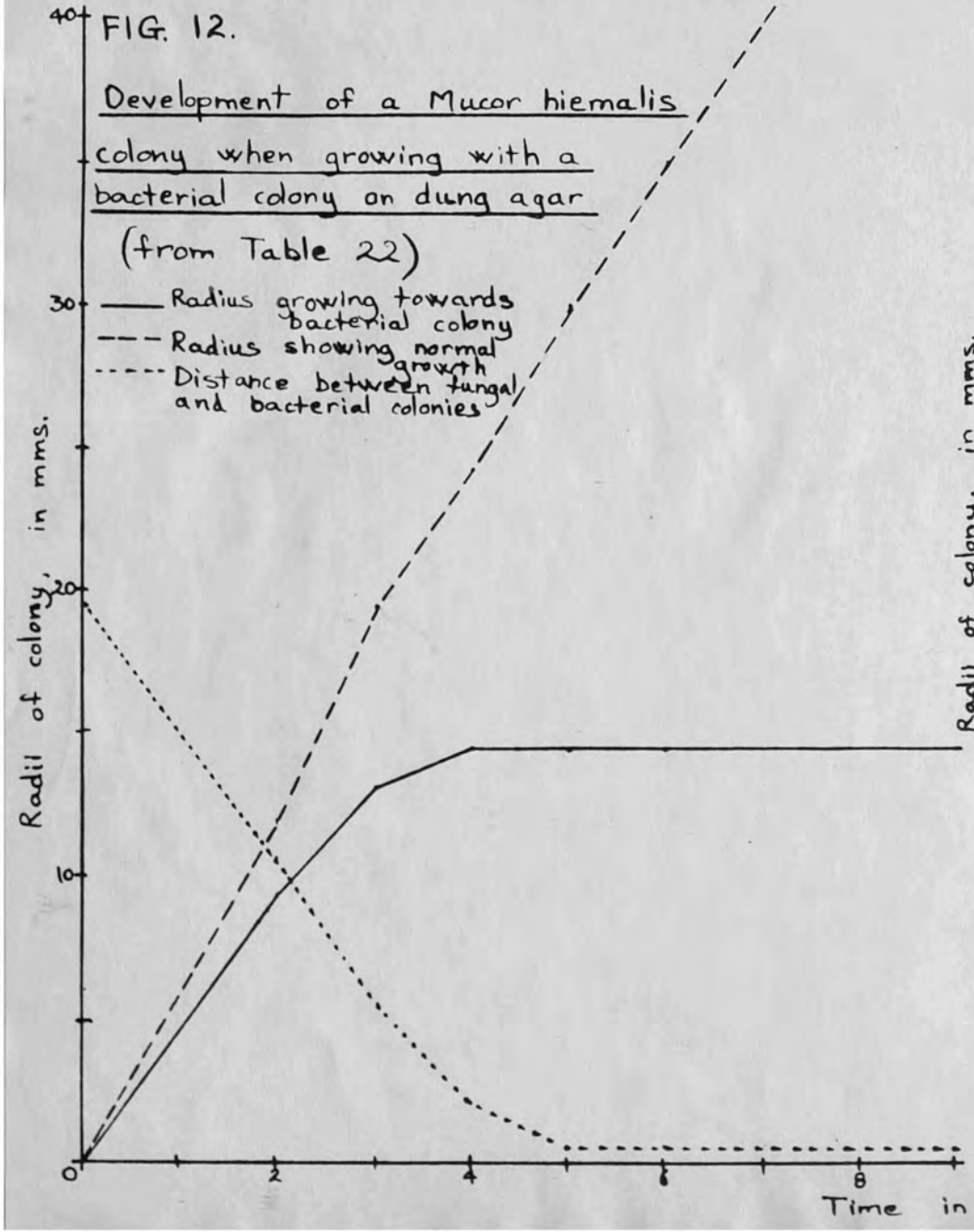


FIG. 13

Development of a Pilaira anomala colony when growing with a bacterial colony on dung agar.

(from Table 22)

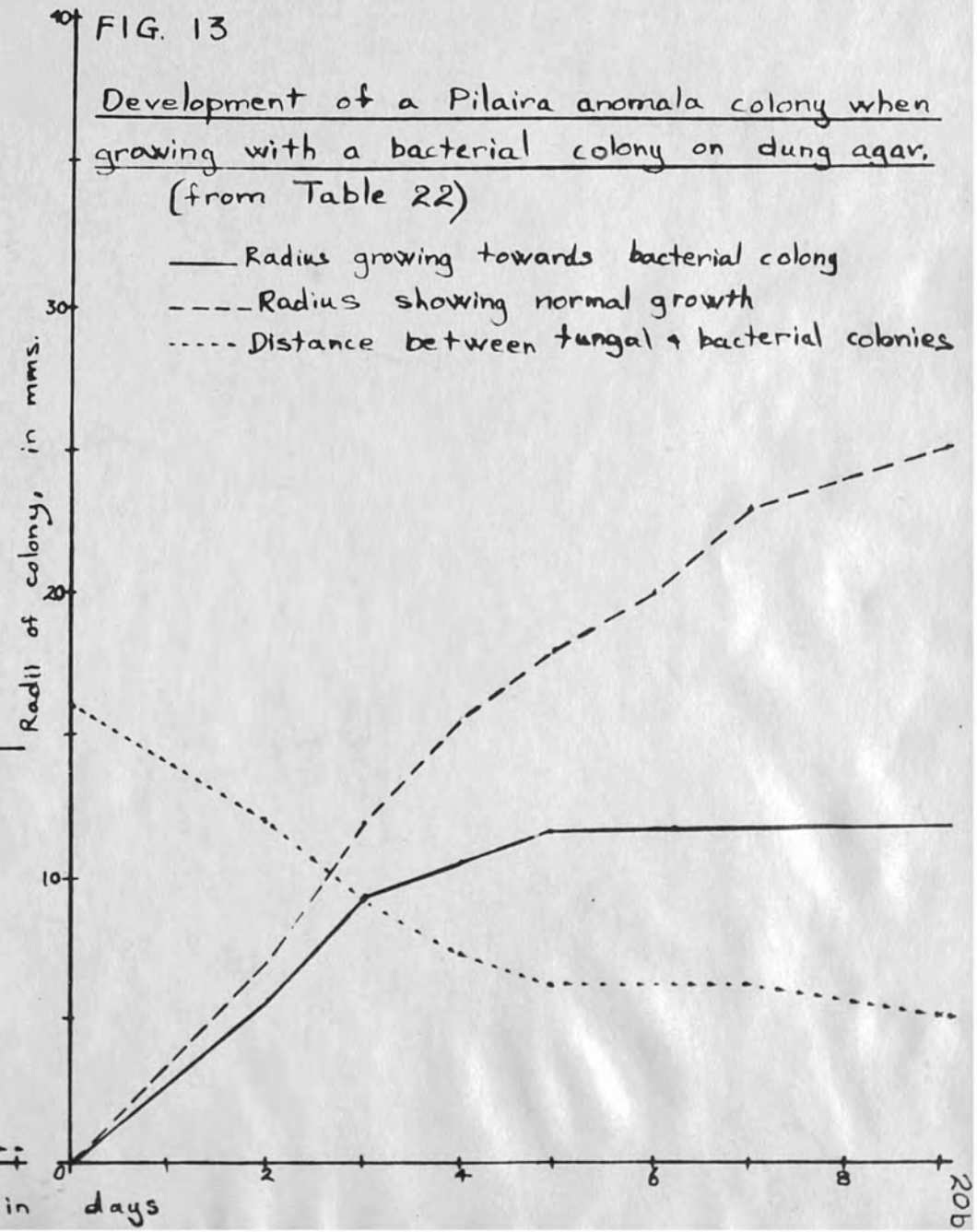


FIG. 14

Development of a Sordaria macrospora colony when growing with a bacterial colony on dung agar (from Table 22)

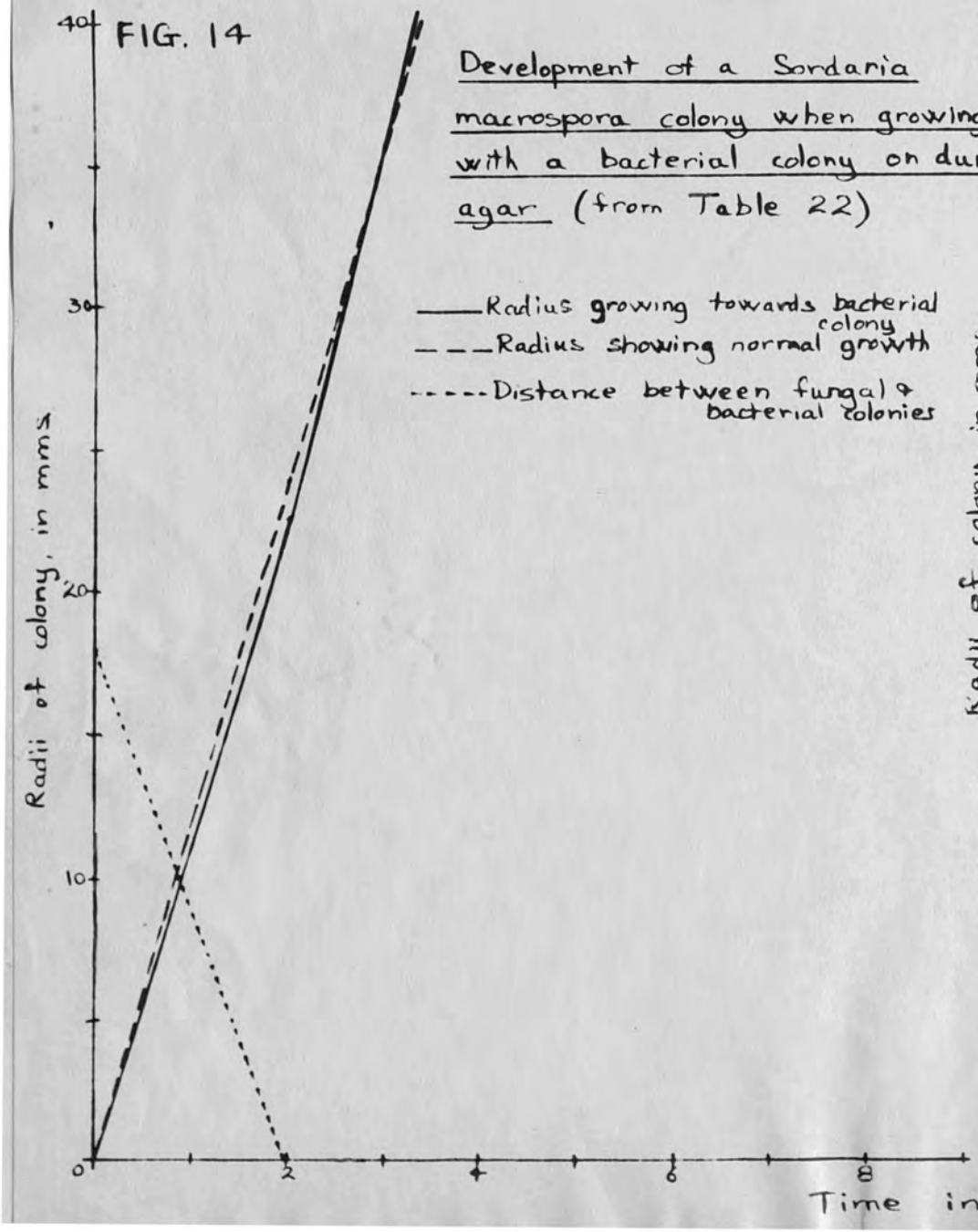
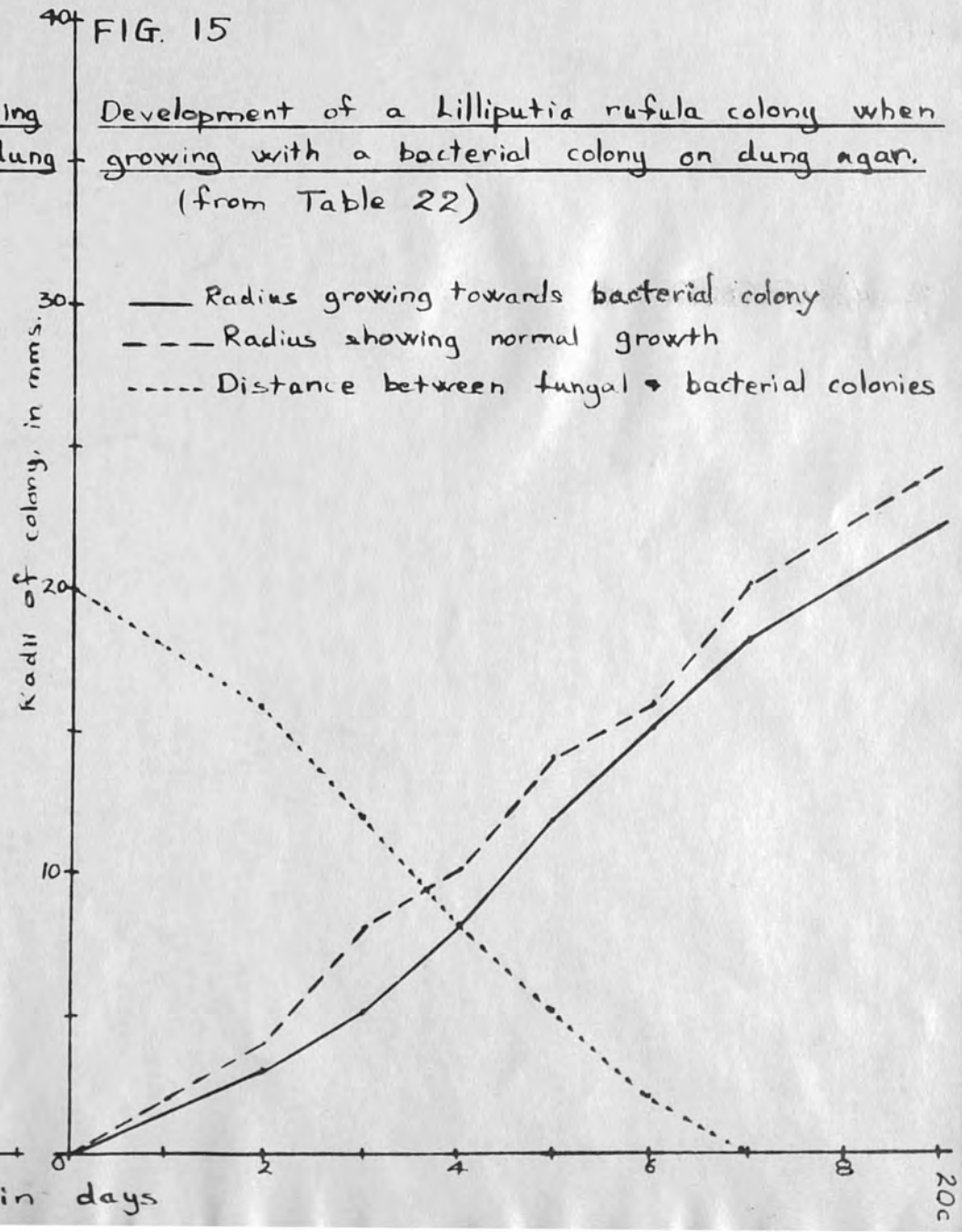


FIG. 15

Development of a Lilliputia rufula colony when growing with a bacterial colony on dung agar. (from Table 22)



was the distance between the edges of the fungal and bacterial colonies as they grew towards each other. Graphic recordings were made of the results. (Table 22 in Appendix and Figs. 12-15.)

The graphs indicate the inhibitory effect produced by the bacteria on the phycomycetes. Growth of the latter towards the bacterial colonies ceased after four days, as it did when they were grown on a medium containing the bacteria (see Experiment 11.) Growth of Sordaria macrospora was completely unaffected, and that of Lilliputia rufula was only slightly inhibited. Both these fungi grew through and under the bacterial colonies. The phycomycetes however were completely inhibited at a distance of 1 mm. from the bacterial colonies with Mucor hiemalis and 6 mms. with Pilaira anomala. A diffusible inhibitory substance must therefore have been produced by the bacteria which prevented growth of the phycomycetes, supporting the suggestion made at the end of the last experiment.

In the two final experiments an effort was made to separate this substance from the bacteria and incorporate it in the fungal media.

Experiment 13. The dung-inhabiting bacteria were inoculated into several flasks containing Cook's no. 11 medium and incubated for two weeks at 24°C, by which time thick suspensions had resulted. These were Seitz-filtered and the sterile liquid added to a warm dung extract solution plus agar which had been sterilised in the autoclave. Plates were then inoculated with the four fungi, and colony diameters measured from day to day. A graphic recording compared growth, as before, with that obtained in Experiment 2. (Tables 4 & 23 in Appendix and Fig. 16).

There was a very marked slackening of growth of the phycomycetes in the presence of the bacterial filtrate, whereas the ascomycetes were unaffected. Indeed, the growth of Lilliputia in this case was slightly increased.

Experiment 14. As in the previous experiment the bacteria were incubated at 24°C in flasks containing Cook's no. 11 medium for 14 days, and the suspension Seitz-filtered. This solution plus

/22

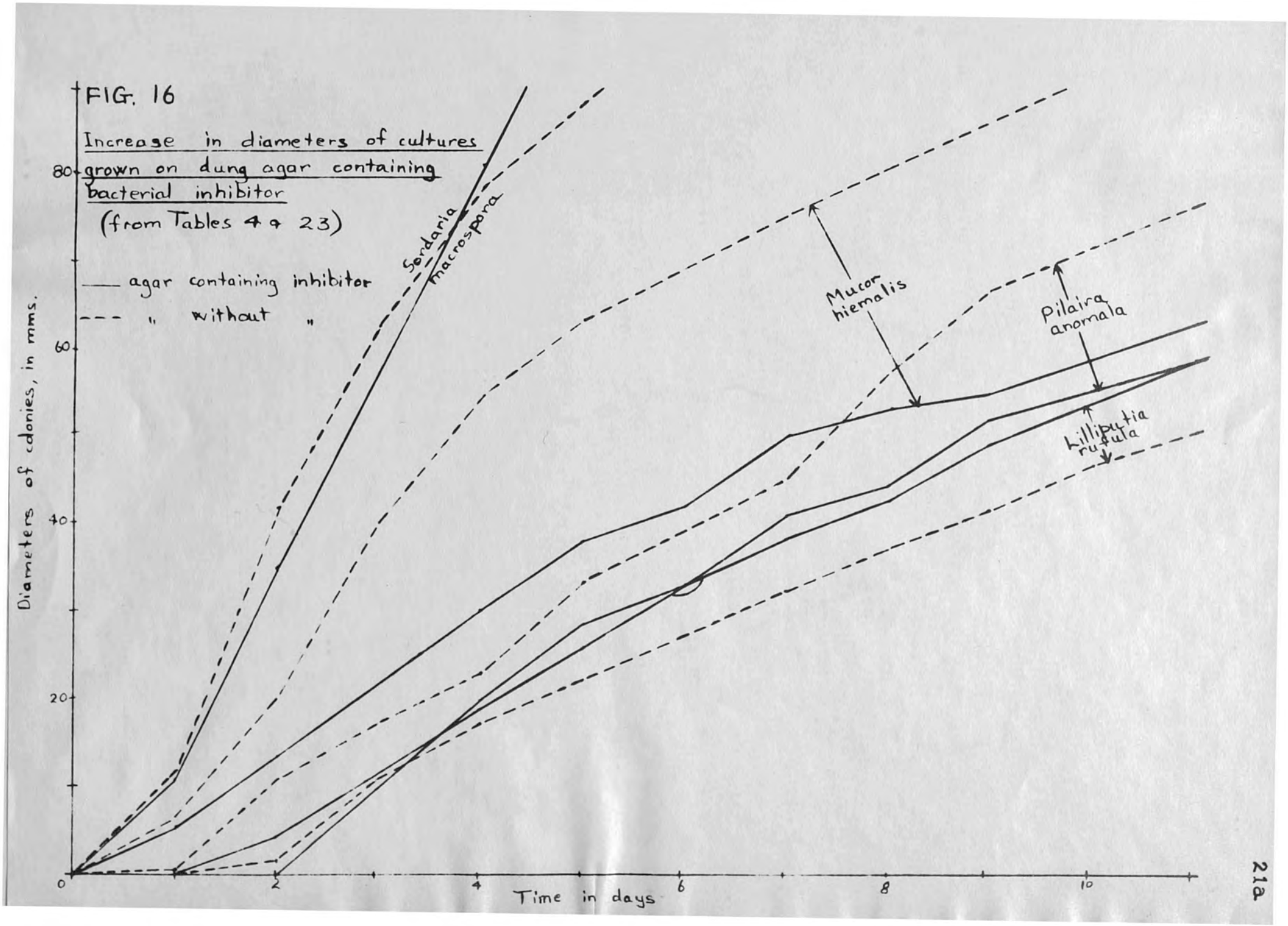
FIG. 16

Increase in diameters of cultures grown on dung agar containing bacterial inhibitor

(from Tables 4 & 23)

—— agar containing inhibitor

--- " without "



distilled water was used to make up 20 mls. of Cook's no. 11 medium in each of four 100 ml. conical flasks. The contents of each flask were subsequently sterilised with the Seitz-filter, and then inoculated with the four fungi. After two weeks the fungal mats were removed, air-dried and weighed. These weights were compared with those obtained in Experiment 9 (see Table 16) when the fungi were grown in untreated Cook's medium. The percentage loss or gain in weight was calculated for each fungus, and the results are shown in Table 24. (in Appendix)

[These last two experiments were repeated at least three times for each petri-dish or flask before satisfactory results were obtained, as it was found to be exceedingly difficult to eliminate all bacterial contamination with the Seitz-filter. To be truly effective a new pad should have been used for each filtration. As this would have proved rather expensive the most satisfactory method was to filter each solution twice, the first operation removing most of the bacteria. The second filtrate was then usually free from contamination.]

