Studies in the Water Moulds of the Royal Holloway College Grounds

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Masters

ABSTRACT

Studies in the Water Moulds of the Royal Holloway College Grounds

The grounds of Royal Holloway College afford habitats for a wide variety of aquatic fungi. These studies have been carried out in two selected families of water moulds, Saprolegniaceae and Pythiaceae.

A vast body of literature on the Saprolegniaceae has accumulated, chiefly in the United States, but comparatively little work on the family has been done in Britain, owing, probably, to the extraordinary difficulties of identification of the members of this group of fungi. A review of the small amount of literature on British Saprolegnaceae emphasises the need for the detailed observation of each species throughout the year. Comparison of a number of species collected, with the corresponding species described by Coker in his monograph on Saprolegniaceae (1923), and with the previous British records, reveals that while some species agree closely with these descriptions, and remain constant throughout a period of culture up to two years in length, other species show differences and a tendency to vary during culture.

An illustrated account is given of Pythium undulatum, and the literature on it is discussed. Apparent departures of this species from the usual conception of members of the genus Pythium necessitate a proper appreciation of the characteristics of the genus as a whole. An account is,

therefore, given of the features of the mycelium and asexual reproduction of those species of Pythium which have previously been described in detail, and the classification of the genus is discussed, in order to find justification for the taxonomic position of Pythium undulatum in the family Pythiaceae.

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NOTE

CHYTRIDIUM LECYTHII (INGOLD) N.COMB.

By E. K. GOLDIE SMITH, *Royal Holloway College*

(With 1 Text-figure)

In March 1943, when working in Dr C. T. Ingold's laboratory at University College, Leicester, I had the opportunity of studying living material of *Rhizophydium Lecythii* Ingold, parasitizing the rhizopod *Lecythium hyalinum*, from the same locality in which the fungus was originally found. The chytrid was described by Ingold (1941), but he failed to observe the discharge of the zoospores. However, in the living material at my disposal I was able to establish that dehiscence of the sporangium is by a lid which may either hinge backwards (Fig. 1, *E–J*) or become completely detached (Fig. 1, *A*) and that the zoospore has a single posterior flagellum.

Since dehiscence is by a lid, it is necessary to transfer this species from the inoperculate genus *Rhizophydium* to the operculate genus *Chytridium*, and the name becomes, therefore, *Chytridium Lecythii* (Ingold) n.comb.

REFERENCE

Ingold, C. T. (1941). Studies in British Chytrids I. *Trans. Brit. myc. Soc.* xxv, 41–8.

(*Accepted for publication 26 November 1945*)

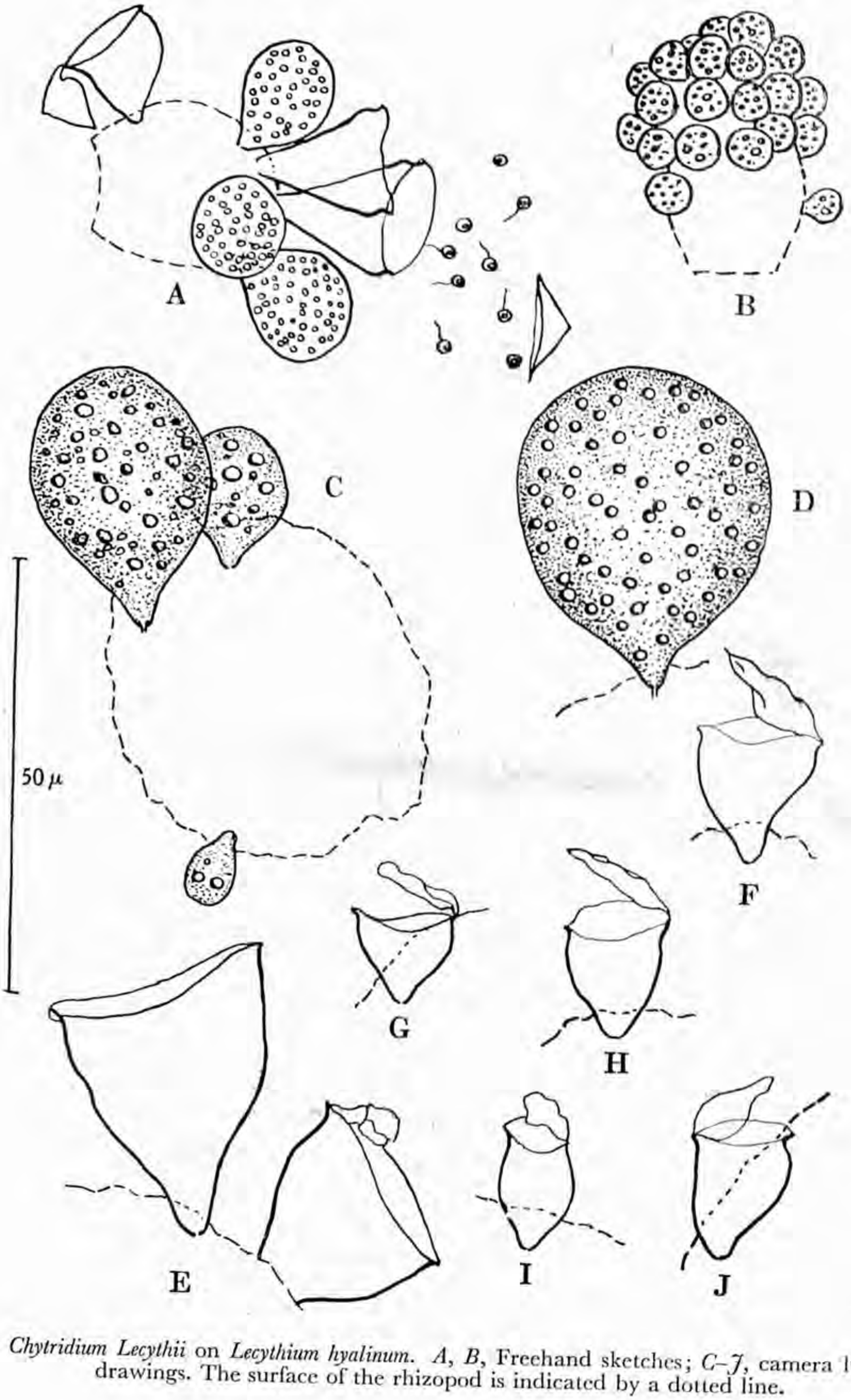

Fig. 1. *Chytridium Lecythii* on *Lecythium hyalinum*. *A*, *B*, Freehand sketches; *C–J*, camera lucida drawings. The surface of the rhizopod is indicated by a dotted line.

STUDIES IN THE WATER MOULDS OF THE ROYAL HOLLOWAY COLLEGE GROUNDS

Contents

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THESIS PRESENTED FOR THE MSc. EXAMINATION, JUNE 1948

BY

E.K. GOLDIE SMITH

ROYAL HOLLOWAY COLLEGE

ENGLEFIELD GREEN

SURREY

STUDIES IN THE WATER MOULDS OF THE ROYAL HOLLOWAY COLLEGE GROUNDS

General Introduction

The ponds and streams in the grounds of Royal Holloway College afford habitat for a wide variety of aquatic fungi, on which observations have been recorded from time to time since 1933.

My interest in water moulds was first aroused in the spring of 1943 by the appearance in jars in the laboratory of growths of Saprolegnia monoica and a species of Achlya, which provided interesting subjects for simple morphological and biological studies. These studies were continued during the Easter vacation, at University College, Leicester, together with observations on some chytrids and aquatic hyphomycetes.

Since 1943, observations on water moulds have been carried on intermittently, including part time work in this laboratory in 1944-5, on aquatic species of Phytophthora. At this time, the work of Dr. Lilian Hawker on the rôle of vitamins in the nutrition of Fungi, and that of Dr. Roper on sexual hormones in species of Achlya, stimulated an interest in physiological problems, and the award of a research studentship at Royal Holloway College for 1946-7 seemed to offer a chance to investigate certain aspects of the physiology of the Saprolegniaceae.

However, preliminary collections of water moulds from the grounds, made with the intention of securing suitable material for such work, revealed the wealth of opportunity offered by this laboratory and the proximity of a variety of ponds and streams, for a more general study of the watermoulds of this locality. These collections were therefore continued, and the execution of the original plan was postponed.

Excluding Chytridiales, representatives of the following families of aquatic Phycomycetes have been found:-

Blastocladiaceae -	Blastocladia
Monoblepharidaceae -	Monoblepharis
Saprolegniaceae -	Saprolegnia, Achlya, Aphanomyces
Rhipidiaceae -	Rhipidium, Sapromyces
Pythiaceae -	Pythium, Phytophthora

After a few weeks, the investigation became narrowed down to a study of the distribution in the grounds and seasonal variation of members of the Saprolegniaceae. The discovery of a relatively large number of species in a single stretch of water, and the many problems connected with their identification, soon made it clear that a thorough knowledge of each species is necessary before such work can be profitably undertaken in this group of fungi. Consequently the more general aspects of the study have been subordinated to the careful observation and recording of a limited number of species cultured under the same conditions. No attempt has been made to discover the entire range of species, even in this small locality.

In the autumn of 1946, a fungus was frequently found in the collections, which, in general form and in the coarseness of its protoplasm, resembled members of the Saprolegniaceae; and, although its mode of discharge from an ellipsoid sporangium into a vesicle suggested its position in the genus Pythium, in its appearance, and in the considerable degree of differentiation reached by the protoplasm before discharge, it seemed remarkably different from other members of the genus collected from the same habitat and observed under similar conditions. After its identification as Pythium undulatum, a study of the limits of this genus as a whole, as well as the characteristics of the species, seemed necessary in order to reach a proper understanding of the taxonomic position of the latter.

The discovery in March, 1947, of a detailed description of P. undulatum, and a discussion of its taxonomic position, which had been published by Drechsler in October, 1946, did not seem to reduce the interest of this work. It was therefore continued, and the two studies, on Saprolegniaceae and Pythiaceae, have been carried out simultaneously during the past two years, according to a plan which has allowed concentration on one or the other at a particular time. The thesis consists, therefore, of two sections.

The first section, on Saprolegniaceae, is chiefly a record of observations on the species which have been isolated. Some account is given of the literature on the Saprolegniaceae in Britain, but no review of the entire enormous field of literature on the subject has been attempted, though much of it has been consulted.

The second section, entitled "Pythium undulatum, and its position in the Pythiaceae," consists of two sub-sections, as follows:-

1. A description of P. undulatum, and a discussion of its taxonomic position.

2. A critical account of the features of the genus Pythium, based on those previous records in which a detailed description is given, showing how far the characteristics of P. undulatum are in agreement with those of the genus as a whole. While particular care has been taken over the accuracy of the quotations, and with regard to keeping them in their proper context, an attempt has been made to weave them into a consecutive account of the vegetative features and asexual reproduction of freshwater-inhabiting species of Pythium. Reference to sexual reproduction is considered irrelevant, since oogonia have not been reported for P. undulatum.

There are two appendices to this subsection:-

Appendix I: a history of the taxonomy of the genus *Pythium*.

Appendix II: a discussion on the relation between the genera *Pythium* and *Phytophthora*.

SECTION I

The Saprolegniaceae in Britain together with a full description of fourteen fungi isolated from R.H.C. ponds.

Contents.

THE SAPROLEGNIACEAE IN BRITAIN TOGETHER WITH A FULL DESCRIPTION OF FOURTEEN FUNGI ISOLATED FROM ROYAL HOLLOWAY COLLEGE PONDS

AN HISTORICAL ACCOUNT OF THE RECORDS OF SAPROLEGNIACEAE IN BRITAIN

Despite the wide distribution of the Saprolegniaceae and their evident occurrence in almost any stretch of stagnant or slowly-moving water, British records of the group are scanty in comparison with the mass of literature that has accumulated elsewhere, chiefly in the United States.

The first list of British Saprolegniaceae was published in 1891 by Massee. Two of the ten species given are synonymous, and two are doubtful members of the group. Eight species are mentioned in the papers by Trow on the cytology of the Saprolegniaceae published in 1895, 1899, and 1904, and it is evident that he had collected several more (1895, p.619); no complete list is given, however. In 1910, the asexual reproduction of an unidentified species of Saprolegnia was described by Lechmere. A new list containing twelve species was published by Ramsbottom in 1916. There were no further publications on British Saprolegniaceae until the 'thirties.

In an address published in 1931, Ramsbottom pleaded for more work on aquatic fungi in Britain; this plea was endorsed by Barnes and Melville in 1932, who also added two species to the list of Saprolegniaceae. Subsequently, lists were published of the Saprolegniaceae occurring in four districts in Britain: Bristol (1933, 1935), Manchester (1935), Cardiff (1934, 1938, 1939), Aberystwyth (1938).

In the collections of aquatic fungi made by Sparrow in 1932-3, attention was concentrated on other groups, and only five species of Saprolegniaceae appear in his list published in 1936. Apart from a note by Berkeley in 1944, on the habit and spore formation of an unidentified species of Saprolegnia, there is no other published record of Saprolegniaceae in Britain.

The lists of the species found are given below. The synonym is stated, in brackets, if the record had been made under that name.

* denotes that explanatory morphological figures are given.

MASSEE. Species included in the lists of "British Fungi", 1891:-

Saprolegnia ferax (Gruith) Thuret. (S. ferax Nees)

*S. monoica Pringsheim, also recorded as Diplanes saprolegnioides Leitgeb.

Achlya cornuta Archer

*A. polyandra Hildebrand

*Aphanomyces stellatus de Bary

Dictyuchus monosporus Leitgeb

Aplanes androgynus (Archer) Humphrey
(S. androgyna Archer)

Two records are included in his addenda:-

*S. philomukes Smith, on which Coker (p.76) has commented:- "Not a Saprolegnia; possibly a Pythium."

*S. elongata n.sp. Coker's comment is:- "Apparently based on mixed material, in part a Pythium and in part a Saprolegnia." The sporangia described and figured under this name bear some resemblance to those of Pythium undulatum.

TROW. Species observed during work on the cytology of Saprolegniaceae, published between 1895 and 1904. These were probably collected in the Cardiff area.

Saprolegnia diclina Humphrey (S. dioica de Bary)

S. ferax (Gruith) Thuret (S. Thureti de Bary)

S. mixta de Bary

*Achlya americana var. cambrica n.var. (1899)

A. de Baryana Humphrey

A. polyandra Hildebrand

A. prolifera (Nees) de Bary

Aphanomyces laevis de Bary

RAMSBOTTOM

Species published in a new list, in 1916:-

Saprolegnia ferax (S. ferax Nees v. Esenb.)

S. torulosa de Bary

[Achlya americana Humphrey]
var. cambrica Trow

A. apiculata de Bary

A. colorata Pringsheim (A. racemosa var. stelligera Cornu)

A. cornuta Archer

A. polyandra Hildebrand

A. racemosa Hildebrand

A. spinosa de Bary

Aphanomyces stellatus de Bary

Dictyuchus monosporus Leitgeb

Aplanes androgynus (Archer) Humphrey (Aplanes Braunii de Bary

Ramsbottom included also "Saprolegnia elongata Mass."

BARNES and MELVILLE

Species mentioned in "Notes on British aquatic fungi,1932":

Dictyuchus monosporus Leitgeb. Forest Row, October 1930

Thraustotheca clavata (de Bary) Humphrey. South London, Autumn,1929

Geolegnia sp. South London

SPARROW

Species of Saprolegniaceae recorded in 1936, which appeared while collections were being made for other groups:-

Saprolegnia asterophora de Bary Suffolk, November 1932

Achlya colorata Pringsheim Cambridge, September 1932

Aphanomyces parasiticus Coker Suffolk, November 1932

Dictyuchus monosporus Leitgeb Cambridge, throughout the year 1932-3

Thraustotheca clavata (de Bary) Humphrey York, September, 1932

The following table shows the results of collections which were made with the definite aim of discovering the full range of species of Saprolegniaceae in four districts in Britain.

TABLE SHOWING THE MAJOR COLLECTIONS OF SAPROLEGNIACEAE IN BRITAIN

District	Bristol	Manchester	Cardiff	Aberystwyth
Collector	Forbes	Forbes	Ivimey-Cook and Morgan	Brown
Period of Collection	Sept.-Mar. 1932-3	Oct.-May, 1933-4	Summer 1933-6	Sept. - Feb. 1935-6
Saprolegnia asterophora de Bary	-	+ *	+	-
S. crustosa, var. II Maurizio.	-	-	-	+ *
S. delica Coker	-	-	-	+
S. diclina Humphrey	(*S. dioica* de Bary)	(*S. dioica* de Bary)	+	-
S. ferax (Gruith) Thuret	+	(*S. ferax* Kutzing)	+	+
S. mixta de Bary	+		+	+
S. monoica Pringsheim	+	+	+	+
S. monoica var. glomerata Tiesenhausen	-	-	+	-
S. paradoxa Maurizio	-	+ *	-	+ *
Isoachlya eccentrica Coker	-	-	+	-
I. monilifera (de Bary) Coker	-	-	+	-
I. toruloides Kauffman and Coker	-	-	-	+
I. unispora Coker and Couch	-	-	+	+
Pythiopsis cymosa de Bary	-	-	+	-
P. intermedia Coker	-	-	+	-

	Bristol	Manchester	Cardiff	Aberystwyth
[A]chlya americana Humphrey	+	–	+	–
[A]. apiculata de Bary	+	+	+	–
[A]. apiculata var. prolifica Coker and Couch	+	–	–	–
[A]. caroliniana Coker	+ *	–	+	–
[A]. colorata Pringsheim	+	+	–	+
[A]. conspicua Coker	–	+ *	–	–
[A]. crustosa Coker	–	–	+	–
[A]. de Baryana Humphrey	+	+	–	–
[A]. flagellata Coker	–	–	+	–
[A]. imperfecta Coker	–	–	+	–
[A]. Klebsiana Pieters	+	–	–	–
[A]. megasperma Humphrey	+	–	–	–
[A]. oblongata de Bary	+	+	–	–
[A]. oblongata var. gigantica (de Bary) Forbes	+ *	–	–	–
[A]. Orion Coker and Couch	+	–	–	–
[A]. polyandra Hildebrand	+	+	+	–
[A]. prolifera (Nees) de Bary	–	+ *	–	–
[A]. racemosa Hildebrand	–	+	+	+
A. radiosa Maurizio	+ *	+	+	+
[A]. recurva Cornu	+ *	+	–	–

	Bristol	Manchester	Cardiff	Aberystwyth
Dictyuchus monosporus Leitgeb	+	–	–	–
Dictychus sp.	–	+	–	–
Aphanomyces eutiches Drechsler	–	–	+	–
A. laevis de Bary	–	–	+	–
A. scaber de Bary	–	–	+	–
A. stellatus de Bary	–	–	+	–
Aphanomyces sp.	–	+	–	–
Leptolegnia sp.	–	+	–	–
Calyptralegnia achlyoides Coker and Couch	+ *	+	–	–
Brevilegnia diclina Harvey	+ *	–	–	–
Thraustotheca clavata (de Bary) Humphrey	–	–	+	–

* denotes figures.