As in the previous experiment the figures indicate that growth of the phycomycetes was inhibited by some diffusible substance or substances produced by the bacteria. Although growth of the ascomycetes was more-or-less unaltered in the presence of this substance, the weights of both increased so it could possibly be stimulatory in this instance.

The results of these last experiments thus offered an explanation as to why the phycomycete population on the pellets began to decline after the first five days, and finally disappeared after less than two weeks of succession.

DISCUSSION

Succession of fungi on dung has been an accepted fact since Massee and Salmon reported their observations in 1901. The graph from Experiment 1 illustrates this well-known phenomenon. There is little doubt that the fungi imperfecti play only a small part in the sequence, and the basidiomycetes an even lesser one, the two most prominent classes being the phycomycetes and ascomycetes. It is almost certain that the fungi imperfecti appear on the dung either as the imperfect stages of some of the ascogenous fungi, or as contaminants arising from wind-bourne spores. The role of the basidiomycetes has not been investigated, as they appear to play an insignificant part in the succession.

Although much detailed work has been carried out inquiring into the properties of wood-rotting and cellulose-destroying fungi, and the activities of many soil fungi, the problem of succession in any of these habitats has never been fully investigated; and, as far as I have been able to ascertain, not at all with reference to coprophilous fungi. Conclusions reached in an attempt to explain succession in one case do not necessarily apply in another, because of the varieties of substrate, environment, and the fungi involved. Thus, although Cook's methods of approach towards the problem of succession on decaying fruits and vegetables (1924, - this paper being the only one I have been able to discover which is entirely concerned with the problem of <u>why</u> succession occurs) proved to be very useful during the present investigations, his final conclusions could not be accepted as applying to coprophilous fungi also.

Before experiments could be carried out in pure culture and the results used to explain the behaviour of the fungi on the pellets, it was necessary to correlate their behaviour on a dung agar medium with that on the pellets. A series of observations made on the appearances of fruiting-bodies is the simplest method by which growth of the fungi can be measured on the pellets. Thus, since fruiting-bodies were found to appear at comparable times in pure culture, it was indicated that conditions for growth are similar in the two media.

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The phycomycetes produced sporangia on the second day of incubation, both on fresh pellets and on a dung agar medium. Appearances of the ascomycetous fruiting-bodies however did not correspond on these two media until cellulose had been added to the dung agar. Some of the ascomycetes growing on the pellets utilised pure cellulose in the form of filter-paper, as a food material, but could not use it as the only nutrient supply. Its presence in a dung-agar medium served only to precipitate the formation of fruiting-bodies so that they appeared in plate culture at the same time as on the pellets.

Mycelial growth of both the ascomycetes and phycomycetes remained unaffected by the presence of cellulose in the medium. There is therefore no reason to suspect that mucoraceous fungi appearing on dung behave any differently to other members of the same group, various workers having shown that the Mucoraceae are well-defined by their inability to utilise cellulose as a source of food supply.

Appearances of fruiting-bodies at comparable times on both the pellets and a dung agar medium indicate that mycelial growth of the phycomycetes and ascomycetes commence at the same time during succession. Cook's theory with regard to fruit-decaying fungi - i.e. the 'first' fungus (phycomycete) dies and subsequently leaves favourable conditions for the invasion of a 'second' fungus (ascomycete)- does not therefore apply in this case, as both are present in a developing mycelial form at the beginning of succession.

Since a dung agar medium appears to produce conditions for growth similar to those present in the pellets, any factors affecting this medium which cause subsequent behaviour of the fungi to correspond to their behaviour on the pellets, can be said to apply also to the medium of the pellets. It has been found that phycomycetes developing from mycelial fragments and spores can be isolated on dung agar from pellets several weeks old and on which all appearances of phycomycetes have ceased. Further growth after their decline during the second week of succession is therefore inhibited,

and not prevented by death of the fungi concerned. Thus any factors causing an inhibition of growth of these phycomycetes in pure culture over a period corresponding with that observed on the pellets can be said to be possibly operating in the pellets themselves.

pH values taken throughout succession remain fairly constant at a neutral value of 7.1. Although the Mucors are unable to attack the large mass of cellulose present in dung, or other complex organic compounds, S.A. Wakeman in his investigations of soil fungi and their activities (1916), found that they can easily attack nitrogen compounds such as peptones, amino-acids, urea and uric-acid, all of which are present in dung. After decomposition ammonia is released, some of which is assimilated by the fungi and built up into proteins. He found also that such members of the fungi imperfecti as Cephalothecium roseum and Stysanus spp. are active ammonifiers. However an accummulation of ammonia in the pellets produced by such activities which could possibly have a harmful effect upon the fungi is not indicated by a change in pH values.

It has been demonstrated that a decrease in nutrient supply is not the explanation for the disappearance of the phycomycetes from the pellets after less than two weeks. Growth of phycomycetes is retarded to the same extent as that of ascomycetes and fungi imperfecti when members of these classes are grown on a dung agar medium made from pellets about four weeks old.

It has also been shown that the formation of harmful staling-products by the phycomycetes is not the explanation of their decline. Cultures on dung agar remained vigorous for at least three weeks, and no harmful substance was extracted from a liquid medium on which the fungi had been growing for two weeks, or from the fungi themselves.

The possibility of some substance being produced by one fungus which could be harmful to another was considered, as this type of interaction is known to occur among some soil fungi (Porter and Carter, 1938). No such interaction was observed however. Cook's experiments with fruit-decaying fungi were largely concerned with this aspect, but although he produced no toxic substance during experimentation, he suggested it may have been destroyed during his

/26.

heat-sterilisation processes. During the present investigations sterilisation, using a Seitz-filter for liquid media or propylene oxide vapour for pellets, still failed to reveal the presence of any harmful substance, whether a diffusible one from media used by the fungi, or an indiffusible one from the fungi themselves.

As well as interaction between fungi in the soil, it is also known that there is a certain amount of competition between fungi and bacteria. Such competition has been found to occur in the pellets to such an extent as to seriously affect growth of the phycomycetes. A diffusible, heat-labile, substance (or substances) has been shown to be produced by dung-inhabiting bacteria which completely inhibits growth of the phycomycetes, while not affecting the ascomycetes. When the bacteria are introduced into a dung agar medium on to which the fungi are inoculated, growth of Mucor hiemalis and Pilaira anomala is slowed down after two days and stopped after four days. These findings are similar to those made during succession on the pellets, where the two species begin to disappear after three and two days respectively (see Table 1). Again growth is inhibited when colonies of these same two species approach colonies of the bacteria, but the ascomycetous colonies are unaffected and continue growing through the bacterial colonies.

Further evidence of this inhibitory action was provided when the filtrate from a liquid medium, in which the bacteria had been growing, was incorporated in either a dung agar or liquid medium for the fungi. Growth of the phycomycetes was considerably slower on the former, and their dry weights were much less on the latter compared with results obtained when untreated media were used. Growth of the ascomycetes remained unaffected by the presence of this diffusible substance, except for a slight but distinct increase in their dry weights. This gain has not been explained, unless it is that the fungi are able to utilise the substance to their advantage.

Although these investigations show that the presence of a substance produced by dung-inhabiting bacteria plays an important part in the succession of coprophilous fungi, much of the mystery

still remains unsolved. There has been no attempt, for instance, to find why the phycomycetes should be so affected while the ascomycetes remain unharmed. Also a very general picture has necessarily to be drawn, as the experiments have been carried out on only a few of the fungi occurring in succession on the pellets. Nevertheless a clue has been provided towards a solution of this little-investigated problem.

SUMMARY

1. During succession on rabbit dung there is a dominance in the fungal flora by the phycomycetes for the first week, followed by their disappearance and dominance by the ascomycetes. Appearance of fungi imperfecti and basidiomycetes is slight, the former probably being contaminants from wind-borne spores.

2. The presence of cellulose in a dung agar medium brings forward the time of formation of the ascomycetous fruiting-bodies to correspond with the times of their appearances on the pellets. Appearances of the phycomycetous fruiting-bodies on the two media correspond without this addition.

3. pH values do not alter significantly during succession, remaining at about pH 7.1.

4. There is no exhaustion of a nutrient supply for the phycomycetes in pellets four weeks old.

5. No harmful staling-products are formed by the phycomycetes for at least three weeks, so their presence cannot provide an explanation of the rapid disappearance of the phycomycetes from the pellets after only one week.

6. There is no interaction between the fungi used in the experiments. No inhibitory substance, diffusible or indiffusible, is produced by any one of these fungi which could affect the others.

7. A diffusible heat-labile substance (or substances) is produced by bacteria isolated from the pellets which inhibits growth of the phycomycetes in culture at a time comparable with their disappearance from the pellets. It does not affect the ascomycetes adversely, but causes a slight increase in their dry weights, in culture.

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REFERENCES

1. BARNETT, H.L. Illustrated Genera of Imperfect Fungi. Burgess Publishing Co., Minn., 1955.
2. BESSEY. Morphology and Taxonomy of Fungi. Constable and Co., London, 1952.
3. BULLER. Researches of Fungi. Vol. 6, p.169. Longmans, Green and Co., London, 1934.
4. CHIVERS. Mem. Torrey Bot. Club 14, 155, 1915 - Monograph of genera Chaetomium and Ascotricha.
5. CLEMENTS & SHEARS. Genera of the Fungi. H.W. Wilson Co., New York, 1931.
6. COOK, M.T. Am. Journ. Bot. 11, 94, 1924 - Succession of Fungi on Culture Media.
7. DALTON, F.H. & HURWITZ, C. Soil Science 66, 233, 1948 - Effect of volatile disinfectants on survival of microflora in soil.
8. GILMAN, C.A. Manual of Soil Fungi. Iowa State College Press, 1945.
9. GWYNNE-VAUGHAN & BARNES. Fungi. University Press, Cambridge, 1927.
10. LANGE. Flora Agaracina Danica. Vol. 4. Published under the auspices of the Society for the Advancement of Mycology in Denmark and the Danish Botanical Society, 1939.
11. MASSEE & SALMON. Ann. Bot. 15, 313, 1901)
12. " " " " 16, 57, 1902)
    - Researches on Coprophilous Fungi.
13. PORTER, C.L. & CARTER, J.C. Bot. Rev. 4, 165, 1938 - Competition among Fungi.
14. RABENHORST. Kryptogamen Flora, Vols. 2, 8 and 9.
15. REESE, E.T. & LEVINSON, H.S. Physiol. Plant 5, 345, 1952. - A Comparative Study of the Breakdown of Cellulose by Micro-organisms.
16. SACCARDO. Sylloge 4, 579, 1886.
17. SEAVER. North American Cup Fungi (Operculates) Seaver, New York, 1928.

/30.

18. SIU, R.G.H. & REESE, E.T. Bot. Rev. 19, 377, 1953 - Decomposition of Cellulose by Micro-organisms.

19. WAKEMAN, S.A. Soil Science, 2, 103, 1916 - Soil Fungi and their Activities.

A P P E N D I X

Tables 1 - 14, 16 - 24
(15 included in text)

Table 1. Numbers of pellets producing each species.

Species	Time in days																								Max No.
	1	2	3	4	5	7	8	9	10	11	12	14	15	16	17	18	19	21	22	23	24	25	28		
Pilaira anomala	50	50	46	40	35	23	6																		50
Mucor hiemalis	50	50	50	43	42	38	19	17	7																50
Chaetocladium brefeldii		1	1	1	2	2	1																		2
Pilobolus kleinii			4	17	32	32	28	26	7																32
Piptocephalis freeseniana			11	15	18	13	12	6	1	6	2														18
Sphaeronaemella fimicola			3	13	13	13	12	15	13	13	13	13	13	11	10	7	6	4	4	2	2	2	1		15
Didymostilbe sp.			2	5	10	8	12	11	10	10	10	9	7	6	6	5	4	4	4	3	2	2			12
Sordaria macrospora				20	28	45	43	46	44	44	49	48	47	45	45	34	20	15	5	4	1	1			49
Saccobolus violascens					1	1	3	3	11	18	21	20	26	16	6	6	4	4	2	1	1	2			26
Syncephalis cordata						1	3	3	2	2															3
Sordaria minuta							13	23	31	39	43	46	47	46	42	30	30	19	14	7	5	2			47
Coprinus fimetarius								2	3	4	4	4	3	1	1	2	2	1	1						4
Sporormia intermedia									9	20	31	40	40	38	27	19	16	13	13	8	4	4			40
Coprinus ephemerus									1	2	2	3	2	3	4	5	3	8	7	7	8	8	10		10
Stysanus medius											1	1	1	1	1	1	1	1							1
Ascophanus carneus												1	6	10	14	22	29	32	35	39	41	41	40		41
Arthrobotrys superba																		1	1	2	2	3	6		6
Oedocephalum lineatum																		2	7	8	6	5	7		8
Chaetomium spirale																					2	2	2		2
Psilocybe coprophila																					1	1	2		2

$\therefore$ 100% coverage $\equiv$ total of maximum numbers = 418

Index of occurrence

Table 2. ~~% coverage~~ for each class per day. (Fig.1.)

Class	Time in days											
	0	1	2	3	4	5	7	8	9	10	11	12
Phycomycetes	0	23.8	24.1	26.7	27.6	30.7	26.0	16.4	12.4	4.1	1.9	0.5
Ascomycetes	0	0	0	0	4.8	6.9	11.0	14.1	17.1	22.6	28.8	34.3
Basidiomycetes	0	0	0	0	0	0	0	0	0.5	1.0	1.5	1.5
Fungi Imperfecti	0	0	0	1.2	4.3	5.4	5.0	5.7	6.1	5.4	5.4	5.7

Class	Time in days											
	14	15	16	17	18	19	21	22	23	24	25	28
Phycomycetes	0	0	0	0	0	0	0	0	0	0	0	0
Ascomycetes	36.9	39.5	36.9	31.9	26.4	23.6	19.8	16.4	14.1	12.9	12.4	10.0
Basidiomycetes	1.7	1.2	1.0	1.2	1.7	1.2	2.1	1.9	1.7	2.1	2.1	2.9
Fungi Imperfecti	5.4	5.0	4.3	4.1	3.1	2.6	2.9	3.8	3.6	2.9	2.9	3.3

Table 3. Diameters of cultures growing on dung agar, in mms.

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Sporormia intermedia	Lilliputia rufula	Penicillium expansum	Cephalothecium roseum	Stysanus medius
0	3	3	4	4	3	2	2	2
1	3.5	9.5	16	4	3	2	2	2
2	13.5	28	46	10	5	7	9.5	4
3	20.5	43	67	16	14	13.5	15	6.5
4	25.5	57	82	21	20	17	21	9
5	36.5	66.5	92	26	26	20	26	11
7	48.5	78.5		37	36	24	34	16
8	60.5	83.5		39	40	26.5	37.5	17
9	69.5			43.5	45	29.5	40	18
10	75			48	49.5	32	42.5	19
11	80			52	53.5	34.5	45.5	19.5

Table 4. Increase in diameters of cultures grown on dung agar, in mms.
(from Table 3)

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Sporormia intermedia	Lilliputia rufula	Penicillium expansum	Cephalothecium roseum	Stysanus medius
0	0	0	0	0	0	0	0	0
1	.5	6.5	12	0	0	0	0	0
2	10	18.5	30	6	2	5	7.5	2
3	7	15	21	6	9	6.5	5.5	2.5
4	5	14	15	5	6	3.5	6	2.5
5	11	9.5	10	5	6	3	5	2
7	12	12		11	10	4	8	5
8	12	5		2	4	2.5	3.5	1
9	9			4.5	5	3	2.5	1
10	5.5			4.5	4.5	2.5	2.5	1
11	5			4	4	2.5	3	0.5

Table 5. Diameters of cultures grown on agar without cellulose, in mms. (controls)

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula	Penicillium expansum	Cephalothecium roseum
0	4	3	3	4	4	3
1	4	8	17	4	4	3
2	14	29	42	6	10	9
3	25	42	60	12	14	15
4	31	60	75	19	16	22
5	35	68	85	25	20	26
7	54	82		36	24	34
8	62	86		40	28	36.5
9	70			45	31	39
10	74			49	33	42
11	78			53	35	44.5

Table 6. Increase in diameters of cultures grown on agar without cellulose, in mms. (from Table 5)

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula	Penicillium expansum	Cephalothecium roseum
0	0	0	0	0	0	0
1	0	5	14	0	0	0
2	10	21	25	2	6	6
3	11	13	18	6	4	6
4	6	18	15	7	2	7
5	4	8	10	6	4	4
7	19	14		11	4	8
8	8	4		4	4	2.5
9	8			5	3	2.5
10	4			4	2	3
11	4			4	2	2.5

Table 7. Diameters of culture grown on agar plus cellulose, in mms.

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula	Penicillium expansum	Cephalothecium roseum
0	3	3	4	3	2	2
1	3	12	23	3	2	2
2	12	28	48	5	8	7
3	20	44	67	16	15	13
4	26	60	77	22	19	20
5	38	68	87	29	21	25
7	50	82		38	25	35
8	57	90		42	27	38
9	66			46	30	40
10	71			50	33	42
11	76			54	35	44

Table 8. Increase in diameters of cultures grown on agar plus cellulose, in mms. (from Table 7)

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula	Penicillium expansum	Cephalothecium roseum
0	0	0	0	0	0	0
1	0	9	19	0	0	0
2	9	16	25	2	6	5
3	8	16	19	11	7	6
4	6	16	10	6	4	7
5	12	8	10	7	2	5
7	12	14		9	4	10
8	7	8		4	2	3
9	9			4	3	2
10	5			4	3	2
11	5			4	2	2

Table 9. pH values during succession

Time in days	pH
0	7.1
1	7.1
2	7.1
3	7.3
4	7.3
5	7.1
7	7.2
8	6.9
9	7.0
10	7.3
11	7.3
12	7.3
14	7.3
15	7.1
16	7.1
17	7.4
18	7.2
19	7.1
21	7.1
22	7.3
23	7.2

Table 10. Diameters of cultures grown on "exhausted" dung medium, in mms.

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula	Cephalothecium roseum	Penicillium expansum
0	7	4	3	4	6	4
1	7	4	13	4	6	4
2	18	19	34	6	6	4
4	35	40	62	19	19	13
5	41	48	74	27	24	15
6	47	58	80	32	28	17
7	56	64	87	35	31	19
8	60	73	92	37	35	20
9	64	78		42.5	39	22
11	71	85		49	44	24

Table 11. Increase in diameters of cultures grown on "exhausted" dung agar, in mms. (from Table 10)

Time in days	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula	Cephalothecium roseum	Penicillium expansum
0	0	0	0	0	0	0
1	0	0	10	0	0	0
2	11	15	21	2	0	0
4	17	21	28	13	13	9
5	6	8	12	8	5	2
6	6	10	6	5	4	2
7	9	6	7	7	3	2
8	4	9	5	2	4	1
9	4	5		5.5	4	2
11	7	7		6.5	5	2

Table 12. Control weights

Fungus	Weight after 14 days
Mucor hiemalis	102.5 mgms
Pilaira anomala	38.5 "

Table 13. Weights of Phycomycetes grown on medium used by *Mucor hiemalis*, in mgms.