METHODS WHICH HAVE BEEN USED IN THE STUDY OF SAPROLEGNIACEAE IN BRITAIN

Collection

The various methods which have been used, in collecting Saprolegniaceae from aquatic habitats and from soil may be summarised as follow:

Medium

1. Aquatic habitats. a) Plant and animal debris is brought from the pond or stream and examined directly, or after a few days in water, for fungi which may be growing on it. (Barnes and Melville, Sparrow, Brown).

b) The debris is placed in jars of water in the laboratory; bait is added, and this becomes infected either by the hyphae of fungi growing on substrata with which it is in contact, or by zoospores released by the fungi since their transfer to the laboratory (Trow, Sparrow).

c) Pond- or stream-water, relatively clear of debris, is brought into the laboratory, and the bait which is added becomes infected by zoospores already present in the water (Forbes, Brown).

There appears to be no record for Saprolegniaceae in Britain of a further possible method, in which bait, in suitable containers, is placed directly in the pond or stream.

2. Soil. Soil samples taken from a depth of two inches are placed in wide dishes in the laboratory and covered with about an inch of distilled water; bait is added, which becomes

infected either by the growth of hyphae or by zoospores. (Barnes and Melville, Ivimey-Cook and Morgan).

Bait

Both animal and vegetable materials have been used as bait. Trow used flies "killed by chloroform to prevent injury to the chitinous coats, moistened with alcohol and washed with sterilised water," but also "found hard-boiled white of egg, cut up into minute oblong pieces and floated on the surface of the water in the jars containing the cultures, to be a very good substitute ...." Lechmere, also, used flies and white of egg, as well as "hard boiled pieces of fish or the skin of fish, and pieces of beef," and made plate cultures on a solid medium requiring "half-a-pound of beef without any fat" to 100 gm. gelatine in a litre of water! "Ants' eggs, cress seeds, rose hips and dead flies" were used by Brown. Recognising the susceptibility of aquatic fungi to changes in their environment, Ivimey-Cook and Morgan, and Forbes, considered it advisable to use the same substratum for all cultures, and chose halved hemp seeds. The use of hemp seeds had been recommended by Harvey (1925); Barnes and Melville, however, preferred cress seeds, as they seemed "less liable to bacterial infection."

At Royal Holloway College, halved hemp seeds, hips, grapes, and flies have constituted the usual bait. After the preliminary stages of collection, halved hamp seeds have beer used throughout, in water cultures.

Methods which have been used at Royal Holloway College.

Most collections have been made from aquatic habitats, and as preliminary experiments showed method 1(c) to be more profitable than 1(a) or 1(b), this method has generally been used. As soon as possible after the dipping has been made, and always within half an hour, the water is tipped into wide, flat dishes and baited. After about twenty-four hours, the bait is washed and transferred to sterilised distilled water, in a number of separate dishes with loose covers. It has become obvious during the course of the work that the best results are obtained when the water is an inch or less in depth.

Only very occasionally have collections been made from soil. The sample of soil is placed in a wide dish, and sterlised distilled water is added until the soil is just covered. The bait is then placed either in the water-film, directly in contact with the soil, or on a piece of muslin, previously wetted, and laid on the surface of the soil. Later, the bait is transferred to clean water, as above.

Subsequent Treatment

Rough cultures have usually been obtained by removing the bait soon after fungal growth on it has become visible, washing, and transferring it to clean water. Sterilised distilled water has generally been used. Brown, however, used the tapwater of the Aberystwyth district, which was sterilised, and aerated by bubbling air through it.

Cleaning.

Brown freed her cultures from bacteria and protozoa by transferring pieces of mycelium to clean water containing fresh bait. Ivimey-Cook and Morgan cleaned their cultures by repeated subculturing on agar. At. R.H.C., agar cultures have been cleaned effectively by Raper's method (1937), in which the bacteria, those commonly encountered being aerobic, are retained on the surface of the agar within a glass ring, while the fungus grows underneath the ring and forms a clean growth outside it. Potato citric agar has generally been used during the process of cleaning followed in subsequent cultures, chiefly by cornmeal agar, also oatmeal and malt agars. Malt and cornmeal agars were used by Forbes, maize agar by Ivimey-Cook and Morgan, and pea agar by Brown. After growth on agar, cultures in water can be obtained by using portions of cleaned mycelium in blocks of the agar placed in water to infect fresh bait; at R.H.C. it has usually been done by allowing the cleaned mycelium to infect directly, halved hemp seeds left on the agar culture for one or two days, before being transferred to sterile distilled water.

Isolation.

The methods which have been used for obtaining pure cultures may be summarised as follow:-

1. A single sporangium is transferred to clean water, where zoospores liberated by it infect fresh bait (Brown).
2. Water containing zoospores is diluted and spayed "through a very fine nozzle" on to an agar plate. After about two days, infections from single spores are transferred to clean agar plates (Brown.)
3. Single hyphae are teased out and transferred separately to agar plates (Forbes).

At Royal Holloway College, single-spore cultures have usually been made as follows:- If the growths appear to be mixed, pure cultures are first obtained by placing hyphal tips, cut off with scissors, separately on agar plates; the pure culture is transferred back to water. Vigorous production of sporangia and liberation of zoospores are then encouraged by frequent washing, and drops of water containing zoospores, diluted if necessary, are distributed on agar by means of a pipette. The growths from single spores are later cut out and transferred to separate clean agar plates.

Maintenance.

Cultures can be maintained in water by periodically using a vigorous growth to infect fresh hemp seeds in clean water. At Royal Holloway College, cultures were at first kept continuously in water. As some species showed a decline in vigour, however, a change was later made to agar, and the stock cultures have since been maintained on agar slopes.

GENERAL OBSERVATIONS

Observations recorded in the Literature

Occurrence in soil.

The first collections of Saprolegniaceae ever made from the soil were described by Harvey in 1925. The first collection from British soil was of Thraustotheca clavata, isolated from garden soil in London by Barnes and Melville, in 1929; a species of Geolegnia was found. In 1933, Forbes isolated Brevilegnia diclina from the soil in a fernpot at Bristol.

The chief records of the occurrence of Saprolegniaceae in the soil in Britain are given in the accounts of Ivimey-Cook and Morgan, which may be summarised as follow:-

Saprolegniaceae of Glamorgan. During 1934-36 an investigation was carried out on the relative occurrence of species of Saprolegniaceae in soil samples taken each month from stations in wet pasture, dry pasture and gardens, in two districts of Glamorganshire. While species of Saprolegnia and Isoachlya were isolated more often from wet than from dry soils, species of Achlya, Pythiopsis Humphreyana, and Thraustotheca clavata were found more frequently in dry soils. From these results it was concluded that those species in which there is "a reduced planetic phase may be more adapted to drier ~~to drier~~ soils than those in which a diplanetic condition obtains," it was also suggested that in certain species, "soil and water may form an alternate habitat." The further generalisation was made in 1934, that "it appears from the present work that the Saprolegniaceae are more widely distributed in the soil than in water, and it seems probable that a revision of our conception of the group will have to be made, and that the old term "Water Moulds" will have to be given up."

Factors affecting distribution.

For the determination of species distribution in soil, Morgan (1938) has considered water content and temperature to be more important than humus content and acidity. No details are given of the sites from which collections of soil were made in Glamorgan.

Commenting on her collections from water of the Manchester district, Forbes wrote in 1934:- "The number and variety of species obtained from the different ponds apparently depended to some extent upon certain environmental factors. As shown by the work at Bristol, watermoulds cannot thrive in foul stagnant waters, probably owing to the reduced supply of oxygen. Thus, relatively few species have been obtained from the Ashburne Hall pond which, for most of the year, contains a quantity of decaying organic material. The presence of a considerable aquatic vegetation in a living condition , however does not appear to hinder the growth of the fungi in any way. The amount of incident light also appears to be of no consequence, for ponds which are completely overhung by trees, or have a surface-covering of Lemna, have proved as fruitful as any of the more exposed ponds. Collections made from rivers or from rapidly-flowing streams, as one might have expected, yielded comparatively few species." Forbes has observed also that "each pond possesses a characteristic fungus-flora, for it is usually from the same ponds that a species is obtained month after month, while it may never appear at all in the other localities."

In 1938, Brown gave an account of the pools and lakes investigated in the Aberystwyth district, but generalisations on distribution can hardly be made on the relatively small number of species that are recorded. The results were as follow:-

Two mountain pools over 1000 ft. above sea level, receiving water drained from a moorland plateau	Achlya racemosa Saprolegnia mixta Saprolegnia monoica
A marshy pond in a sheep pasture, at 500 ft. with some water weeds. "The water was deep and clear, although the substratum was muddy."	Isoachlya toruloides
Two marshy ponds on the river flats, at sea level, with a rich vegetation of water weeds, and surrounded by marshy grassland	Achlya racemosa Achlya colorata Saprolegnia delica Saprolegnia monoica

A slowly-moving stream almost at sea level	*Saprolegnia crustosa*
A marshy pond on golf-links at sea level, possibly affected by saline conditions	*Saprolegnia ferax*
A roadside ditch near the sea; very muddy	*Saprolegnia paradoxa*

Morphological variation.