Treatment of medium	Mucor hiemalis	Pilaira anomala
Used + basal ingredients	122	78
Used only	6	10
Fungal medium	6	4

Table 14. Weights of Phycomycetes grown on medium used by *Pilaira anomala*, in mgms.

Treatment of medium	Mucor hiemalis	Pilaira anomala
Used + basal ingredients	138	69
Used only	47	4
Fungal medium	6	4

Table 16. Control weights taken after the first inoculations.

Fungus	Weight after 14 days
Mucor hiemalis	102.5 mgms.
Pilaira anomala	38.5 "
Sordaria macrospora	104.5 "
Lilliputia rufula	28

Table 17. Weights of fungi grown on the medium used by *Mucor hiemalis*, in mgms.

Treatment of medium	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula
Used + basal nutrients	78	122	130	58
Used only	10	6	16	22
Fungal medium	4	6	5	5

Table 18. Weights of fungi grown on the medium used by *Pilaira anomala*, in mgms.

Treatment of medium	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula
Used + basal nutrients	69	138	144	42
Used only	4	47	12	3
Fungal medium	4	6	10	3

Table 19. Weights of fungi grown on the medium used by *Sordaria macrospora*, in mgms.

Treatment of medium	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula
Used + basal nutrients	56	116	130	43
Used only	12	42	10	6
Fungal medium	10	5	8	4

Table 20. Weights of fungi grown on the medium used by *Lilliputia rufula*, in mgms.

Treatment of medium	Pilaira anomala	Mucor hiemalis	Sordaria macrospora	Lilliputia rufula
Used + basal nutrients	65	117	131	35
Used only	41	60	89	19
Fungal medium	3	6	6	5

Table 21. Growth of cultures when grown on a medium containing bacteria, in mms.

Time in days	Mucor hiemalis	Pilaira anomala	Sordaria macrospora	Lilliputia rufula
0	0	0	0	0
1	5	0	11	0
2	13	11	53	5
4	15	17	78	20
5	15	17		25
6	15	17		27
7	15	17		31
8	15	17		35
11	15	17		48

Table 22. Radii of fungal colonies growing towards bacteria, in mms.

Time in days	Mucor hiemalis			Pilaira anomala			Sordaria macrospora			Lilliputia rufula		
	N	R	B	N	R	B	N	R	B	N	R	B
0	0	0	19	0	0	16	0	0	18	0	0	20
2	12	9	11	7	6	12	24	23	0	4	3	16
3	19	13	6	12	9	9	36	36	0	8	5	12
4	24	14	2	16	11	7	48	48	0	10	8	8
5	30	14	1	18	12	6				14	12	5
6	35	14	1	20	12	6				16	15	2
7		14	1	23	12	6				20	18	0
9		14	1	25	12	5				24	22	0

N - normal radius

R - radius of fungus growing towards bacteria

B - distance between fungus and bacteria

Table 23. Diameters of fungi growing on media containing bacterial filtrate, in mms.

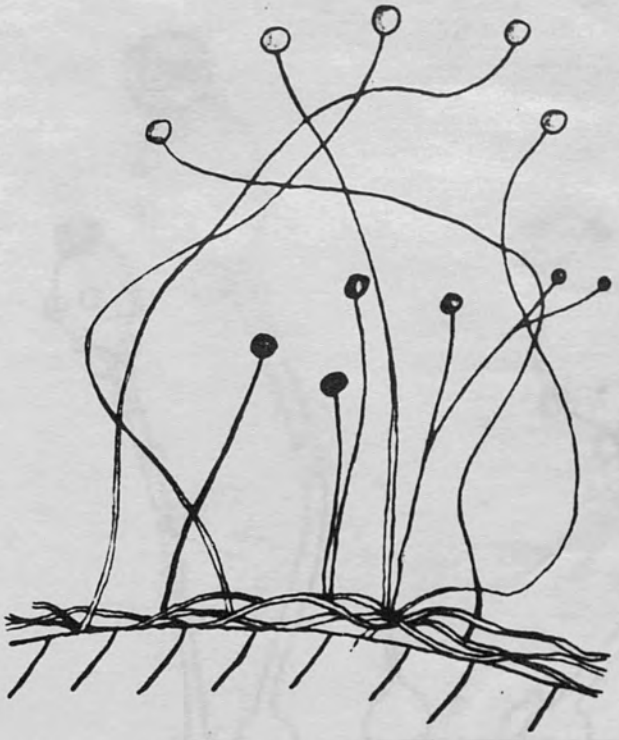
Time in days	Mucor hiemalis	Pilaira anomala	Sordaria macrospora	Lilliputia rufula
0	0	0	0	0
1	6	0	11	0
2	13	0	35	4
4	30	20	81	19
5	38	28		26
6	42	33		33
7	50	41		38
8	53	44		43
9	55	52		49
11	63	59		59

Table 24. Change in weight on medium containing bacterial filtrate.

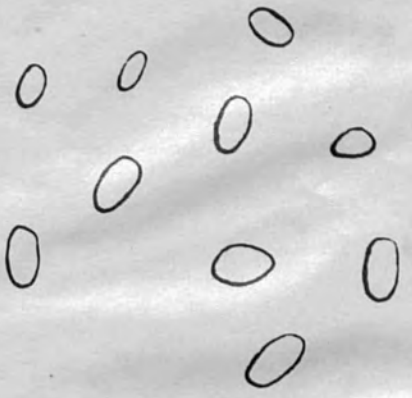
Fungus	Weight on bacterial medium	Weight on untreated medium	Change in weight	Percentage change
Mucor hiemalis	65 mgms.	102.5 mgms.	37.5 mgms. loss	36.5 % loss
Pilaira anomala	17 "	38.5 "	21.5 " "	55.9 % "
Sordaria macrospora	112.5 "	104.5 "	8.0 " gain	7.6 % gain
Lilliputia rufula	30.5 "	28 "	2.5 " "	8.9 " "