Ivimey-Cook and Morgan (1934) have commented on "the variation of individuals from different localities which appear to belong to the same species, and also the variation which appears as a result of continued cultivation under laboratory conditions." As it seemed possible to trace a whole series "from one species to another and then to a third," where "size of structures is the criterion of species segregation, as is generally the case," these investigators have expressed the view that "a more satisfactory method of separation of species will have to be found if their identification is to be made possible to anyone except an expert on the group."

Seasonal periodicity.

Morgan (1939) has observed that "certain species are characteristic of the spring months, others the summer, while still othes occur most commonly in the autumn and winter," but no data are given.

In the months between September and May, when the collections were made both at Bristol and at Manchester, Forbes observed "a marked periodic variation in abundance" of species collected from each pond, which she has explained as being due to alternating periods of activity and quiescence; during the quiescent period, the fungus is "probably in the form of oospores or gemmae," and does not infect the bait. Two graphs are given in which percentage abundance is plotted against time of year. The first shows a rise in the abundance of Achlya radiosa and Achlya racemosa to a peak in the early months of the year, January to March, after which there is a decline; it is pointed out that other species, e.g. "Prolifera"-group of Achlya, Saprolegnia diclina, reach the peak of their activity in the early winter (November). The second graph shows a reciprocal relationship between Saprolegnia ferax and Saprolegnia monoica; the former has a peak in its activity in November, the later in March; Forbes has considered these results as supplying evidence in support of the view that S. ferax and S. monoica are growth-forms of the same species.

General Observations on the Saprolegniaceae of Royal Holloway College Grounds

This study of the Saprolegniaceae of the grounds was first undertaken with the aim of discovering the factors that determine the distribution of the species in the various ponds, throughout the seasons; regular measurements of the temperature and pH of the ponds were made, and a special method was devised for studying seasonal periodicity, as follows:-

1. At about 10 a.m. twice a week (Tuesdays and Saturdays) a dipping was made in each of two ponds, the botany garden lily pond and southwest pond.

2. Each sample of pondwater was placed in a wide, flat dish on the laboratory bench, and baited with five half hemp seeds.

3. After twenty-four hours, each half hemp seed was transferred to a separate dish containing sterile distilled water.

4. The water was changed after a week, and, subsequently, examinations were made at intervals; if identification had not been possible within three weeks of making the collection, owing, usually to the absence of oogonia, the cultures were laid aside, or thrown away.

In this way, forty separate cultures were obtained from each of the two ponds every month, and it was hoped that, by calculating in what percentage of each monthly set of collections a particular species had appeared, a graph could be plotted showing its relative abundance throughout the year.

However, it soon became clear that this method was quite impracticable, owing to the difficulties of identification and it was therefore modified, as follows:-

1. Collections were made only once a week, instead of twice, but from three ponds, the third being the southeast pond.

2. Three half hemp seeds were used instead of five, in each sample.

3. Larger vessels with a diameter of about three inches were used, as the growths had not been found entirely satisfactory in the rather small ones which had been used previously, and more care was taken over using water of the same depth - about ½ to ¾ inch.

The collections have been carried out as follow:-

During the autumn and winter, 1946-7, intermittently from a number of ponds. A variety of bait was used, and the cultures were grown in dishes of different diameter and depth.

From 1st March to 15th July, 1947, twice a week from southwest pond and the botany garden lilypond, and the cultures were all kept under the same conditions, according to the first method described above. Collections from the other ponds were continued intermittently.

During the autumn, 1947, once a week from southwest pond, southeast pond and the botany garden lilypond, according to the modified method described above. Collections from the other ponds were made occasionally.

From January to March, 1948, once a fortnight, otherwise as in the autumn.

March to May, 1948, only occasionally.

Discussion.

The chief problem in a study of the Saprolegniaceae is the fundamental one of identification. As insufficient confidence has been felt in identification by the features of the hyphae and sporangia alone, it has been necessary to wait for the appearance, in each culture, of oogonia. In many species, the production of oogonia under laboratory conditions is sporadic, and no method is known which can be relied upon to induce it in all species; the apparent absence of a species from the collection therefore justifies either of two conclusions:-

1. The species has not been collected, presumably because it is in a quiescent state in the pond, in the form of gemmae or oospores, and is not liberating zoospores. (the bait cannot, then, be infected.)
2. Its presence in the collection has been missed, because oogonia have not been formed.

For some time, observations were made on single-spore cultures of all the species which had been isolated during the work, parallel with those on the growths from these periodical collections, cultured under the same conditions. An apparent decline in the frequency of a particular species in these collections might by this means have been correlated with a decline in the production by it of oogonia, as shown in the isolate. It became evident, however, that some of the isolates were declining in vigour as a result of continuous culture in the laboratory, and the comparison was recognised as useless.

It is clear that a study of seasonal periodicity in the Saprolegniaceae requires a special method of approach. A start might well be made by selecting, for continuous observation throughout the year, a species with characteristic, easily-recognised oogonia, which can be relied upon to produce them readily under constant laboratory conditions, throughout the year. Achlya radiosa may be such a species. In other species, the physiological conditions necessary for the production of oogonia must first be investigated, so that oogonium-formation can be easily induced, and the presence in a collection, of a particular species, or its absence from it, can be confidently determined.

In the present study, therefore, particularly as the method has been changed during its course, so that month with month comparisons cannot easily be made, emphasis has not been laid on comparative observations on the seasons of appearance of the various species, in the collections. However, the following general observations have been made;-

The distribution in the grounds of the various species.

The lists given below of the species which have been collected from the different ponds are not intended to be exhaustive.

The botany garden lilypond. An oblong artificial pond lined with concrete, sunk in the lawn of the botany garden; supplied by rainwater mainly, but kept filled, in summer, by water from a tap; more or less overhung by trees on the south side; pH between 6.9 and 7.3.

S. ferax	A. racemosa
S. monoica var. glomerata	A. imperfecta
"S. paradoxa"	A. flagellata
S. diclina	
S. delica	

Southwest pond. A large pond fed by springs whose source of water is from higher ground; partly shaded by trees; pH 6.0.

S. ferax	A. colorata
S. monoica var. glomerata	A. racemosa
S. delica	A. radiosa
Aphan. laevis	A. apiculata
Aphan. stellatus	A. oblongata ?
	A. recurva
	A. flagellata

Southeast pond. A smaller pond receiving water from southwest pond, and drainage from the roofs of the College buildings, and some spring water; there is a surface covering of pondweeds; pH between 5.9 and 6.3.

S. ferax	A. colorata
S. anisospora	A. racemosa
S. delica	A. radiosa
Aphan. laevis	A. apiculata ?
Aphan. stellatus	

Hazel's pond. A small round pond only a few inches deep, sometimes almost dry, fed by three pipes bringing water drained from higher ground; shaded by trees; pH between 6.3 and 6.8.

S. ferax	A. racemosa
S. monoica var. glomerata	A. radiosa
S. paradoxa	

Engineer's pond. A pond fed by water from higher ground, but also receiving caustic "boiler fluid" from the engine house; pH between 7.2 and 7.4.

S. ferax	A. flagellata
S. monoica var. glomerata	

Samples of the soil near the inlet of the drainage water contained
A. colorata
A. racemosa
A. radiosa

Seasonal periodicity. No definite conclusions can be drawn, but some slight indications of seasonal periodicity made themselves felt. In collections from the botany garden lilypond in the spring, 1947, Saprolegnia monoica var. glomerata appeared frequently, while in the autumn, 1947, Saprolegnia ferax was more abundant. A similar observation on these two species was made by Forbes (1935-6). There have been periods when one particular species appeared predominantly in the collections. Thus, Achlya colorata was predominant in the collections from the southwest pond, in the spring, 1947, but was succeeded by Achlya flagellata in the summer, 1947; and Saprolegnia ferax was predominant in collections from the botany garden lilypond, and Saprolegnia delica in collections from the souteast pond, in the autumn, 1947. A dependence of oogonial production upon temperature may be directly concerned in this apparent periodicity, since the presence of oogonia in the culture encourages the identification of the fungus, while the absence of oogonia may cause a fungus, present in a mixed collection, to be overlooked. False conclusions with regard to the relative abundance in the pond of the particular species may thus be drawn as a result of changes in temperature in the laboratory.

Conditions of culture.

Most species of Saprolegniaceae are strongly aerobic. It has become obvious that thedepth of the water in the culture dishes is an exceedingly important factor in determining the vigour of the cultures. The best result are obtained when the water is an inch or less in depth, and exposes a large surface to the air. In a dish containing rather deep water, a culture on a portion of bait floating at the surface of the water forms a stronger, more extensive growth than a culture on the bottom of the dish. In a shallow dish, such as a Petri dish, the hyphae of some species, e.g. Achlya oblongata grow up to the surface and form an extensive mycelium hanging under the surface-film.

Continuous culture in water, on the same kind of substratum, seems to result in a decline in the vigour of some species, e.g. A. racemosa, S. ferax, but the vigour may be partly restored by growth for some time on agar. This decline may be accompanied by slight morphological changes, as in A. racemosa (which see), but it has not been observed to cause a loss of the specific characteristics.

The production of oogonia.

Remarkable variety occurs among the different species in their readiness to produce oogonia in cultures on hemp seeds in water. While A. radiosa forms them abundantly after a few days, A. apiculata forms them very sporadically, and A. recurva has formed them only once during a year of culture. Similarly, while S. ferax often forms oogonia in large numbers after four days, S. diclina has been kept for months without their appearance in the cultures.

Gemmae.

The gemmae formed by the species of *Achlya* isolated at Royal Holloway College differ markedly from those formed by the species of *Saprolegnia*. They are not formed by *Aphanomyces*. In *Achlya* species, they are cylindrical, formed by the segmentation of the hyphae without any change in their width. The segments may be abstricted actively, as in *A. racemosa*, or may remain in position until they are dislodged by movements of the water, or by other mechanical means.

In *Saprolegnia* species, the gemmae usually consist of swollen portions of the hyphae. In *S. ferax*, they are somewhat filamentous, and irregular. In *S. diclina* they are mostly spherical or pyriform, and are often found in chains. These chains may reach an extraordinary length in old cultures. The gemmae of *Saprolegnia* species do not usually become detached from the hypha bearing them.

In both *Achlya* and *Saprolegnia*, the gemmae may by refreshment of the water be readily induced to act as sporangia and liberate zoospores through terminal or lateral openings. The ready detachment of the gemmae of species of *Achlya*, however, seems to indicate their special efficiency as organs of vegetative propagation, which may become widely distributed by currents or moving objects in the water.

There is a reciprocal relationship between gemmae and oogonia. Cultures in which oogonia are rare or absent usually contain a large number of gemmae.

SUMMARY OF OBSERVATIONS AND CONCLUSIONS

1. Thirteen fungi isolated from Royal Holloway College grounds have been identified with certainty as species of Saprolegniaceae which are described in Coker's monograph. A fourteenth has been identified only tentatively on account of the scarcity of oogonia in the cultures. Two others have been identified, but detailed comparisons of their features with those described by Coker have not been made.

2. Isolates of these fungi have retained their specific characteristics throughout a period of culture up to seventeen months in length. The slight variations shown by some of them have seemed to be related to the vigour of the cultures.

3. The isolates have also retained their generic characteristics. Thus, secondary sporangia are formed typically by proliferation in species of Saprolegnia and by subsporangial branching in species of Achlya. Although proliferation is found frequently in A. colorata, subsporangial branching is more characteristic; and although the zoospores of some species of Achlya e.g. A. apiculata, A. imperfecta, often show the behaviour which is characteristic of species of Aplanes, by encysting and sprouting into hyphae, while still within the sporangium, typically they swarm out of the sporangium

and encyst at its mouth according to the usual behaviour of species of Achlya.

4. The features of some isolates, in single-spore culture, have shown a range which covers those of more than one form or variety of the species concerned. Thus, the Royal Holloway College isolate of S. ferax, like that of Forbes, seems to include the two forms distinguished by von Minden; and the Royal Holloway College isolate of A. oblongata seems to show the features of both the species and the "variety globosa."

5. The similarity between the Royal Holloway College fungi identified as Saprolegnia monoica var. glomerata Tiesenhausen and "Saprolegnia paradoxa Maurizio" do not seem to justify the erection of a species for the latter. On the other hand, the constancy of certain differences throughout a period of culture of eleven months offers considerable evidence against its interpretation as a "growth form" of S. monoica, according to the suggestion of Forbes. It is proposed, therefore, that this fungus should be called Saprolegnia monoica var. paradoxa.

6. One species has been found, S. anisospora, of which there is no published record in Britain.

AN ACCOUNT OF THE FUNGI ISOLATED FROM THE ROYAL HOLLOWAY COLLEGE GROUNDS.

The fungi have been identified by reference to Coker's monograph (1923). Other accounts have sometimes been consulted, and comparisons have been made with previous British records, but the full list of literature on each species has not been investigated. Rather, effort has been concentrated on making a careful record of the particular fungi which have been isolated from these grounds.

The entire range of species occurring in the grounds has not been explored, and this list, therefore, is not exhaustive. Species of Dictyuchus and Pythiopsis have been found by other collectors, and possibly a large number of other species might be added to the list by using a greater variety of bait, different depths of water for culturing, investigating the soil from various parts of the grounds, and by promoting the formation of oogonia in unidentified fungi which appear to be sterile.

Oogonia are necessary in order to identify a species with confidence. Most species form oogonia within three weeks of culture, but some cultures have been kept for several months before oogonia have appeared, and some have remained sterile. Casual experiments have been carried out with haemoglobin, peptone, and other nutritive solutions, but no systematic attempts to induce the formation of oogonia have been made.

Fourteen fungi have been isolated and identified according to Coker's list as follow:

1. *Achlya colorata* Pringsheim p.41
2. *A. racemosa* Hildebrand p.47
3. *A. radiosa* Maurizio p.52
4. *A. imperfecta* Coker p.57
5. *A. apiculata* de Bary p.62
6. *A. oblongata* de Bary p.68
7. *A. recurva* Cornu p.72
8. *Saprolegnia ferax* (Gruith) Thuret p.75
9. *S. monoica var. glomerata* Tiesenhausen p.82
10. *S. paradoxa* Maurizio p.88
11. *S. diclina* Humphrey p.92
12. *S. anisospora* p.94a
13. *Aphanomyces laevis* de Bary p.97
14. *Aphan. stellatus* de Bary p.100.

While such detailed observations have been made on thirteen of these fungi as to establish their identity with certainty, observations on the fungus identified as *Achlya recurva* have been somewhat incomplete owing to the rarity of the oogonia.

Achlya flagellata Coker and *Saprolegnia delica* Coker have also been isolated, but are not included in the following detailed accounts.

The Records

Hemp seeds have been used throughout the present study as the substratum for the growth of the fungi. The records of the fourteen species which are described in detail consist of the following items:-

1. Drawings, made usually with the aid of the camera lucida.
2. Measurements: On account of the great variability of these fungi, the measurement of the enormous numbers necessary in order to obtain results of statistical value is impracticable. Measurements of hyphae and sporangia have not been made, and measurements of only a small number of oogonia and oospores have been made, by ascertaining the upper and lower limits of the range in size and taking twenty to thirty measurements between them.
3. Photomicrographs taken on (a) Ilford ordinary plates.
   (b) Kodaline rapid paper, according to a modification of the method described by Chesters (1939)
4. Living cultures: these are liable to undergo changes.
5. Preserved material (a) slides
   (b) cultures, preserved in bulk.
6. Data concerning the distribution of the various species, and the places of origin of the isolates which have been described.
7. References to records of previous collections at Royal Holloway College which have been made from time to time

since 1935 and kept in the form of slides, preserved material and observations in a book. These records, however, are slight, as few complete identifications have been made.

8. References to other records of species in Britain.

9. Careful comparisons with the accounts given in Coker's monograph "The Saprolegniaceae" (1923).

Fungus 1: identified as Achlya colorata Pringsheim
(Plate 1)

Outstanding features:

Oogonia with a warted or irregularly spiny wall,
yellowish in colour, and 1 - 5 oospores.

Antheridia 1 - 3, arising on the oogonial stalk
usually close under the septum, quite
often above it.

Hyphae stout, somewhat extensive; sometimes undulating (fig.A 11). A conspicuous rhizoidal system has been noticed several times, especially on hips, which seem to be a particularly favourable substratum for A. colorata.

Gemmae (figs.A 9,10) formed by the segmentation of the hyphae into cylindrical portions which are usually thicker distally, and often are abstricted (fig.A 9). Fig.C shows the base of a gemma resting on a stump of the hypha from which it has been abstricted; the broken wall is clearly visible, projecting beyond the end of the gemma. Quite frequently, a gemma develops one or several outgrowths, usually at the distal end, which, if freshwater is added, or if the conditions are otherwise favourable, serve as exit tubes for the escape of the zoospores formed within the gemma.

Sporangia (figs. A1 - 8; B 1 - 3) cylindrical, about the same width as the hyphae; an empty sporangium usually has a well-marked collar (figs. B1- 3). Renewed both by subsporangial branching (figs. 3 - 5) when a portion of the hypha immediately below the primary sporangium often forms part of the secondary sporangium (figs.A 4,5) and by proliferation (figs.7,8), later-formed sporangia usually exceeding earlier ones in length, and completely filling up the cavity (cf. species of Saprolegnia). Rings of empty spore-cysts can often be seen around the sporangia in the region of the constrictions marking the position of the openings of previous sporangia (fig.8)

Oogonia (figs. D1 - 5; E 1 - 3) on lateral branches; variable in size - 50µ - 80µ, mostly about 60µ. Wall sometimes relatively smooth (fig. E 2); usually with a number of blunt warts; sometimes unusually thick, and produced into blunt curved spines (fig. E 3); a solid upgrowth from the septum is often present (figs. E 1 - 3)