ILLUSTRATIONS

Mucor hiemalis

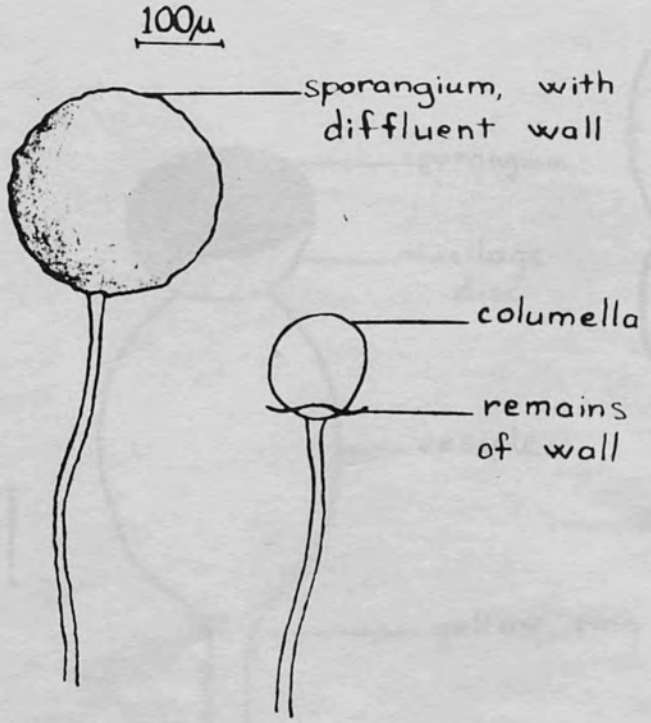


SPORANGIOPHORES

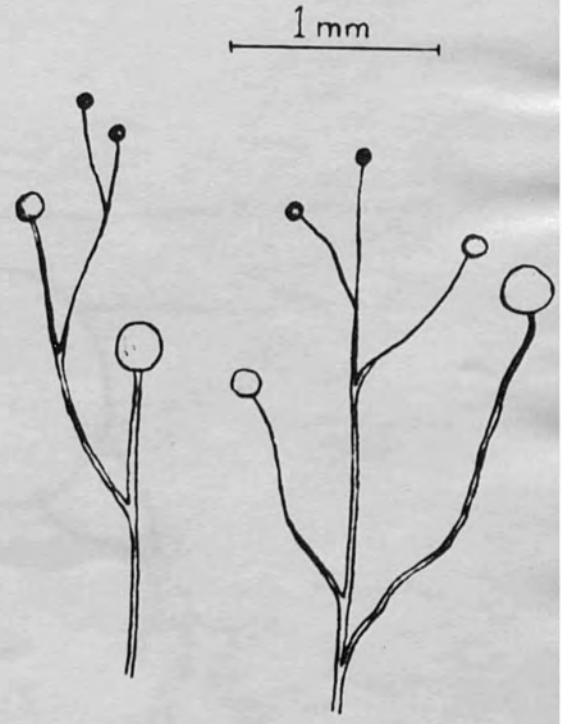


SPORES

7μ x 5μ



SPORANGIA

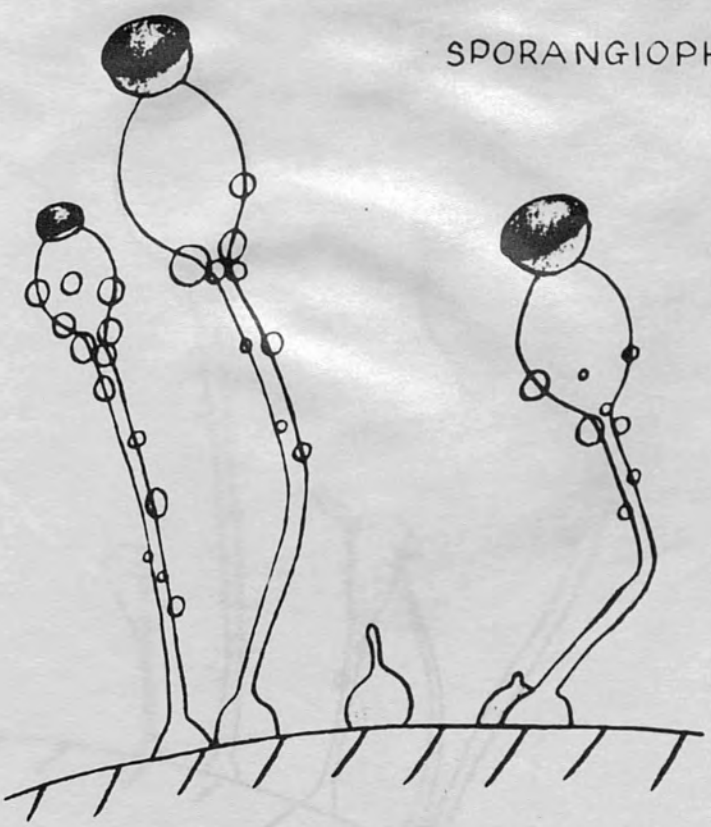


HABIT

Pilobolus crystallinus

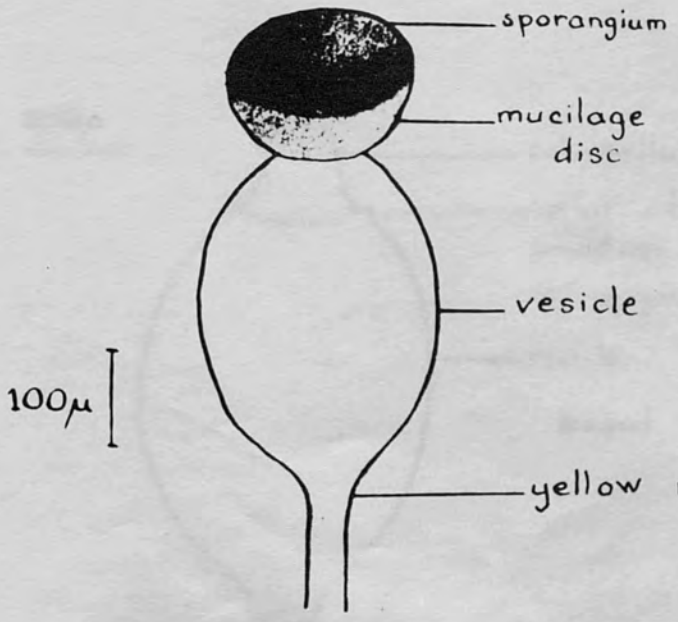
100μ

SPORANGIOPHORES

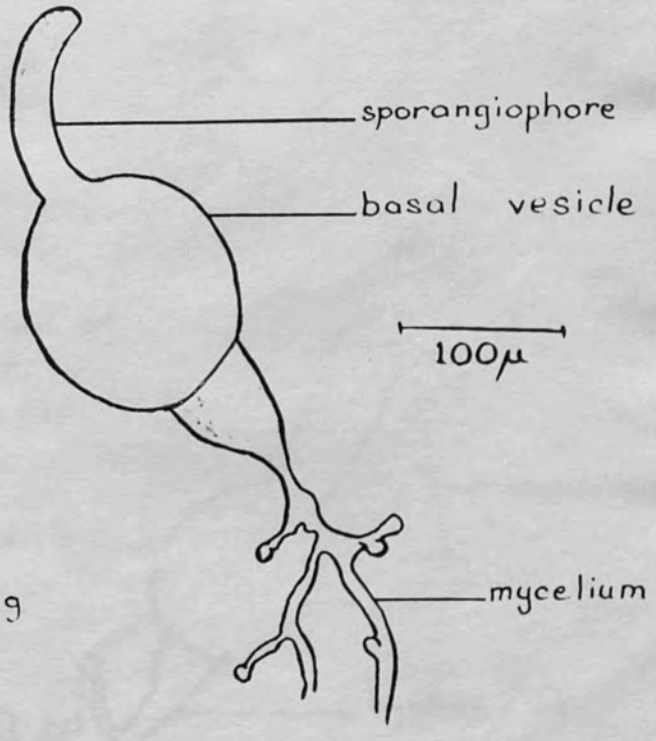


SPORES

8μ × 6μ

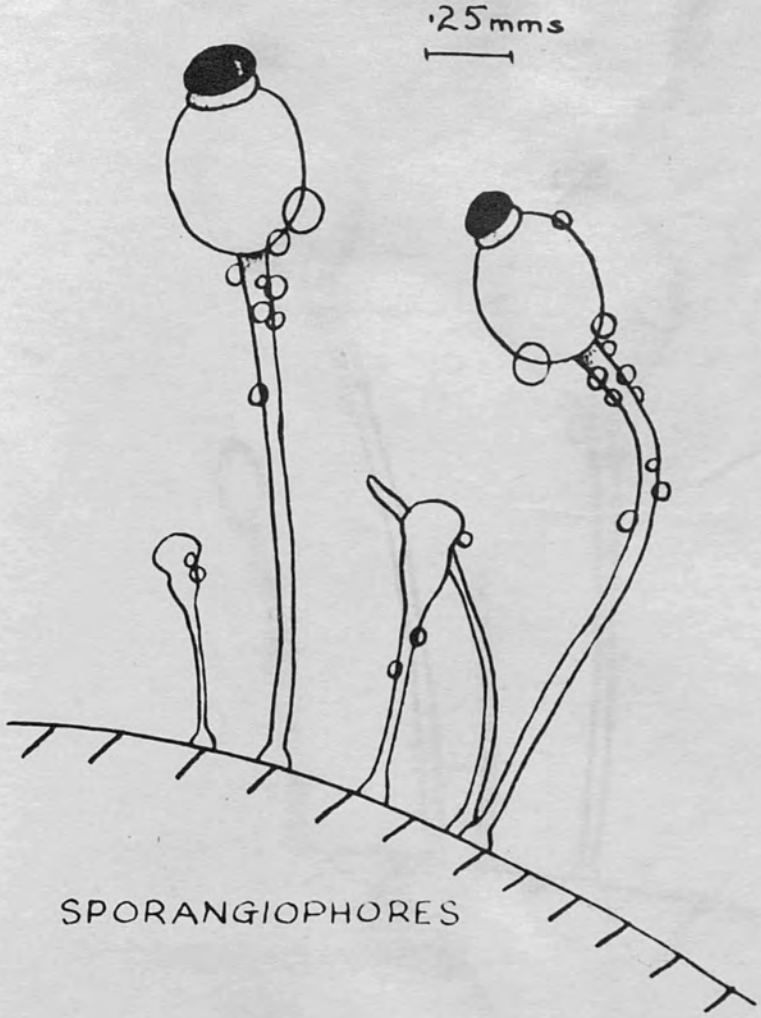


SPORANGIUM



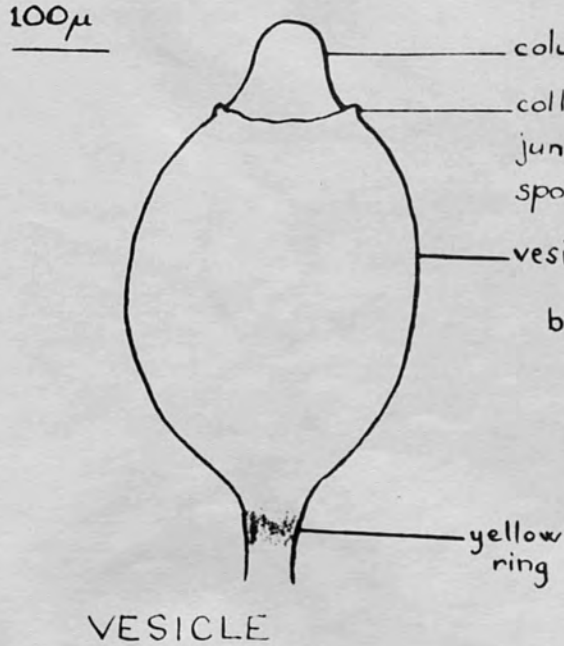
YOUNG SPORANGIOPHORE

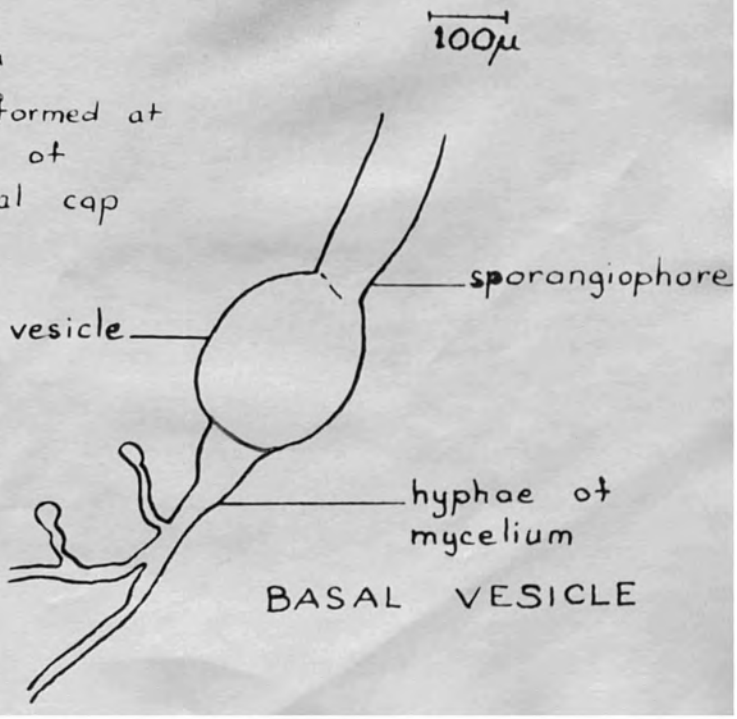
Pilobolus kleinii



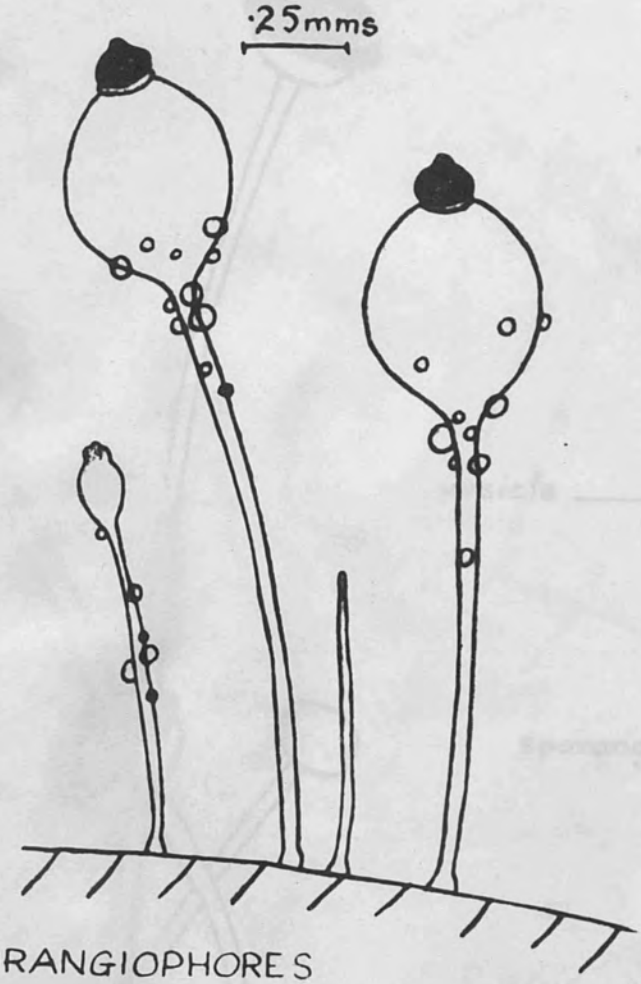
SPORES

10μ × 8μ



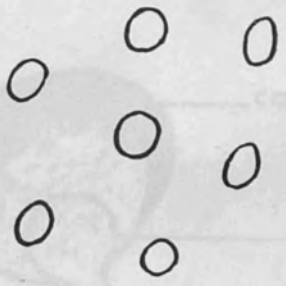


Pilobolus umbonatus



SPORANGIOPHORES

SPORES



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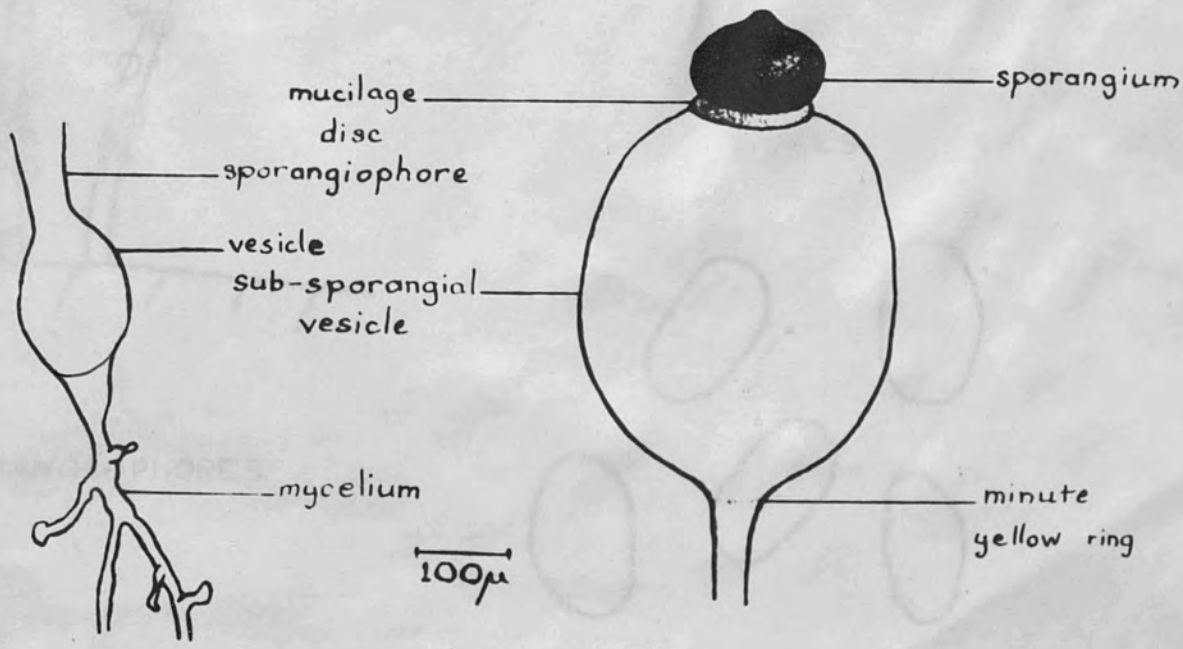
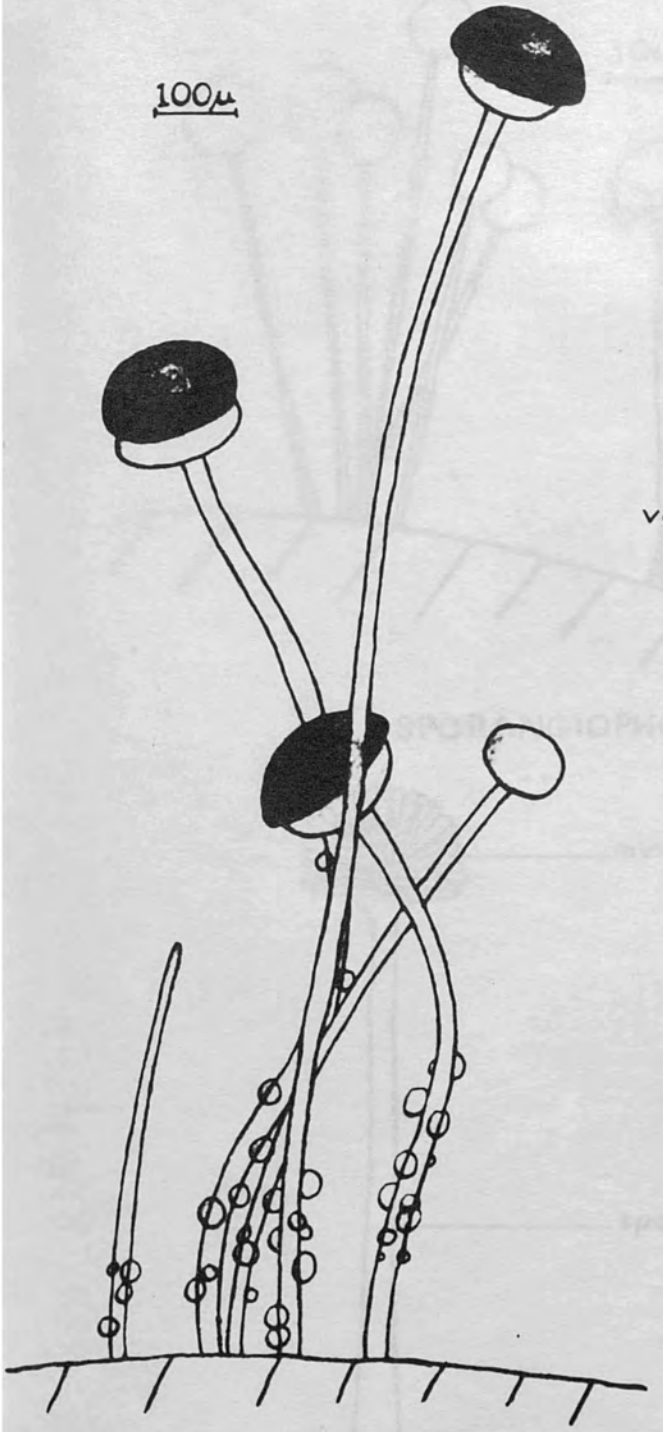


BASAL VESICLE

SPORANGIUM

Pilaira anomala



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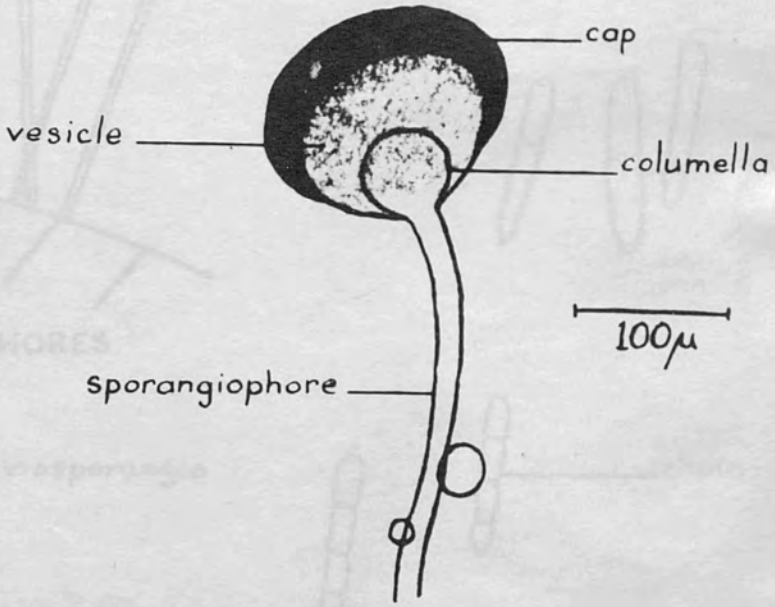

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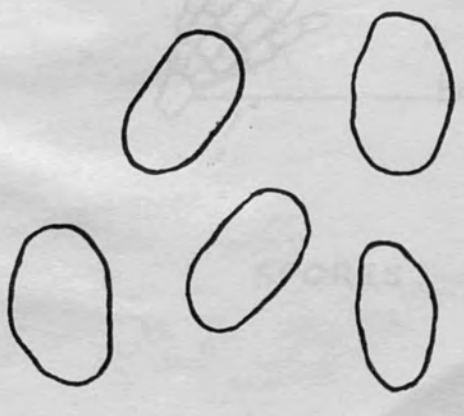
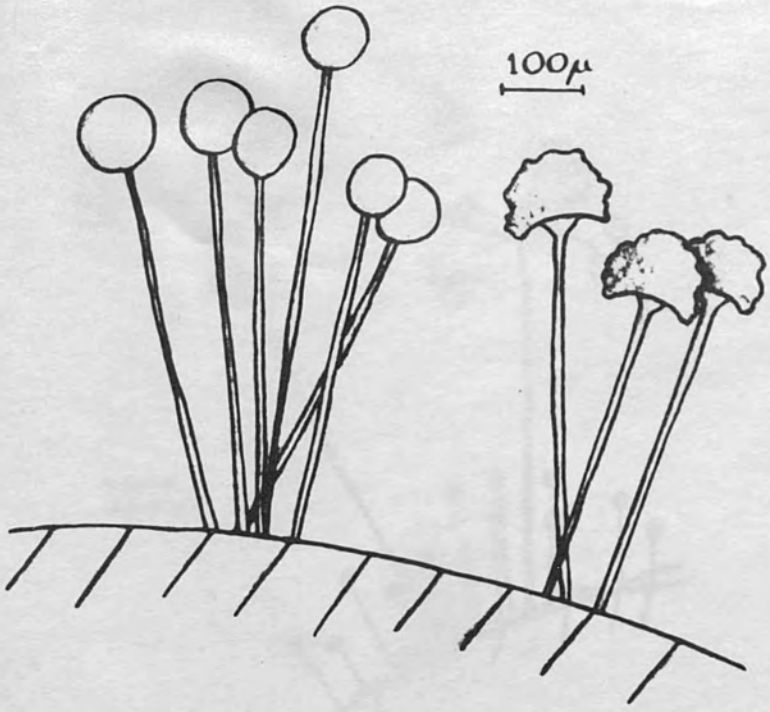


9µ x 5µ

Syncephalis sphaerica



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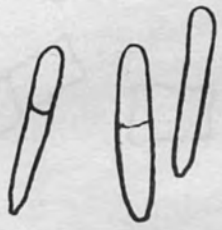
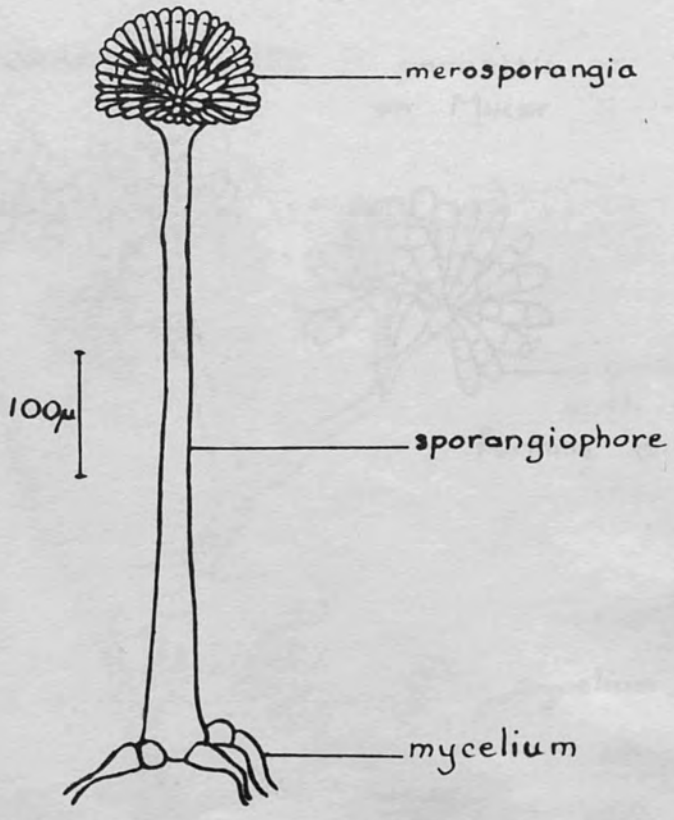
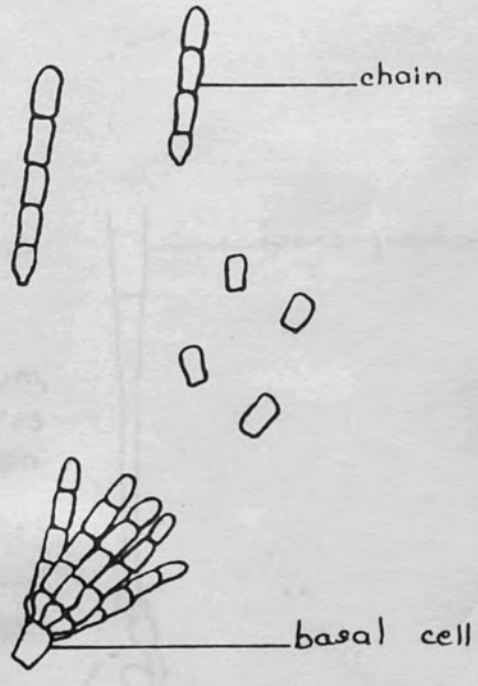
MEROSPORANGIA