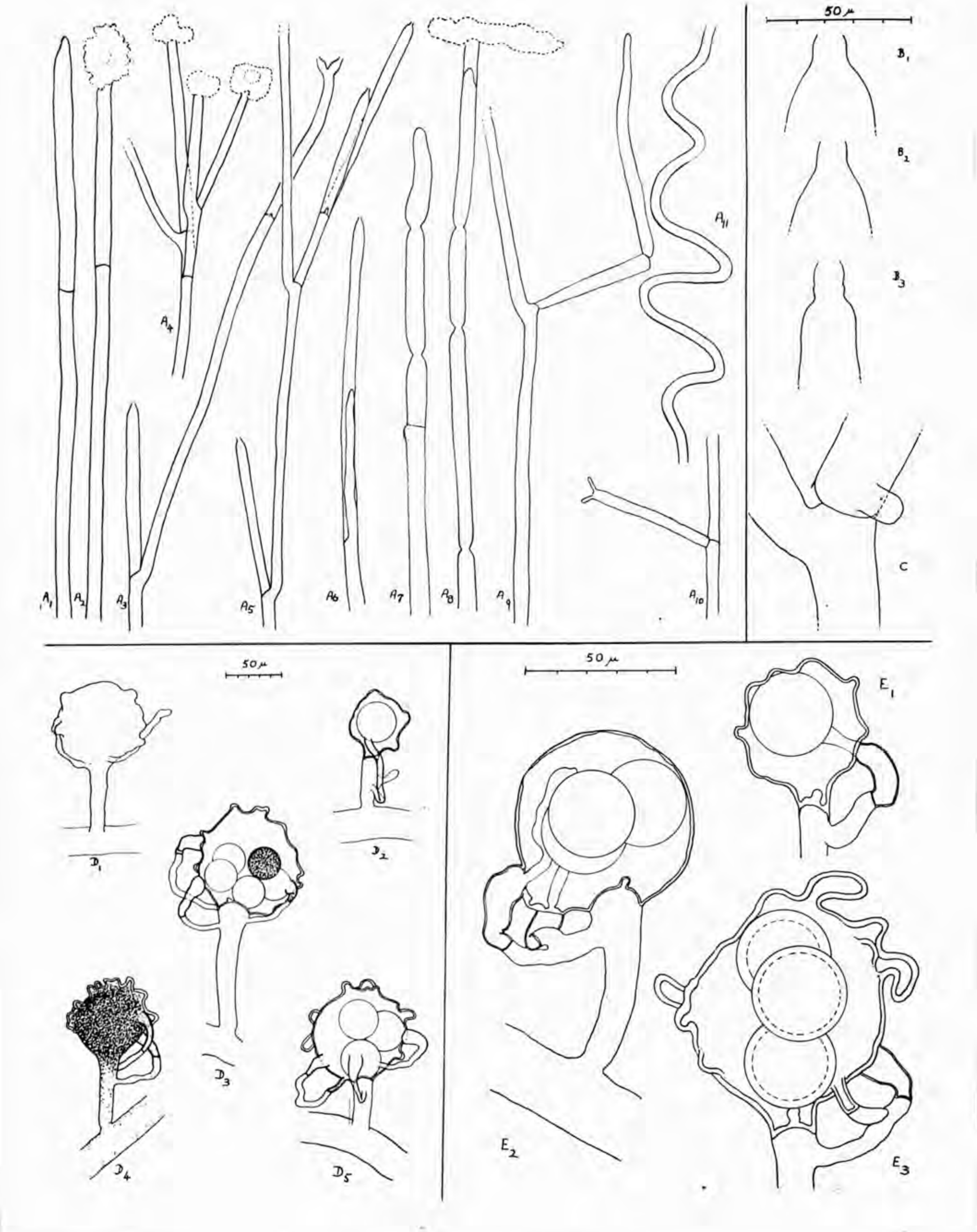

50 μ
B1
B2
B3
A11
A4
C
A1
A2
A3
A5
A6
A7
A8
A9
A10
50 μ
50 μ
E1
D1
D2
D3
D4
D5
E2
E3

PLATE 1

Antheridia on 1, 2 or 3 short branches from the oogonial stalk, which occasionally are branched (fig. E 2); these quite often arise above the oogonial septum (fig. D 5); antheridial tubes often seen (figs. E 1, 2).

Oospores about 27μ - 38μ, mostly about 32μ (many have not been measured) 1 - 5, usually 2 or 3; centric.

Achlya colorata (continued)

Occurrence in the Grounds.

Southwest pond. A.colorata has been collected frequently from this pond. It is in the department records of October, 1935 (on slide and as preserved material) and November 1938. During the present study, it was collected in November and December 1946, and in October (twice), November (twice), and December 1947. It appeared abundantly in almost every collection, from 1st March to 10th June, in 1948, and was found in April 1948.

Southeast pond. November 1938 (slide; preserved material)
November 1940
May, June, November (twice) December (twice) 1947.

In January 1948, it was found in a sample of soil taken at the side of the small stream entering the Engineer's pond at the southwest corner.

Hips seemed to have been the most successful of the bait used.

Origin of cultures I.not recorded; probably southwest pond, in March; certainly before June 1947, when drawings were made of it in single-spore culture.

II.Southwest pond 25th November 1947; single-spore culture made in December 1947.

Observations and drawings of the oogonia of A. colorata in single-spore culture have been few, as neither isolate has produced oogonia in any quantity since December 1947 when a fair number appeared in a culture of II, though a very few (described as "rare") were observed in a culture of I in January 1948.

Discussion. Coker (1923, p.108) has commented that "smooth or nearly smooth oogonia may appear rarely in this species," but these are easily distinguished from the oogonia of A. racemosa by the oospores which are larger than in the latter. Although a large number of observations on the Royal Holloway College isolates has not been possible owing to the scarcity of oogonia, it is clear that this species shows considerable variation in the form of the oogonium, since both spiny and almost smooth-walled oogonia have been observed in single-spore cultures of isolate I.

Coker (1923) has made no comment on the renewal of the sporangia by proliferation, in A. colorata; he has written of A. imperfecta, "It was in this species that we observed the only two cases of proliferation through empty sporangia that we have found in Achlya," and also, "we know of no reference in the literature to internal proliferation of any kind in Achlya except by Petersen," and has quoted Petersen's statement in which he says that he has seen "zoosporangia which had proliferated in undoubted species of Achlya."

A.colorata is included in Ramsbottom's list (1916); it has been found at Cambridge by Sparrow (1936), and at Bristol and Manchester by Forbes (1935 a and b), and at Aberystwyth by Brown (1938).

Summary. 1. A. colorata Pringsheim can be collected at most times of the year from the south ponds; it is also present in the soil around the Engineer's pond; there is some slight evidence of its particular abundance in spring.

2. Both spiny oogonia and oogonia with walls that are almost smooth may be formed by the same isolate, in single-spore culture; (the spines, however, are blunt, and more irregular than those of A. radiosa).

3. The sporangia are renewed by proliferation as well as by subsporangial branching.

4. The conditions of culture during the present study have not favoured the production of oogonia, as cultures from recent collections have been observed to produce them abundantly, while the isolates have remained sterile.

Fungus 2: identified as Achlya racemosa Hildebrand.
(Plate 2)

Outstanding features:

Oogonia with smooth, yellowish wall, and 1 - 9 oospores.

Antheridia 1 to several, arising close under oogonium.

Hyphae stout, fairly extensive.

Gemmae (figs. C 1 - 3) formed by the segmentation of the hyphae into cylindrical portions which are actively abstricted, or liberate zoospores through lateral openings while still in position (fig. C 3)

Sporangia (figs. A 1 - 5; B 1 - 4) cylindrical, sometimes slightly widening towards the tip (fig. A 5). Secondary sporangia are formed by sympodial branching and sometimes include a portion of the hypha immediately under the previous sporangium (figs. A 3-5)

Oogonia (figs. D 1 - 3; E 1, 2) usually terminal on short lateral branches, occasionally intercalary (fig. D 1), spherical. The wall is smooth and unpitted, and becomes distinctly yellowish; it seems to vary in thickness in different cultures. A slight solid or hollow upgrowth from the septum is often present (figs. E 2, D 2) 42μ - 60μ.

Antheridia borne on branches which usually arise close under the oogonium, below the septum (fig. E 2) but sometimes arise above the septum (figs. D 1 - 3). Occasionally, an antheridial branch which arises under one oogonium passes to another oogonium (fig. D 3). The number of branches is usually 1 to 3, but in some cultures there are up to eight rather long, slender branches on a single oogonium (fig. D 3). Antheridial tubes are frequently seen (figs. E 1, 2; D 1)

Oospores 1 - 9, frequently 5 or 6, centric when mature. 22μ - 28μ, mostly about 23μ - 25μ.

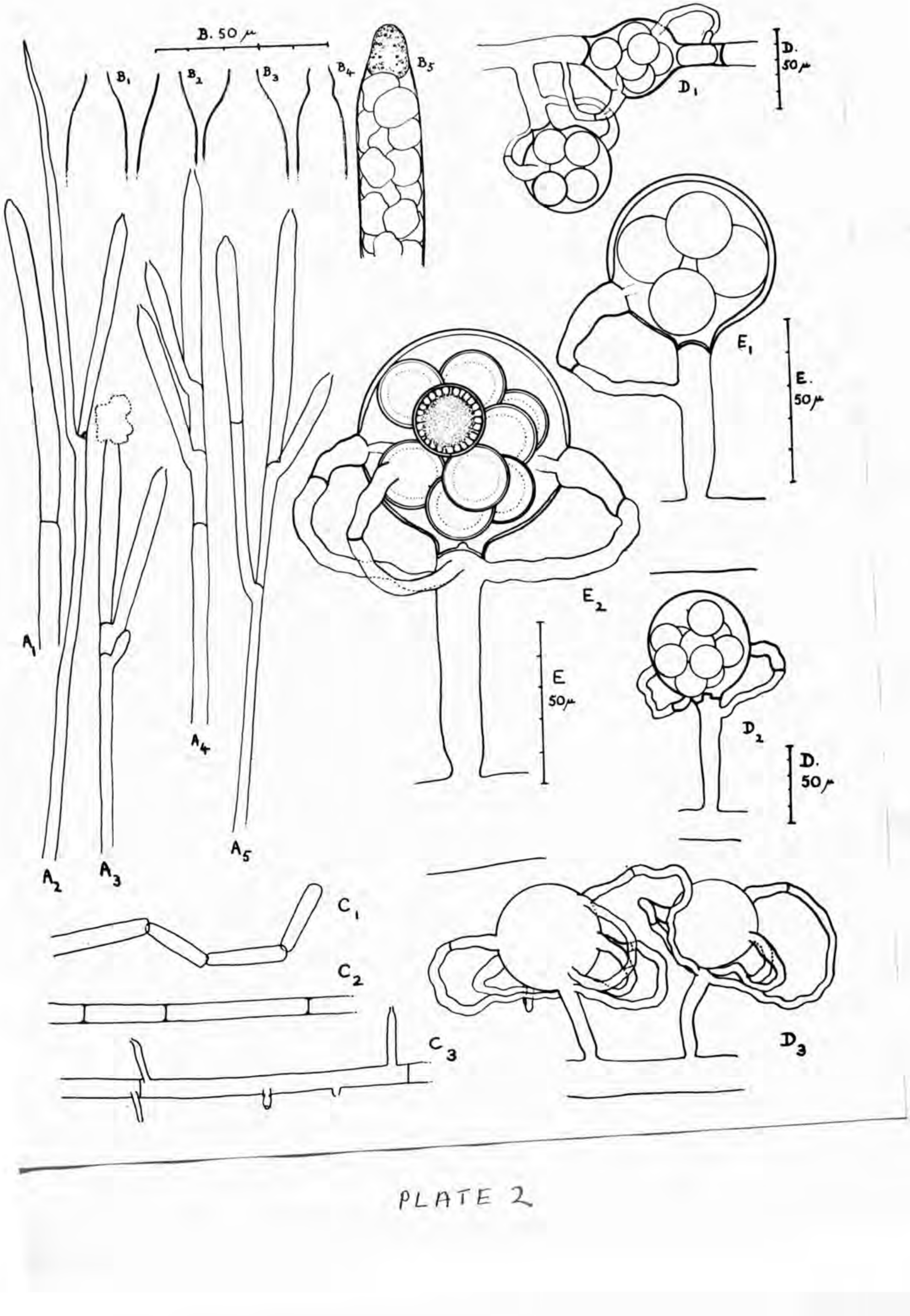

B. 50 μ
B1
B2
B3
B4
B5
A1
A2
A3
A4
A5
D1
D. 50 μ
E1
E. 50 μ
E2
E 50 μ
D2
D. 50 μ
C1
C2
C3
D3

PLATE 2

Achlya racemosa (continued)

Occurrence in the grounds.

Southwest pond. Collected in March (3 times) and May (4 times) 1947
January and April 1948

Southeast pond. Collected in May and June 1947
January 1948

Hazel's pond. Collected in December 1946
January and February 1948

Botany garden lilypond. Collected in March 1947

This species was also found in a sample of the soil beside the small stream which enters the southwest corner of the Engineer's pond.

It is often found with A. colorata and A. radiosa on hips which have been used as bait.

Place of origin of isolate. Hazel's pond (collected 12th December 1946). A single-spore culture was made on 25th May, 1947. This isolate has changed slightly during the period of culture. It seemed as though continuous water culture caused a decline in vigour, as oogonia were produced only sporadically throughout most of 1947. The oogonia had thinner walls and there were a large number of rather long, slender antheridial branches. Some months of culture on agar slopes seem to have partly restored the original vigour, for cultures in 1948 have produced oogonia more readily, with thicker walls and fewer antheridial branches. The hot weather may have contributed towards the production of a remarkably large number of oogonia in the culture in May 1948. Fresh cultures examined soon after their collection from the pond have been found to produce normal thickwalled oogonia with 1 - 3 antheridia, as was shown in a collection from the Southwest pond in April 1948.

Discussion. The characteristic features of the Royal Holloway College isolate show clearly that it is A. racemosa Hildebrand, but it differs in certain features from the description given by Coker. Coker describes the oogonia as "plentifully developed in all cultures," and the gemmae as "usually few." The abundance of oogonia relative to that of gemmae is probably related to the vigour of the culture, for the formation of gemmae by the Royal Holloway College isolate has been particularly noticeable in cultures in which oogonia were rare or absent. The oospores in Coker's description, which average 22 μ in diameter, are smaller than those of the Royal Holloway College isolate, which are mostly 23 μ - 25 μ, but Coker comments that they are "variable in size," and range from 16.6 μ - 27.7 μ in diameter. With regard to the number of oospores in an oogonium, Coker writes:- "1 - 8 in an oogonium (Humphrey says 1 - 10), in most cases 2 - 5." Thus, the differences between the Royal Holloway College fungus and the material described by Coker are negligible.

A. racemosa is included in Ramsbottom's list (1916) and has been collected at Manchester by Forbes (1935b) and at Aberystwyth by Brown (1938). It has been collected from the soil in Glamorgan by Ivimey-Cook and Morgan (1934).

Summary. 1. The Royal Holloway College isolate is undoubtedly *Achlya racemosa* Hildebrand.

2. Some slight changes shown by the Royal Holloway College isolate during its period culture of seventeen months, seem to be related to the decline in vigour caused by continuous water culture. It has retained its characteristic features.

Fungus 3: identified as *Achlya radiosa* Maurizio
(Plates 3 and 3a)

Outstanding features:

Oogonia produced readily and abundantly after a very short sporangial phase. Densely covered with sharp spines. Usually containing a single oospore.

Antheridia formed on 1 - 3 branches from the oogonial stalk.

Hyphae fairly stout, not extensive.

Gemmae absent.

Sporangia (fig. A 1) cylindrical, not much wider than the hyphae; secondary sporangia not formed often.

Oogonia (figs. A 1 - 3; B 1 - 6) exceedingly abundant and readily formed, appearing after a few days; on lateral branches which gradually widen towards the oogonium, and occasionally branch; covered densely with spines which arise as hollow papillate projections, with thickenings of the wall; the protoplasm gradually withdraws from the spines to form the oosphere (figs. B 1 - 3). The spines are usually sharply-pointed (fig. B 5), sometimes slightly bent (fig. B 3); sometimes they arise in incompletely-divided pairs (fig. B 6); it appears as though they may take the latter form in certain oogonia chiefly in one plane.

Antheridia on one, two or occasionally three short branches usually from the oogonial stalk, very occasionally from the main hypha; antheridial tubes frequently observed (fig. B 3)

Oospores usually one, rarely two. 35μ - 45μ, mostly 38μ - 40μ. Very dark at first (fig. B 3), lighter, with a centric structure when mature (fig. B 5), the central protoplasm being surrounded by a single layer of relatively large oil globules, which appear in surface view in Fig. B 4.