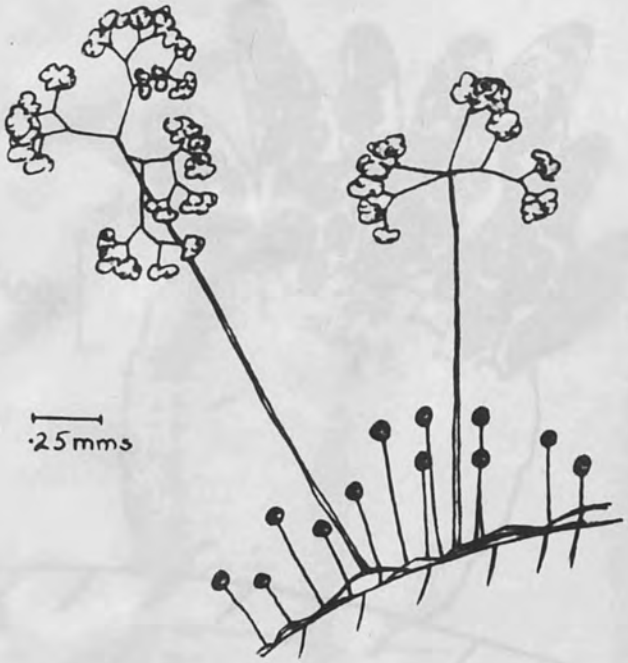
SPORANGIOPHORE



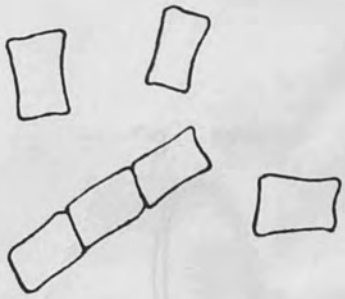
SPORES

8μ × 3μ

Piptocephalis freseniana

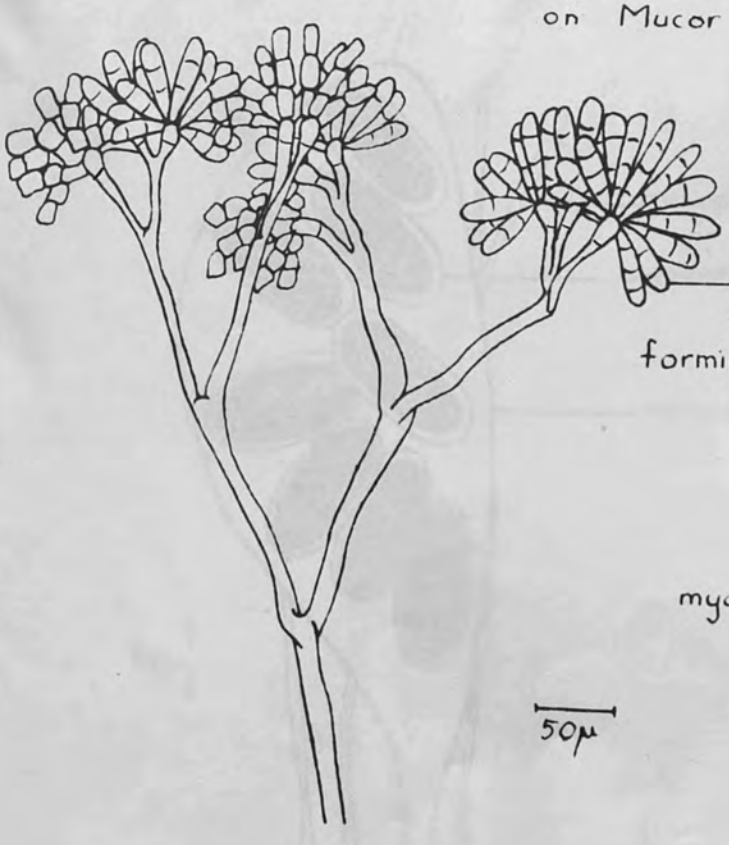


SPORES



5μ × 3μ

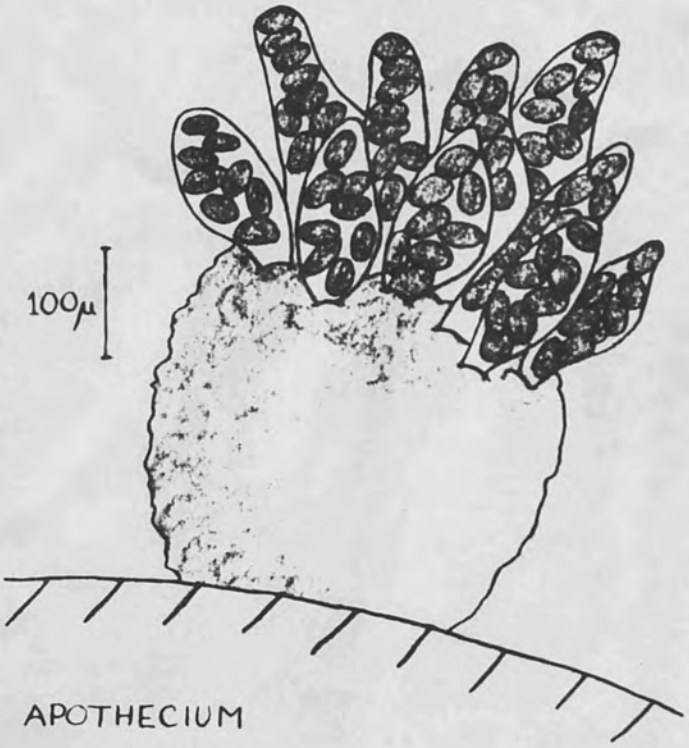
SPORANGIOPHORES — parasitic on Mucor



SPORANGIOPHORE

MYCELIUM

Ascobolus immersus

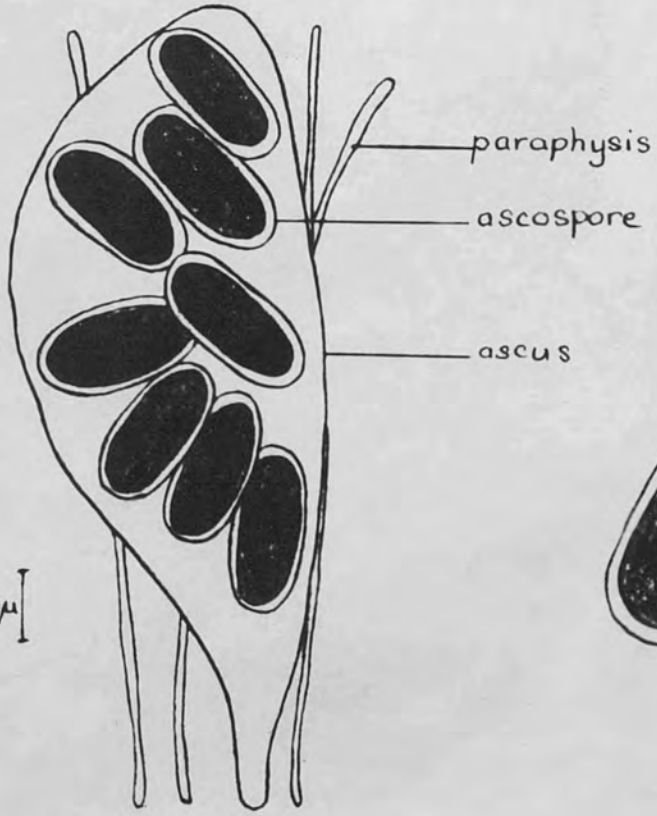


APOTHECIUM

young spores

SPORES

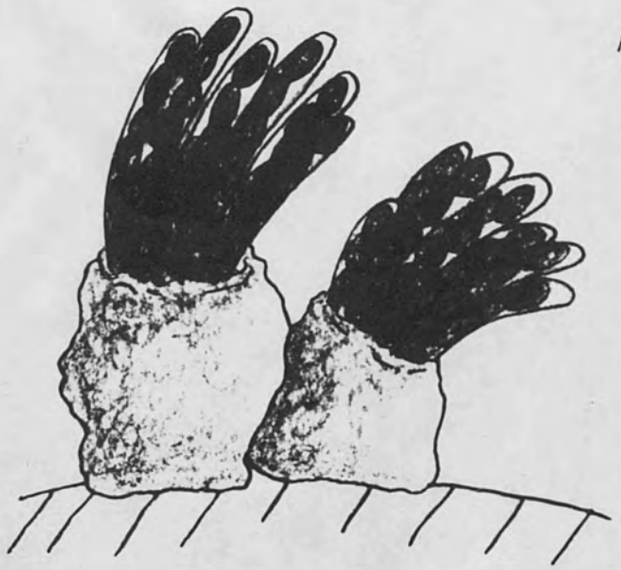
58μ x 26μ



ASCUS

old spores

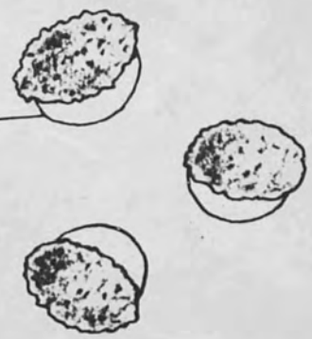
Ascobolus leveillei



APOTHECIA

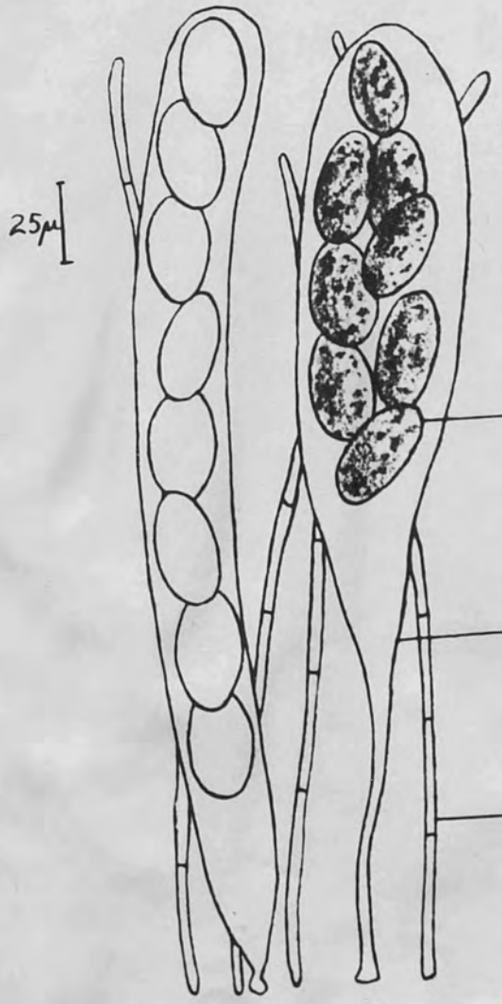
100μ

SPORES



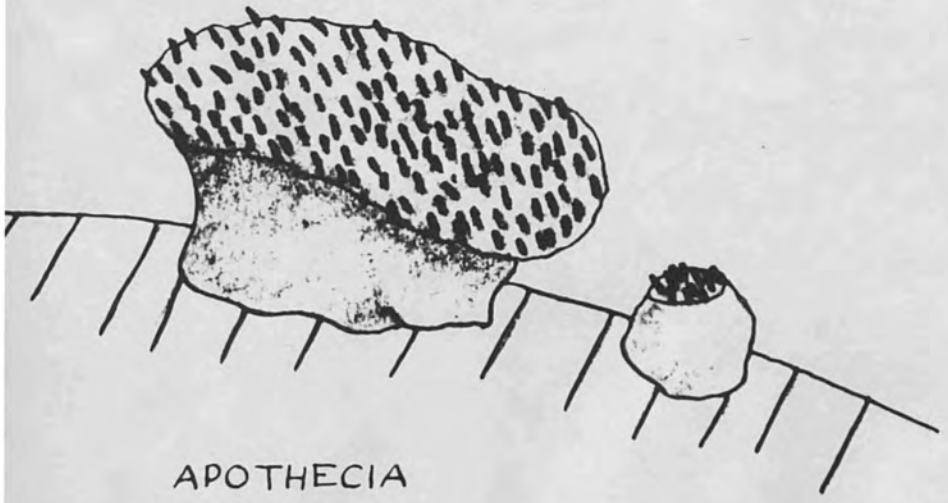
24μ × 14μ

ASCI



Ascobolus stercorarius

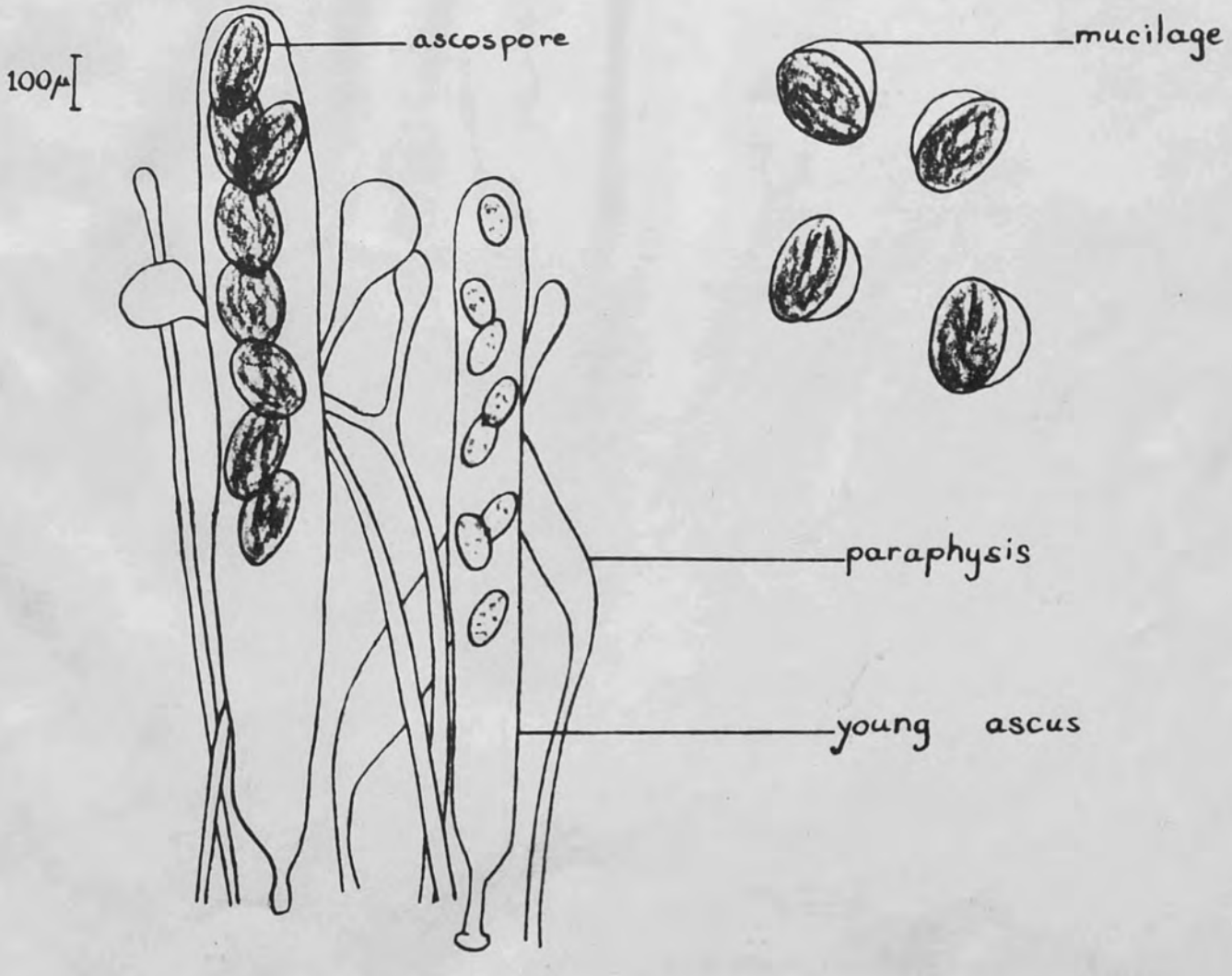
·25mms



APOTHECIA

SPORES

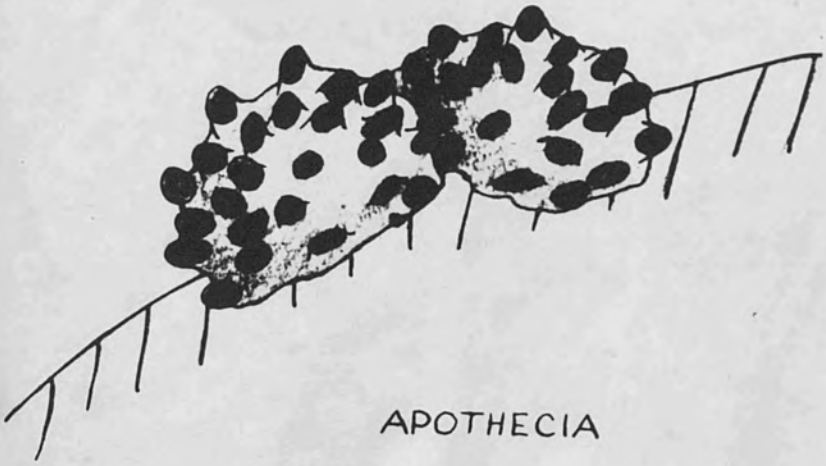
20μ × 11μ



ASCI

Saccobolus violascens

100μ

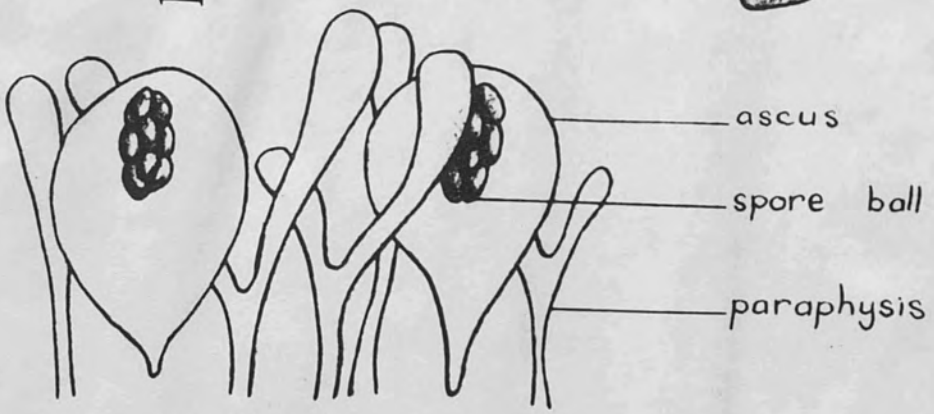


APOTHECIA

SPORES

10μ x 6μ

10μ

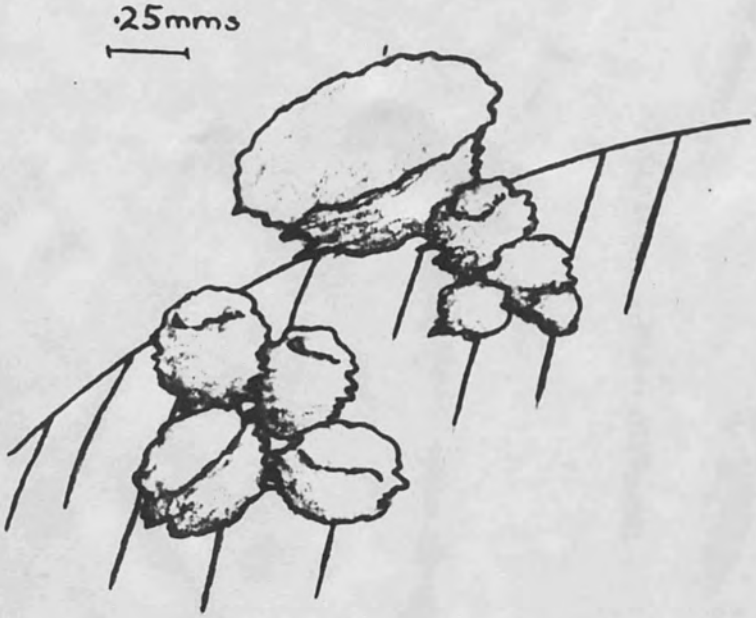


ASCI

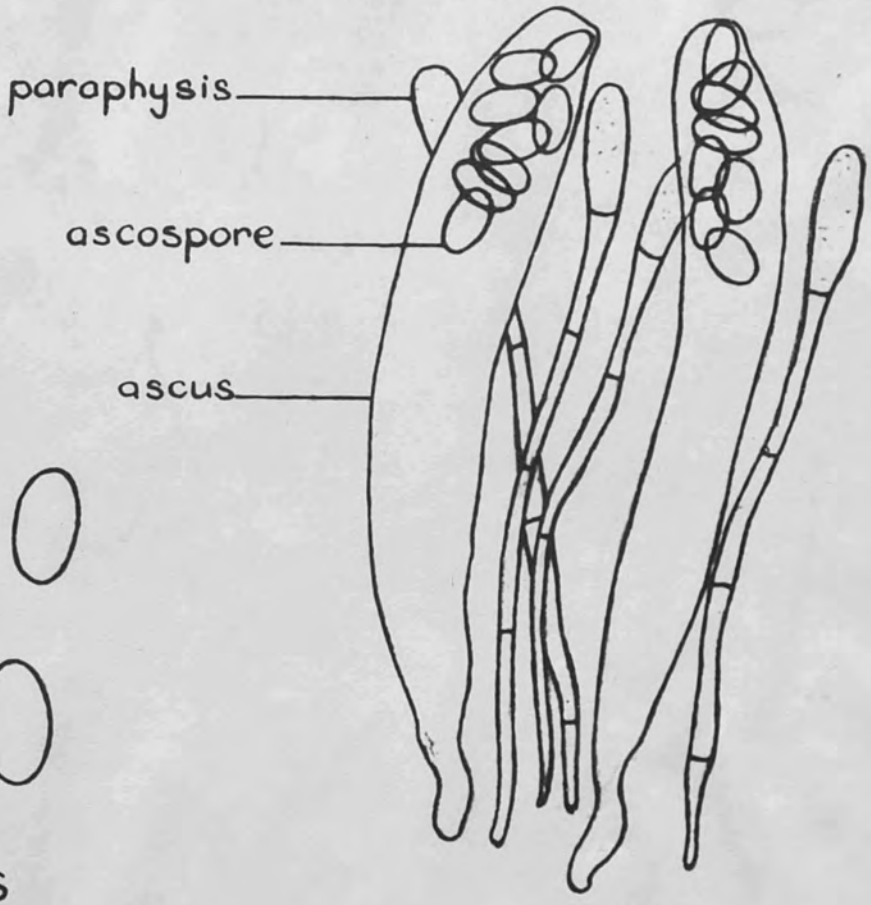
10μ

SPORE BALLS

Ascophanus carneus



APOTHECIA

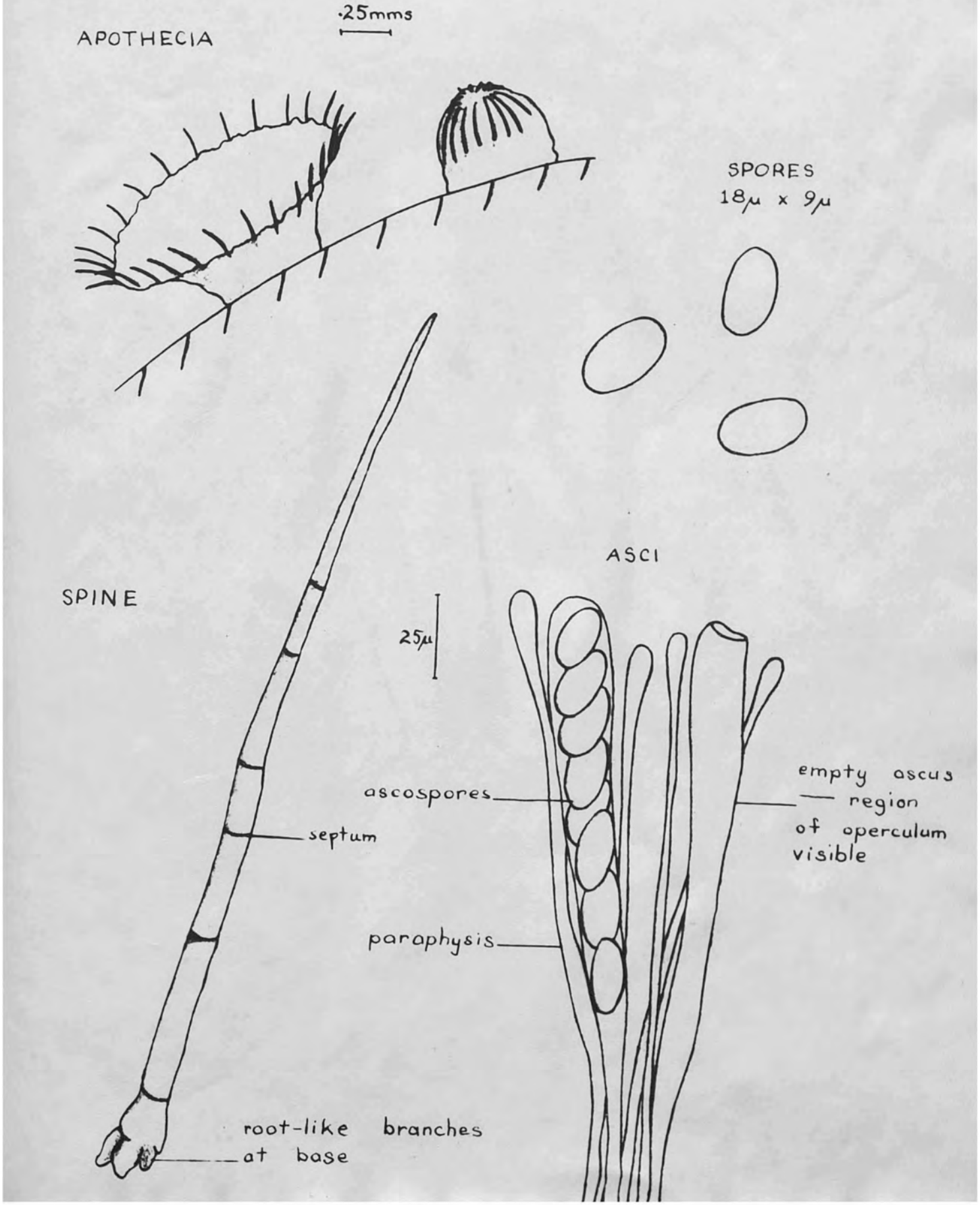


SPORES

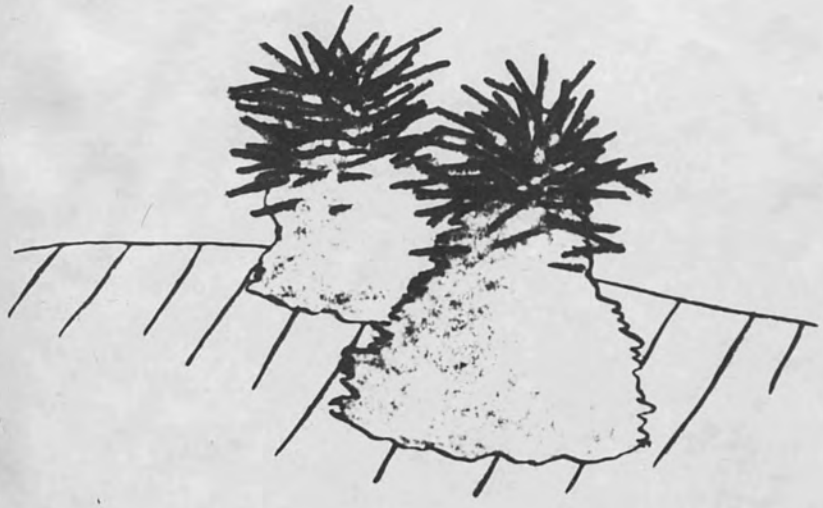
17μ x 11μ

ASCI

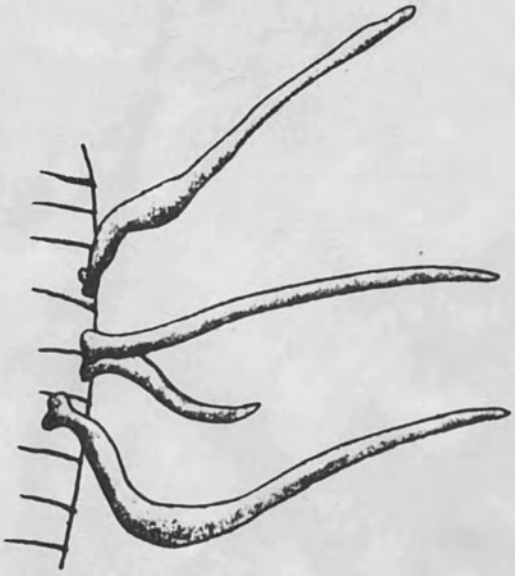
Patella coprinaria



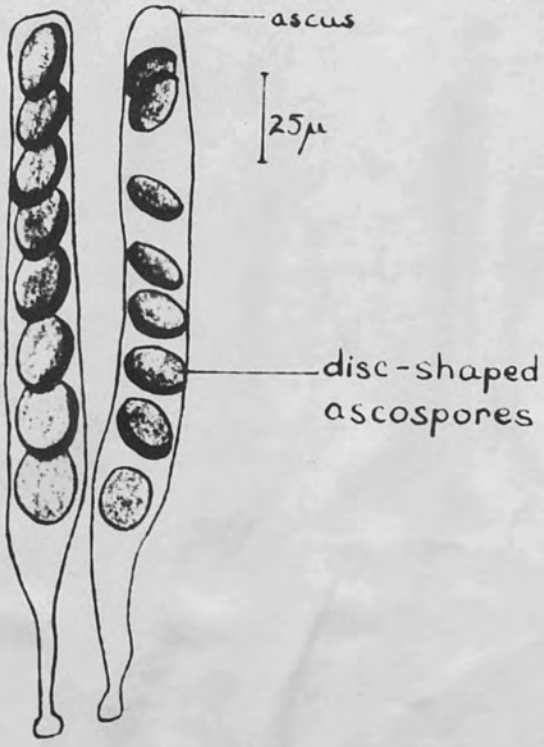
Sordaria discospora



PERITHECIA



SPINES

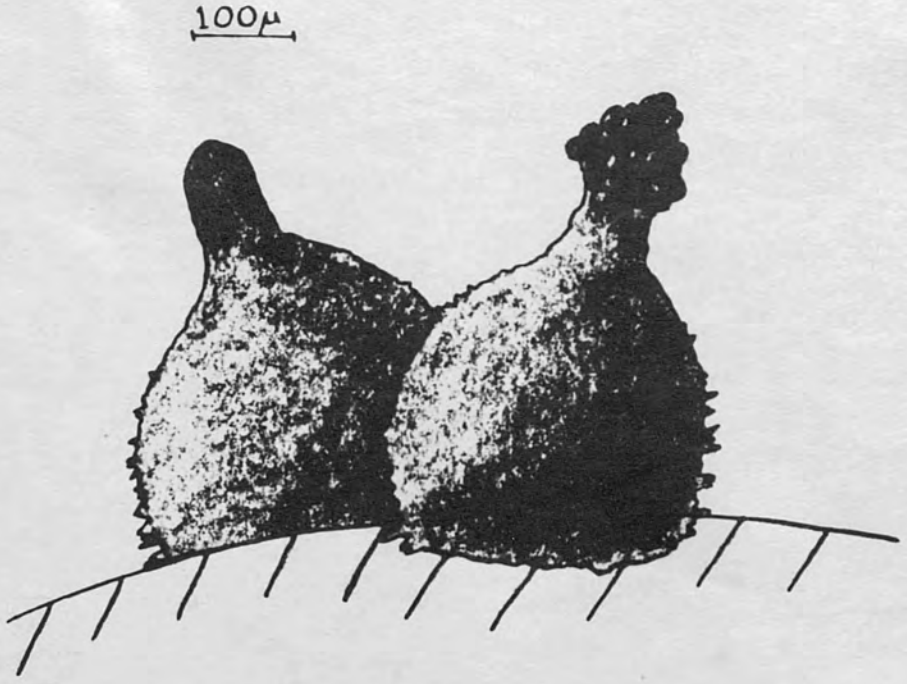


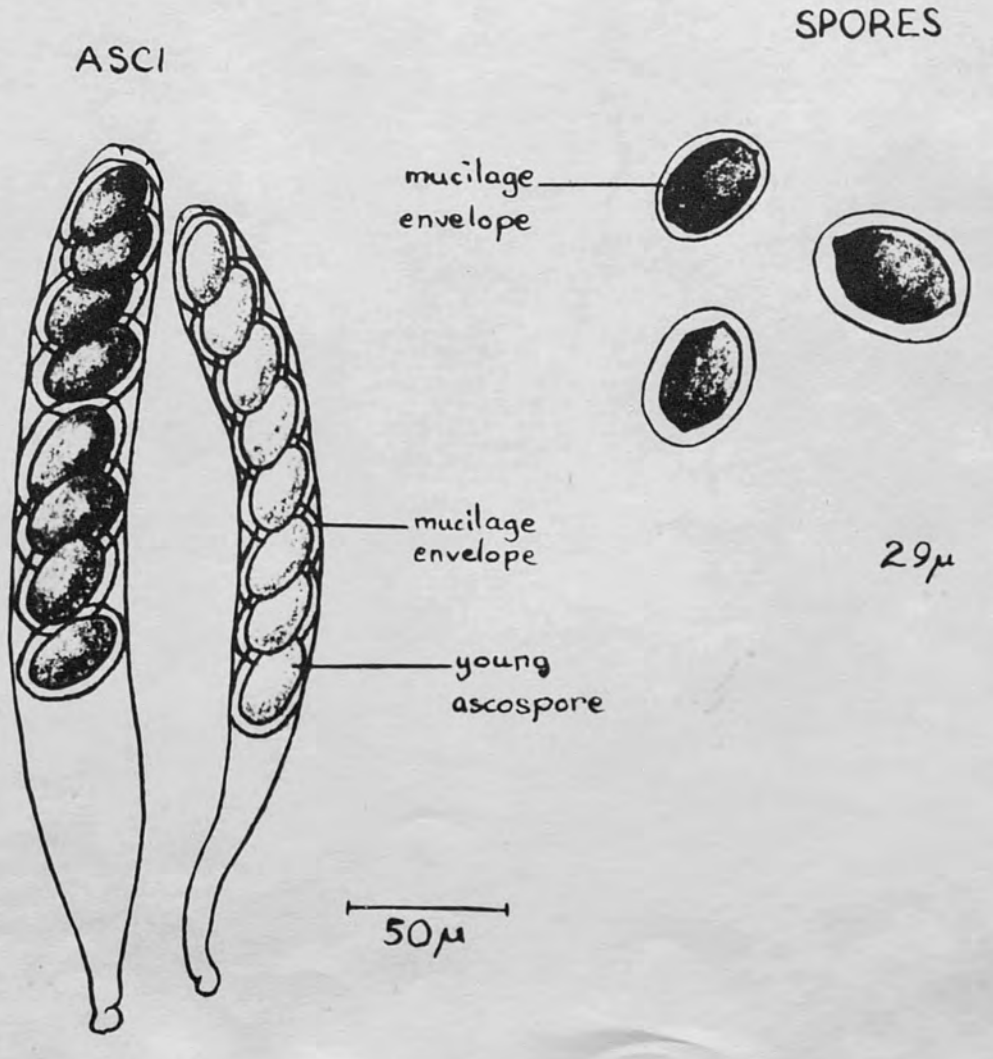
ASCI

SPORES

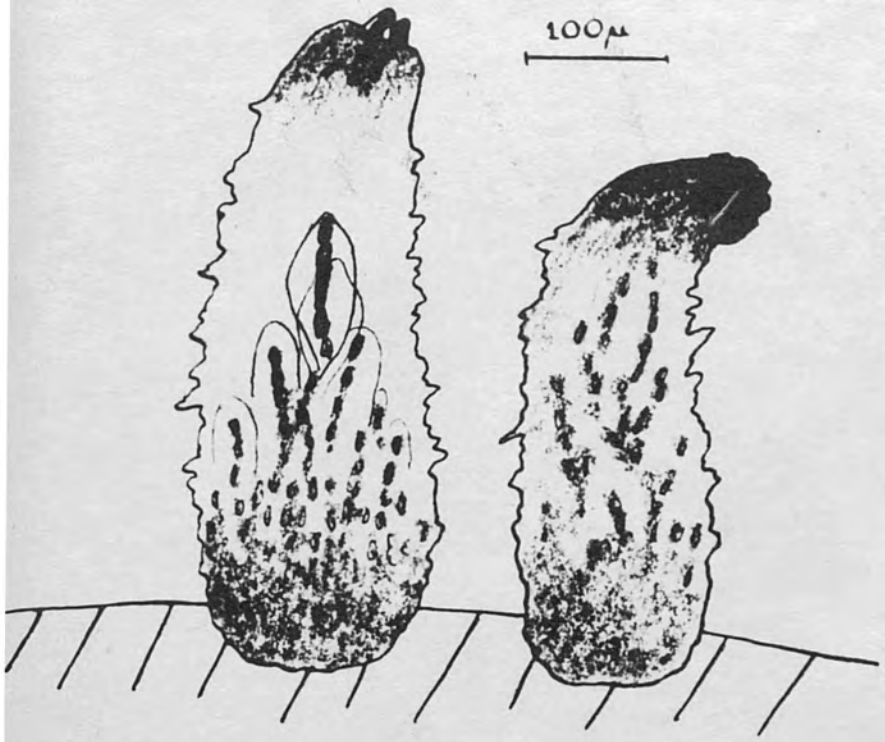
19μ × 15μ

Sordaria macrospora





Pleurage minuta

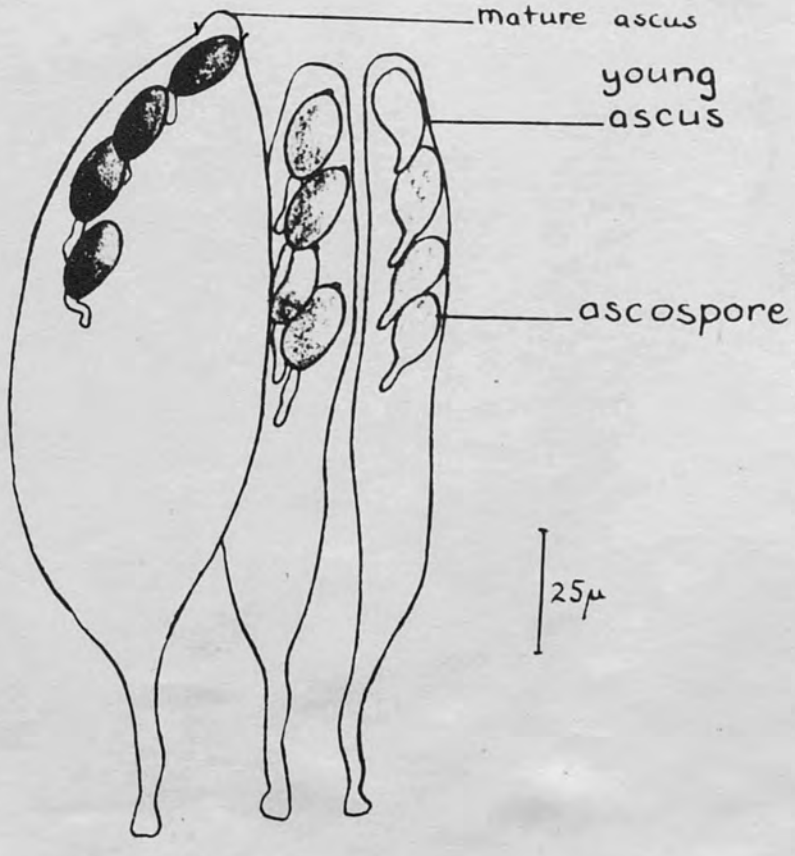


PERITHECIA

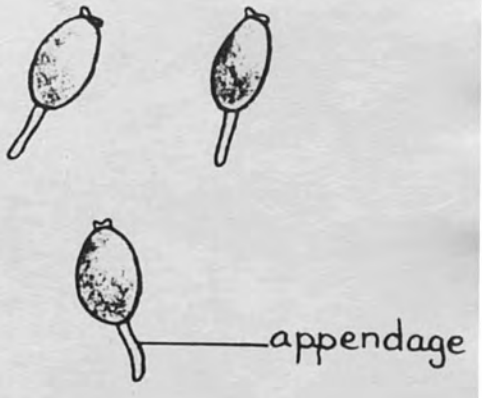
SPORES

20μ × 11μ

mature

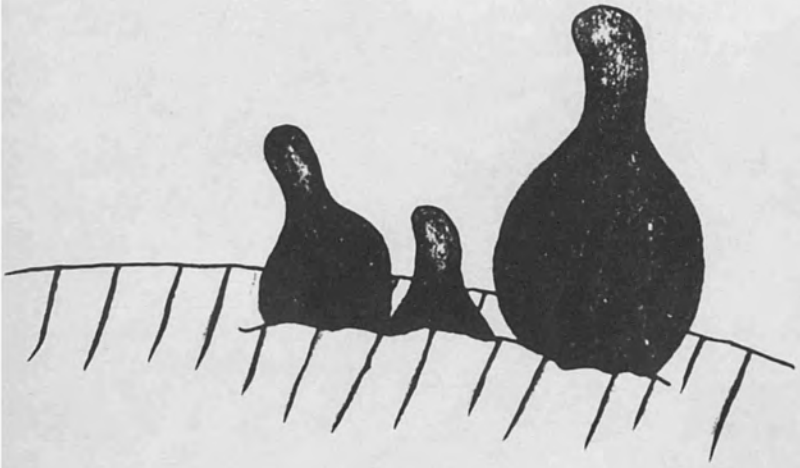


ASCI



young

Sporormia intermedia



50μ

PERITHECIA

extended ascus

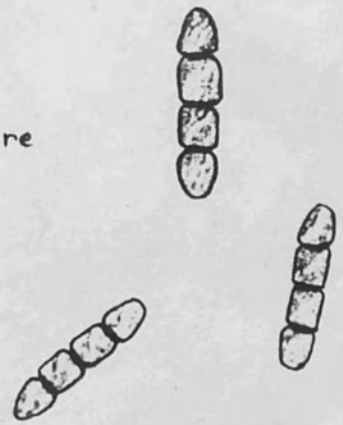
25μ

ascospore

tunic

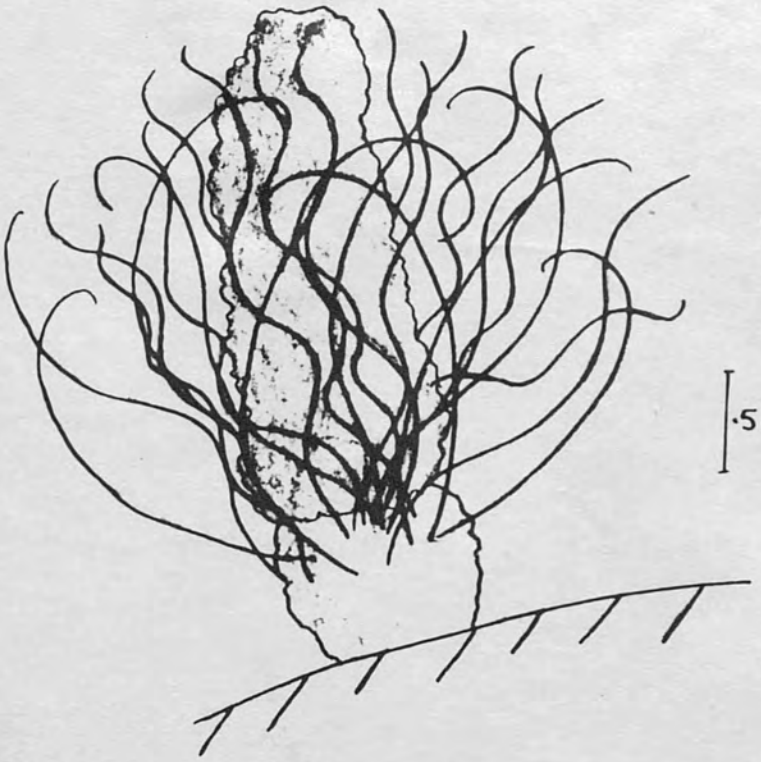
SPORES

38μ x 7μ



TUNICATE ASCI

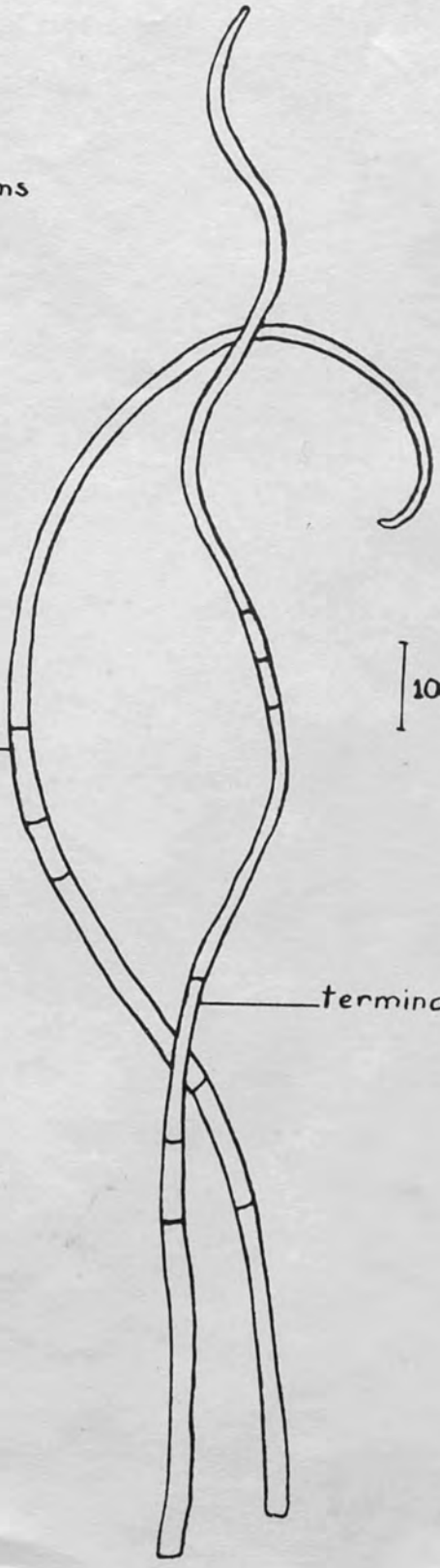
Chaetomium globosum



.5mms

PERITHECIUM

HAIRS



100μ

lateral

terminal

ascospore

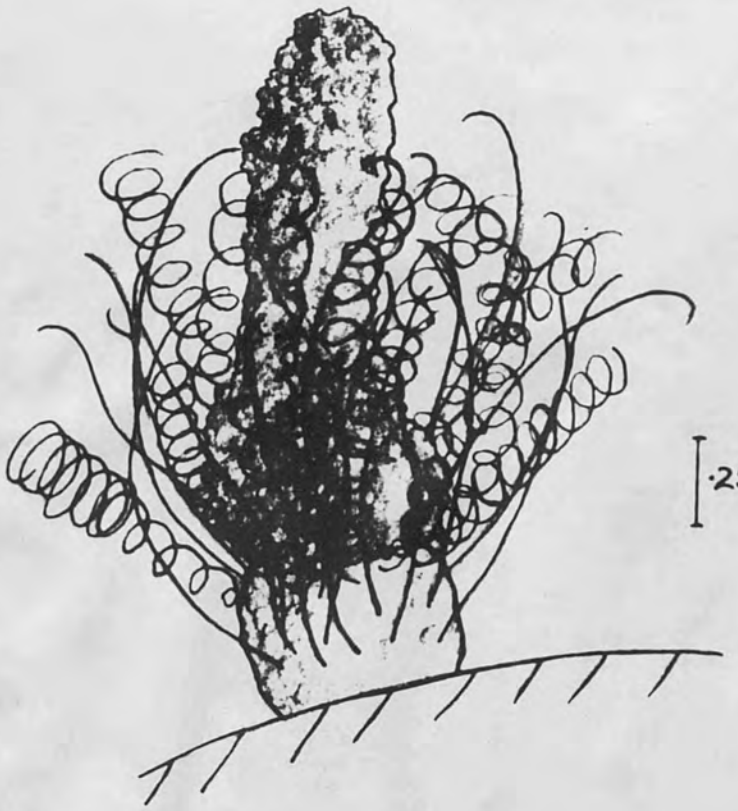
10μ

ASCUS

SPORES

12μ x 9μ

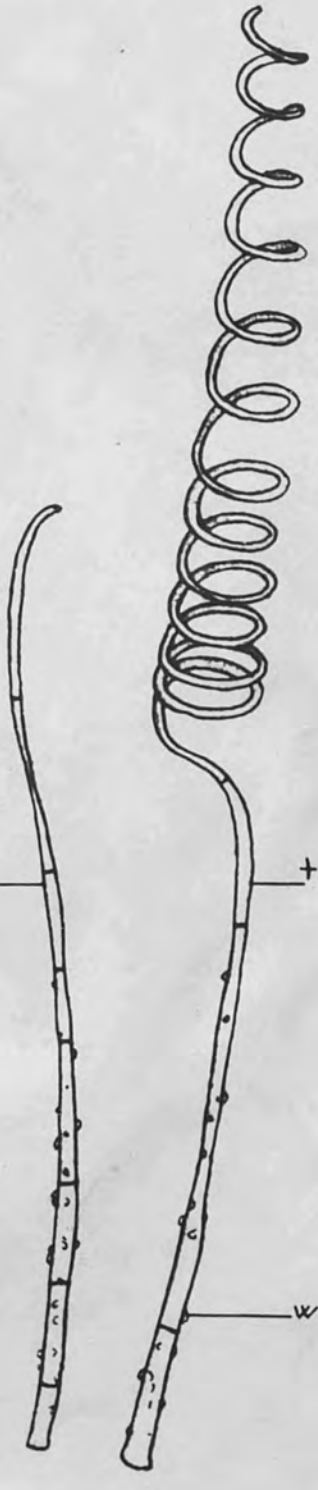
Chaetomium spirale



.25mms

PERITHECIUM

HAIRS

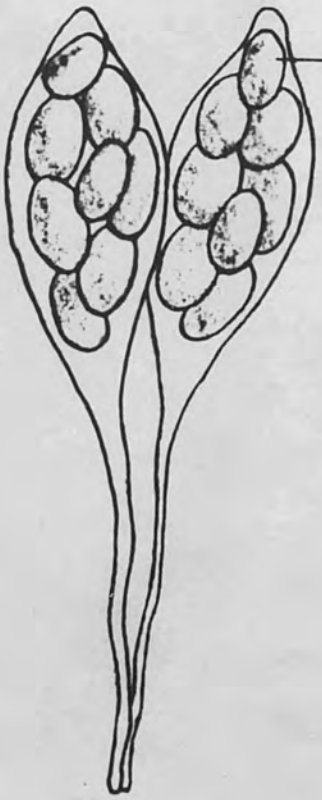


50μ

lateral

terminal

warts



ascospore

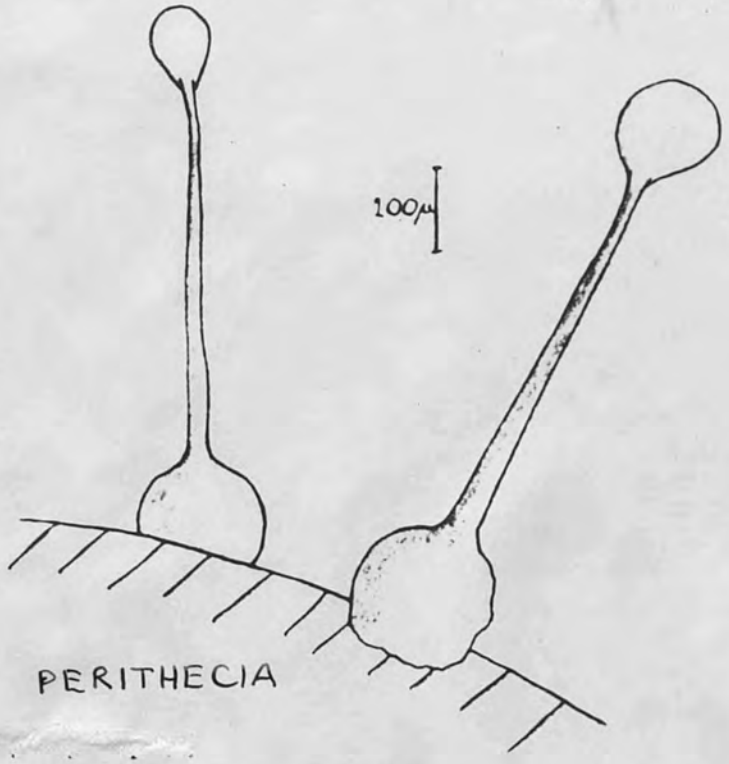
10μ

ASCI

SPORES

7μ × 5μ

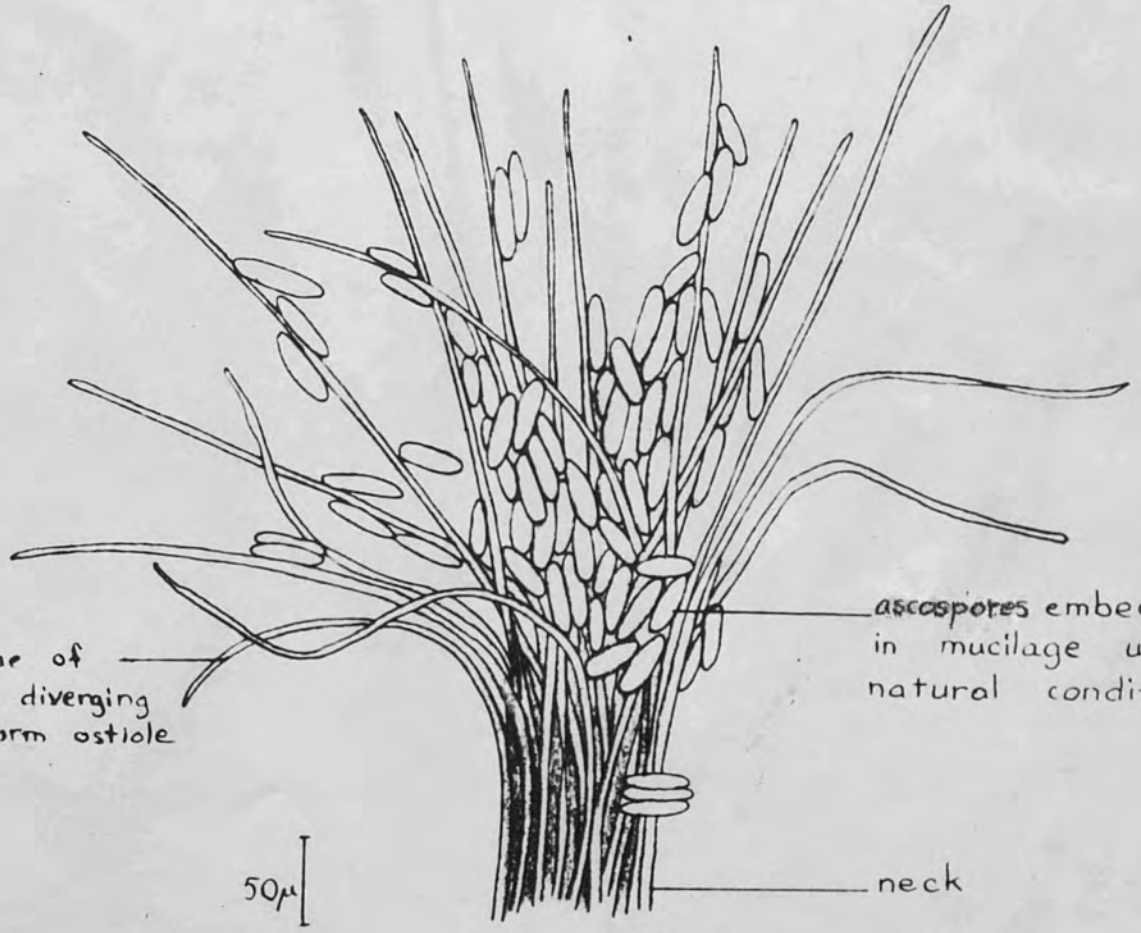
Sphaeronaemella fimicola



PERITHECIA

ASCOSPORES

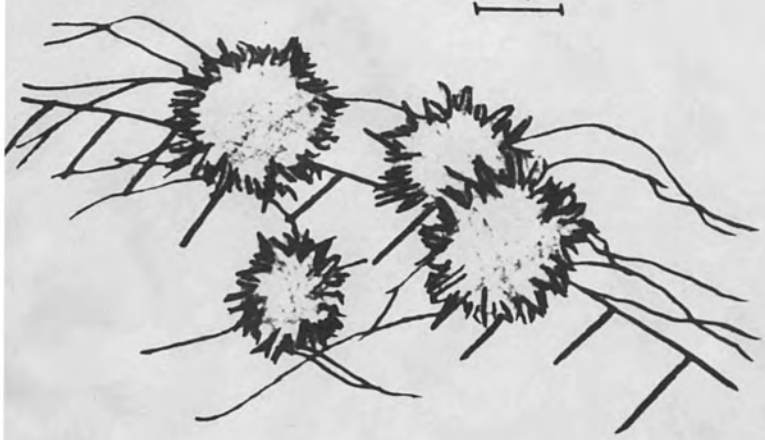
8μ x 2·5μ



NECK and OSTIOLE

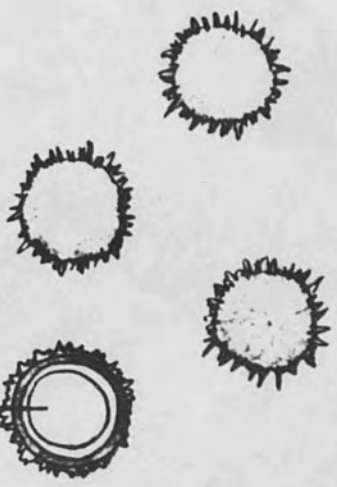
Lilliputia rufula

50μ

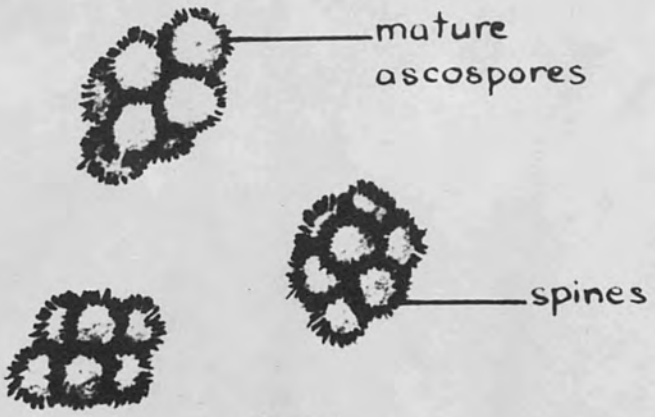


CLEISTOTHECIA

SPORES



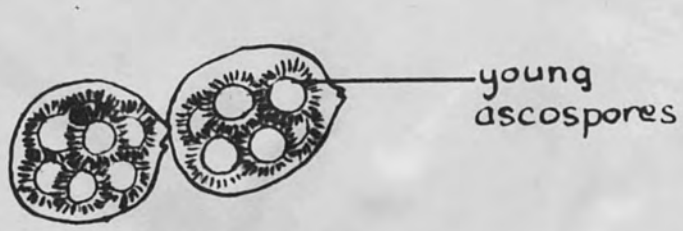
ASCOSPORE GROUPS



19μ x 19μ

25μ

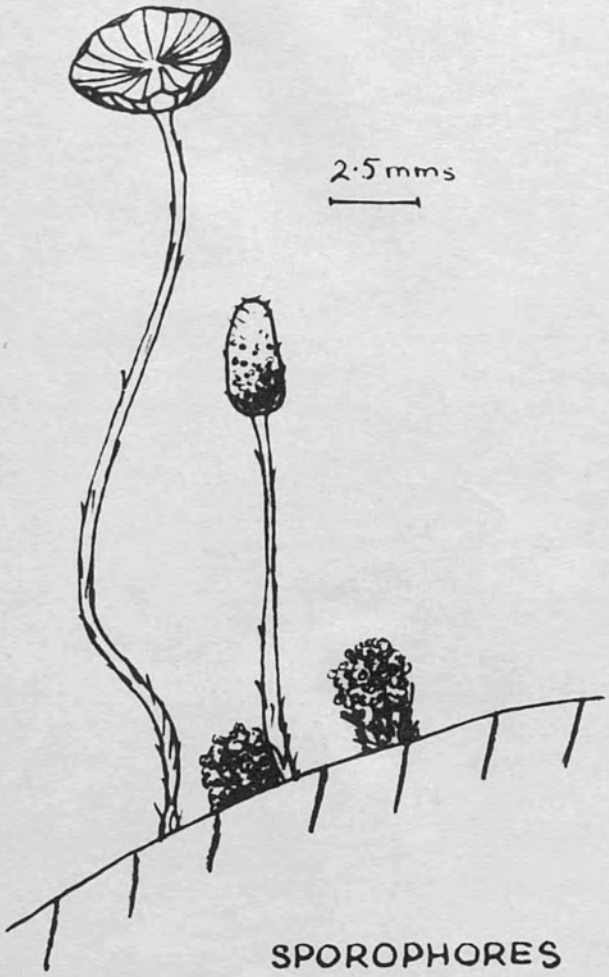
IMPERFECT STAGE



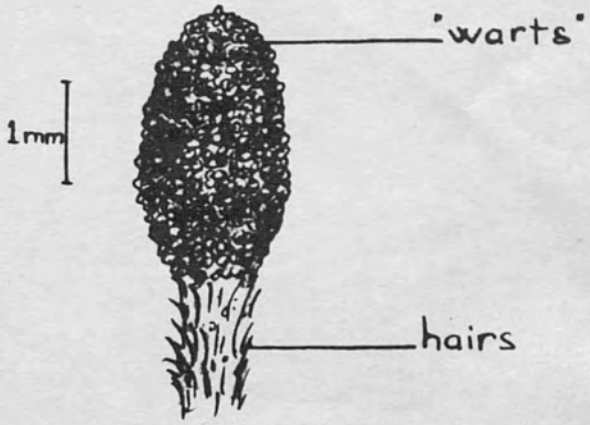
Gliocladium penicillioides

ASCI

Coprinus ephemerus

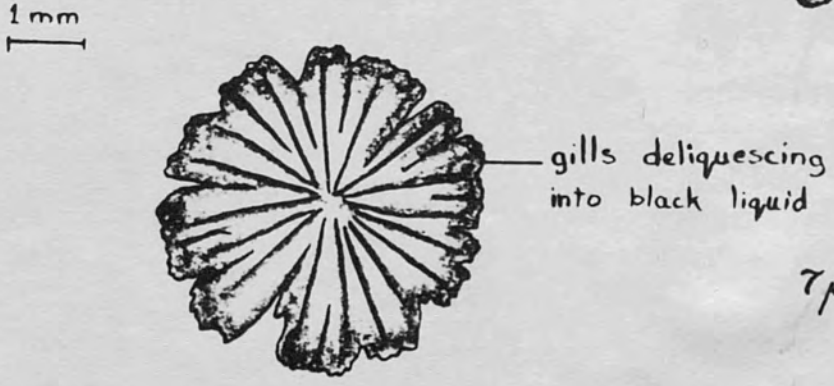


SPOROPHORES



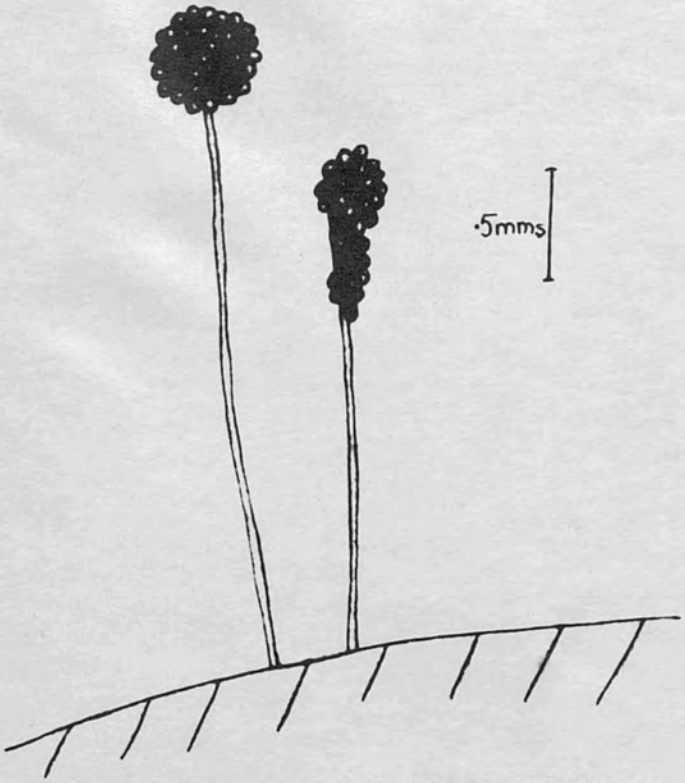
SPORES

7μ × 4μ



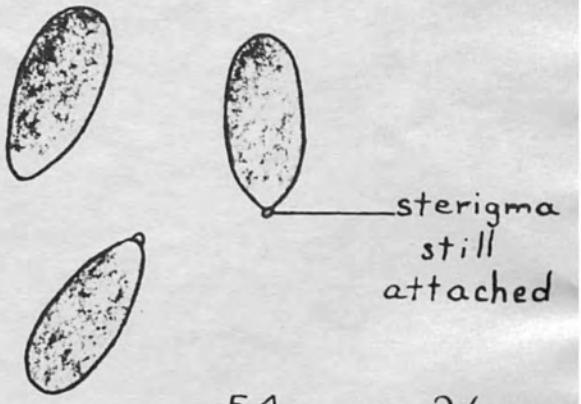
ARRANGEMENT OF GILLS

Rhopalomyces elegans

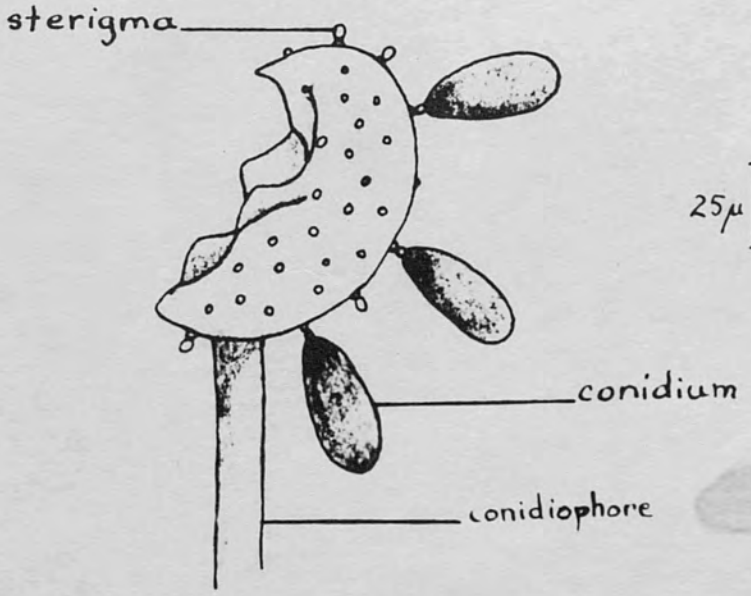


CONIDIOPHORES

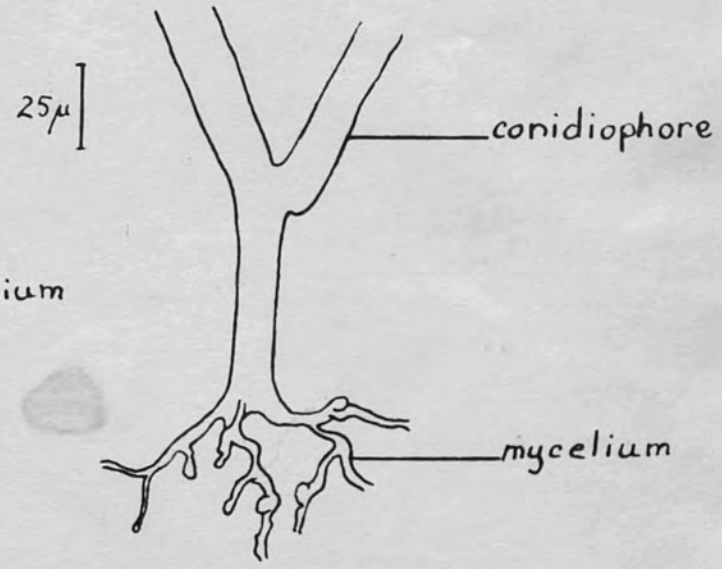
CONIDIA



54μ x 26μ

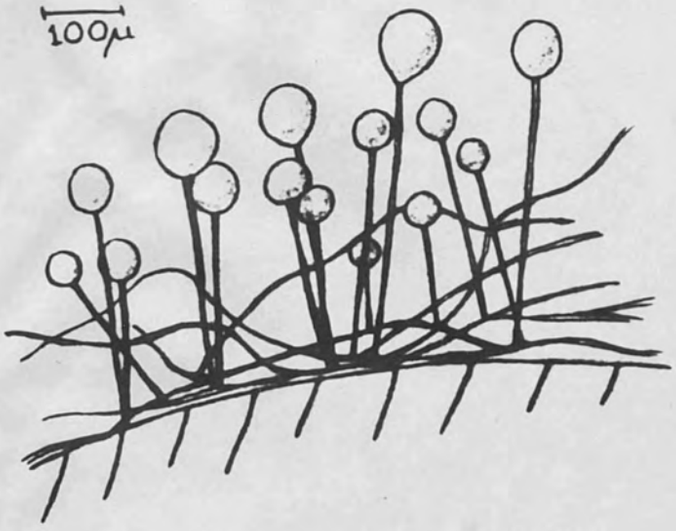


CONIDIOPHORE HEAD



CONIDIOPHORE BASE

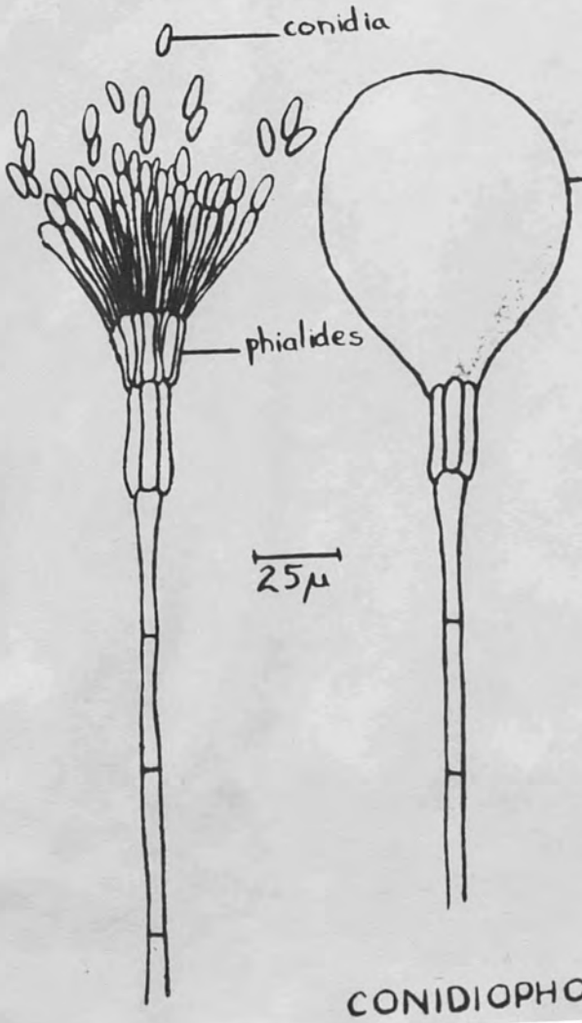
Gliocladium penicillioides



CONIDIOPHORES

CONIDIA

7μ × 3μ

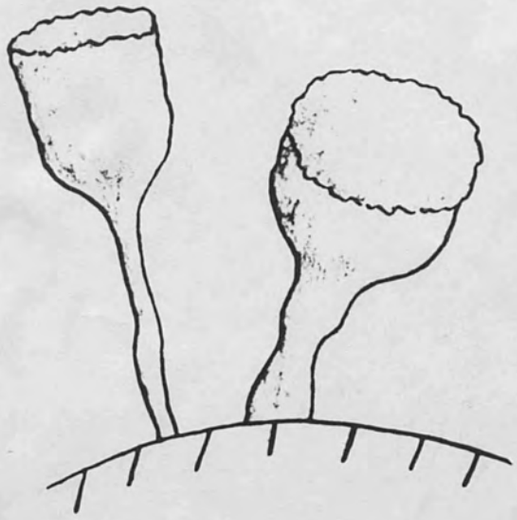


CONIDIOPHORES

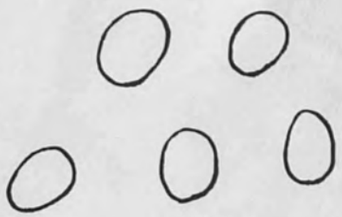
PERFECT STAGE

Lilliputia rufula

Penicillium expansum

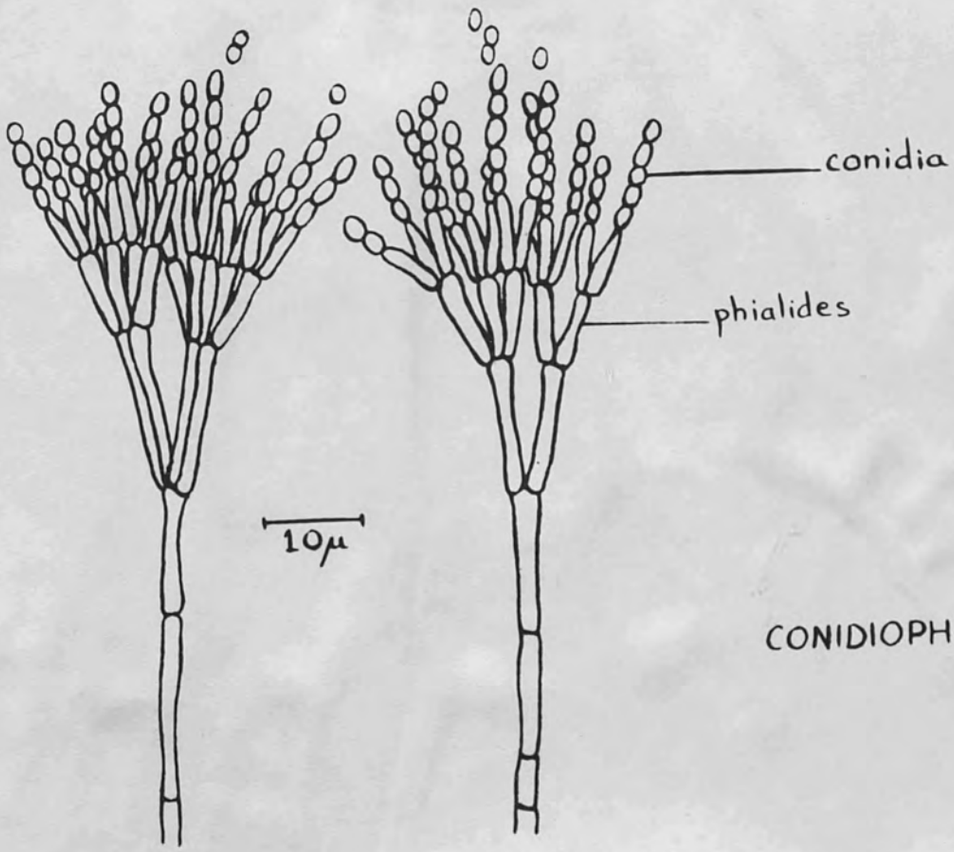


COREMIA



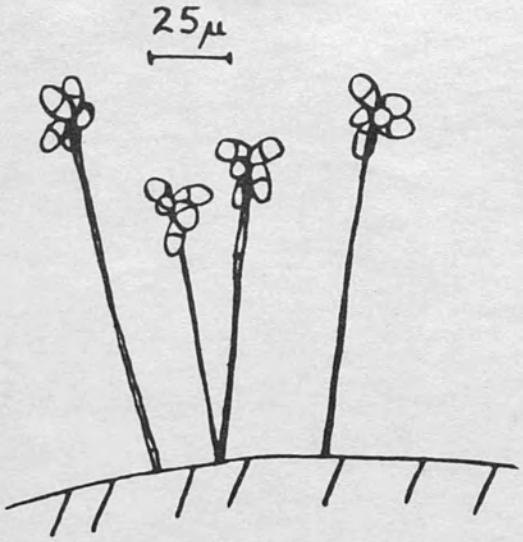
CONIDIA

3μ × 2·5μ



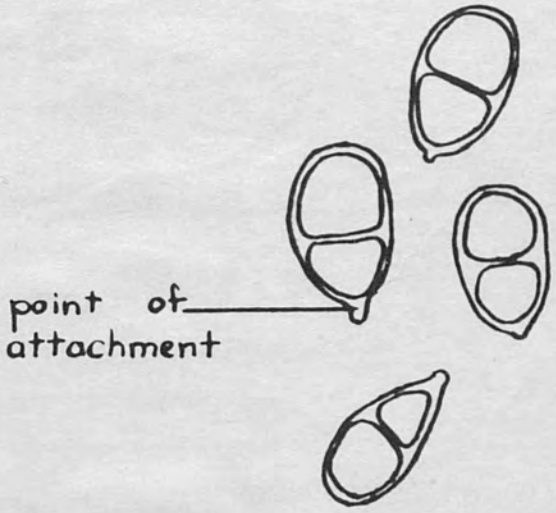
CONIDIOPHORES

Cephalothecium roseum

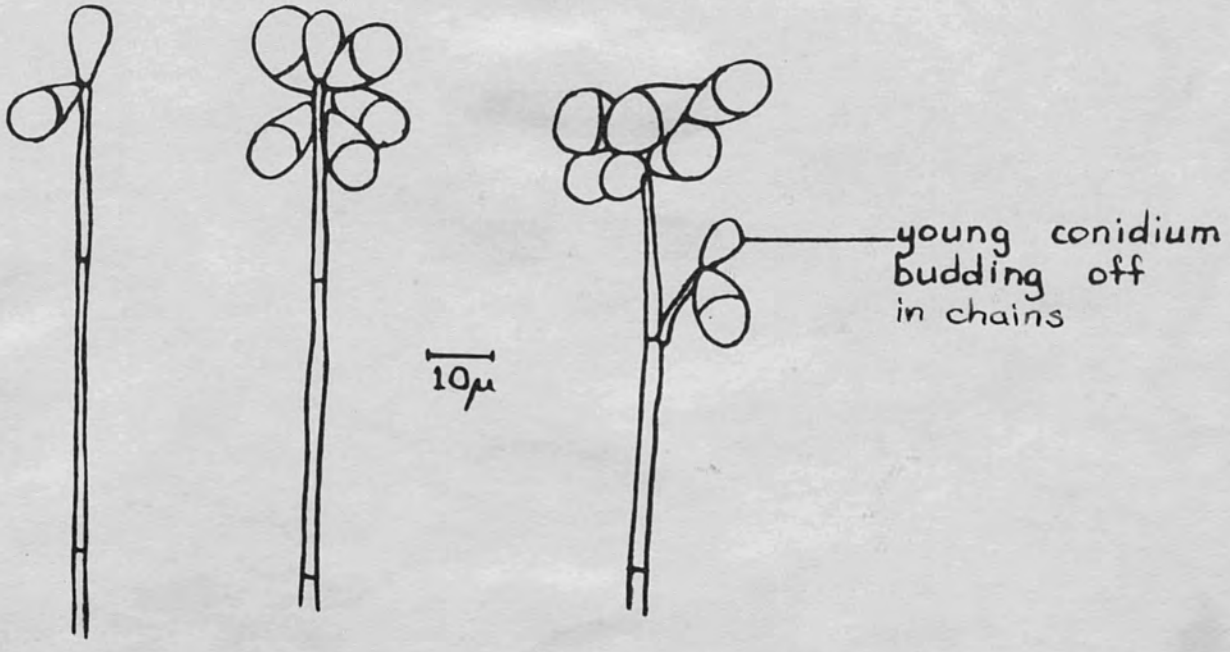


CONIDIOPHORES

CONIDIA

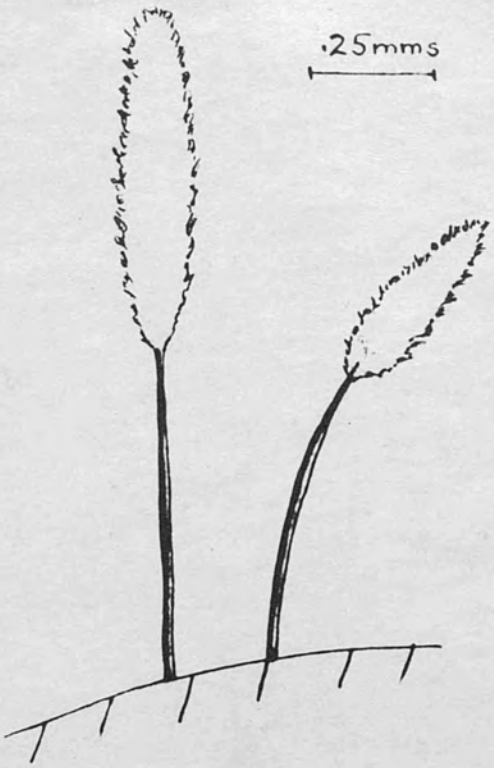


16μ x 8μ

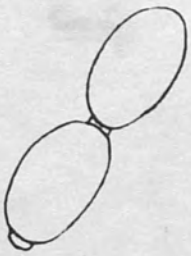


CONIDIOPHORES

Stysanus medius

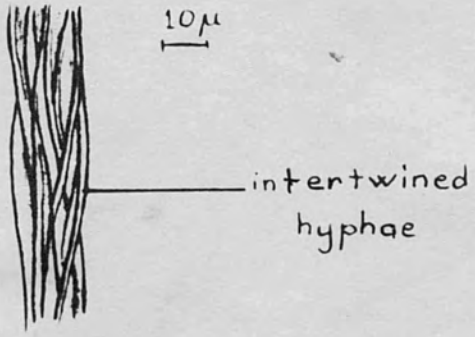


COREMIA

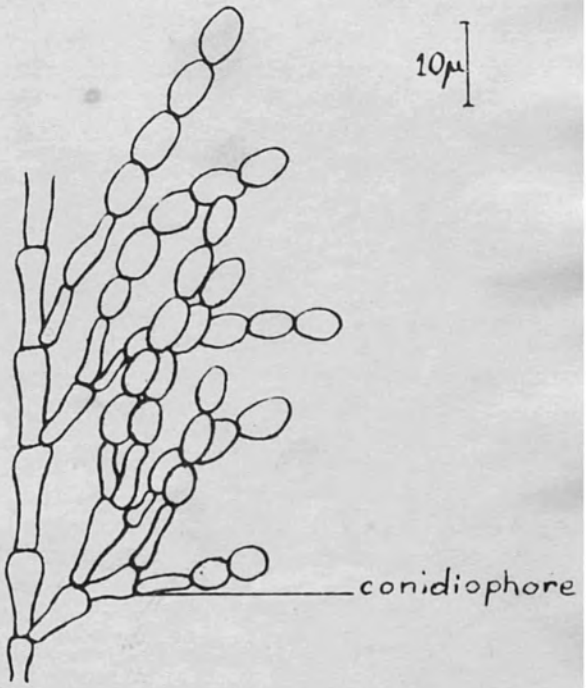


CONIDIA

6μ x 3·5μ

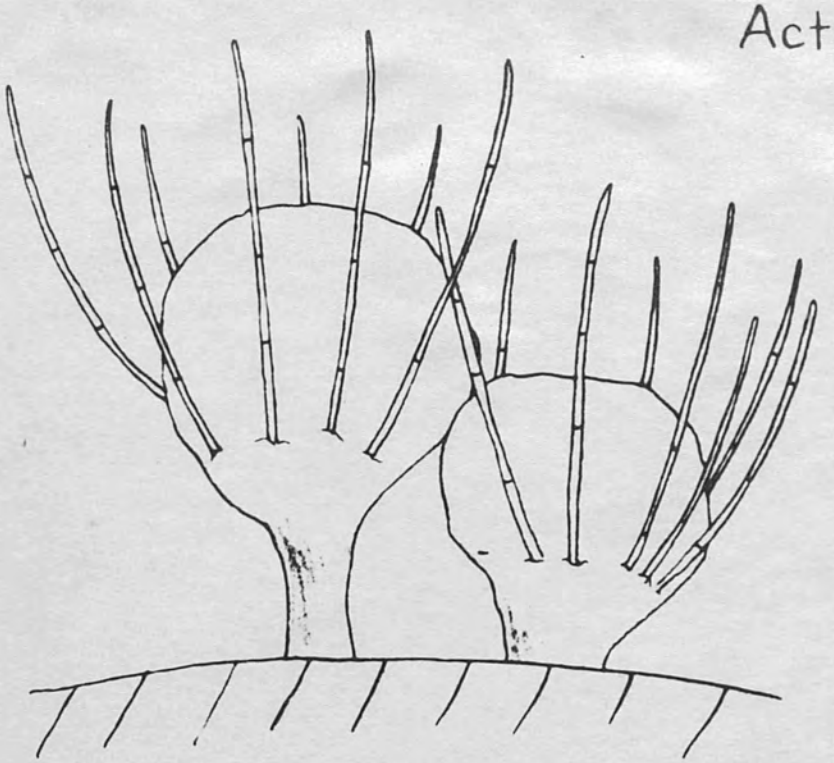


COREMIAL STALK



CHAINS of CONIDIA

Actiniceps thwaitesii



50μ

COREMIA

CONIDIA

4μ × 2·5μ

25μ

.5μ

conidium

septum

conidiophore

5μ

septate

CONIDIOPHORES

SPINES