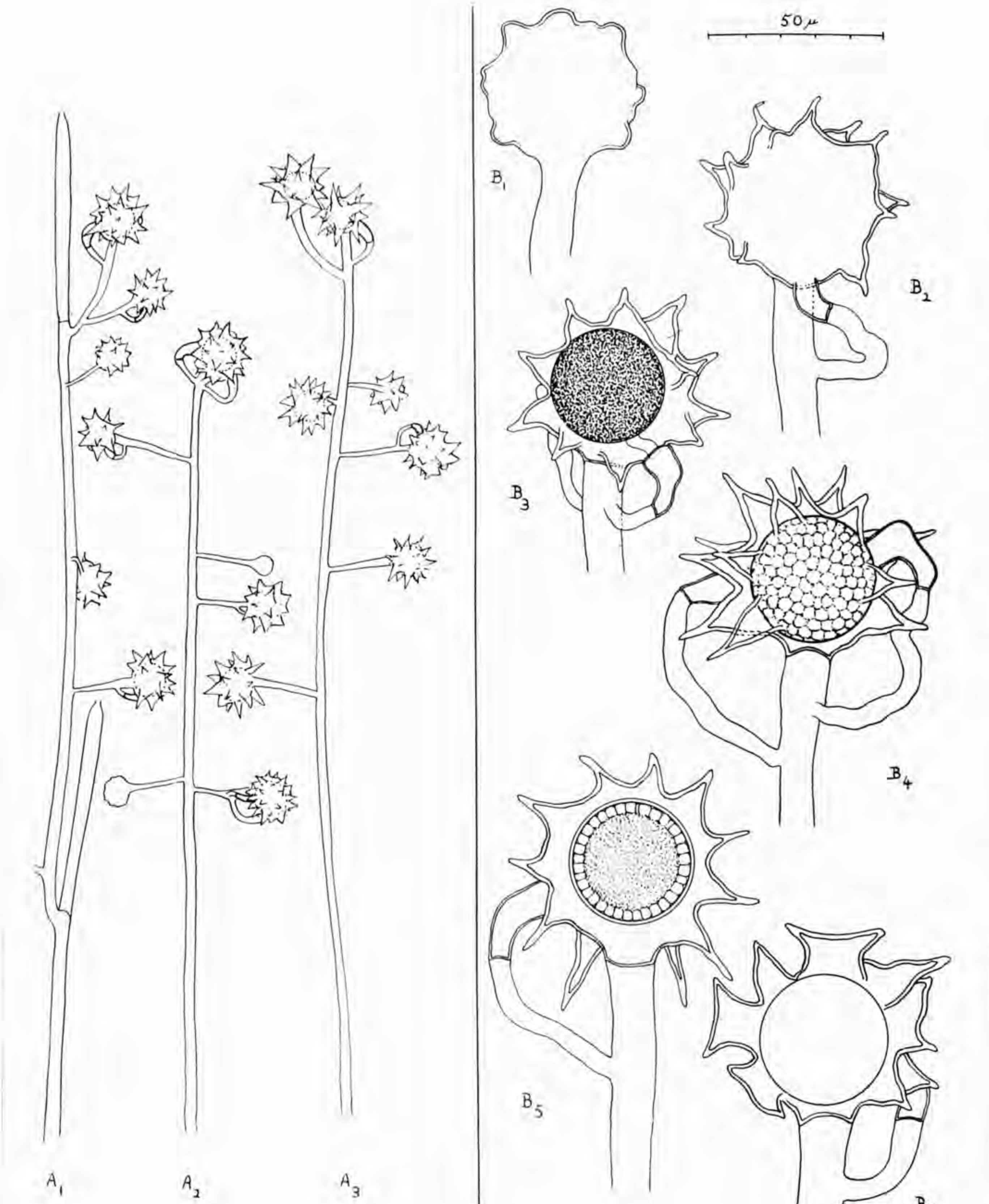


PLATE 3

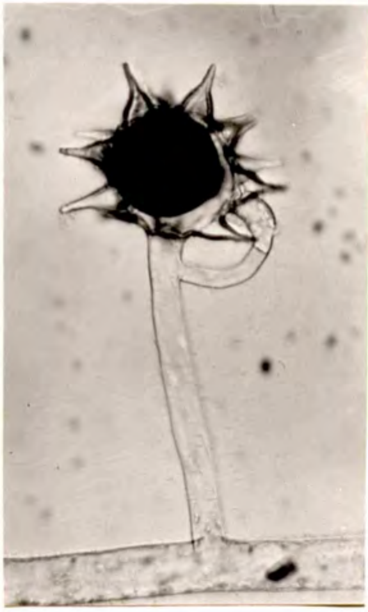


PLATE 3 a

A. radiosa. Oogonium. x 320

Achlya radiosa (continued)

Occurrence in the grounds.

Hazel's pond: April 1945 (slide); and almost every collection that has been made during the present study; thus it has been found in:

September and December, 1946

March, May and June, 1947.

Southeast pond November 1940 (slide).

Southwest pond November 1946

Despite the frequency of the collections from southwest and the botany garden ponds, it has not been recorded again from the former, and does not appear in the lists of the latter.

In January 1948 it was found in two separate samples of the soil around Engineer's pond, taken from either side of the small stream entering the pond at the southwest corner.

A. radiosa is often found together with A. colorata and A. racemosa on the hips used as bait.

Place of origin of isolate. Hazel's pond (collected 2nd December 1946). A single-spore culture has not been obtained. The drawings and observations have been made from single-hypha cultures.

Discussion. There is no doubt as to the identity of this fungus as Achlya radiosa Maurizio. The sharp spines are very characteristic.

Fungus 4: identified as *Achlya imperfecta* Coker
(Plate 4)

Outstanding features:

Sporangia even stouter than the hyphae.

Oogonia abundant, on short lateral branches, often disorganising without forming oospores.

Antheridia on slender antheridial branches which are often much branched.

Oospores eccentric, mostly 23μ in diameter.

Hyphae stout

Gemmae formed by the segmentation of the hyphae into short, sometimes swollen portions.

Sporangia (figs. A 1 - 4; B 1 - 3) plentiful, rather thicker than the hyphae, sometimes widening considerably in the region about a third of the length of the sporangium behind the tip (fig. A 2); sometimes particularly stout, and rather short. Secondary sporangia often consist partly of a portion of the hypha immediately below the previous sporangium (fig. A 3). Sometimes a hypha segments in portions which act as sporangia and discharge by lateral openings. (fig. A 3)

Oogonia (figs. A 4, 5; C 1 - 5) abundant; terminal on short lateral branches which are sometimes bent, especially in young cultures; (fig. A 5) spherical, sometimes with a neck. 44μ - 100μ or more, but mostly 58μ - 63μ; Remarkable variety in the amount of pitting of the wall is shown. Thus, some oogonia are apparently unpitted (figs. C 1, 4), some have a few or several pits (figs. C 2, 3) and a considerable proportion, especially the very large oogonia, have exceedingly numerous very small pits (fig. C 5). An upgrowth from the septum is often present (fig. C 3). The first oogonia to be formed are often rather small, and without antheridia. In older cultures there is usually quite a large proportion of very large oogonia. Many of the oogonia degenerate without forming oospores; sometimes a second oogonium grows out from the first, using the contents of the latter, which is left empty (fig. 5).

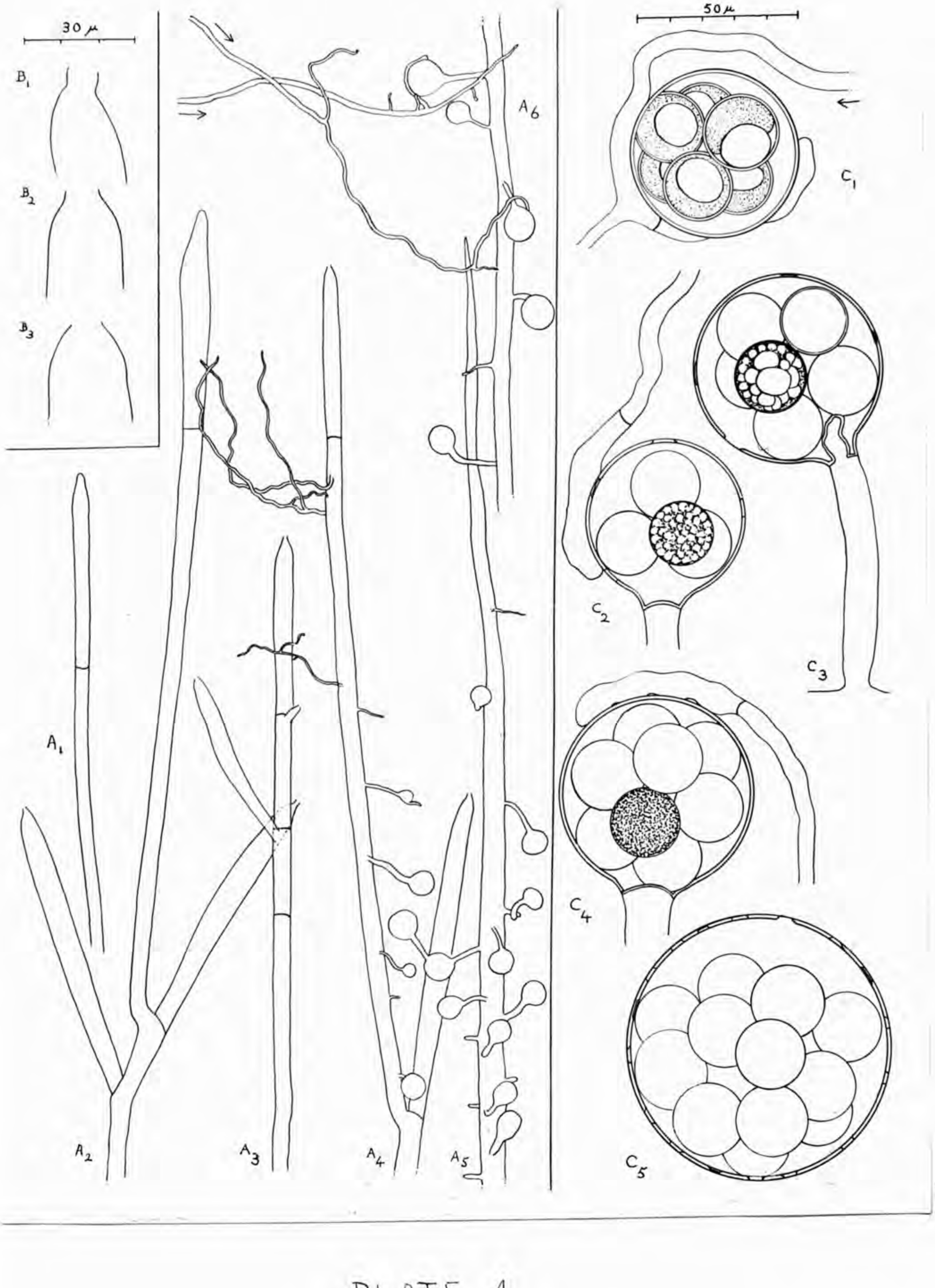


PLATE 4

<u>Antheridia</u> borne on narrow branches which originate from the same main hypha as the oogonial stalks or from different hyphae. Often these branches arise in tufts towards thetips of the main hyphae in young cultures (fig. A 4) but they may be found springing from almost any region of the hyphae in older cultures. They are often much branched, though not as much so as in <u>A. flagellata</u>.

<u>Oospores</u> 3 - 30, or rarely more, usually 6 - 12; 21μ - 23.5μ The number of the oospores is related to the size of the oogonium, in this species (cf. <u>A. apiculata</u>). Small oogonia have few oospores (fig. C 2) while large ones have many (fig. C 5). Newly formed oospores are very dense and dark (fig. C 4) but become lighter as the oil droplets separate out (fig. C 2). Mature oospores are eccentric, with a thick wall. (The oospores represented in fig. C 1 are unusually thin walled). Many oospores fail to reach maturity, becoming disorganised, and forming a number of oil globules of various sizes (fig. C 3)

Achlya imperfecta (continued)

Occurrence in the grounds. This species has been observed several times in collections from the botany garden lilypond, but as it is difficult to distinguish from A. flagellata in mixed cultures, the dates of its appearance have not been recorded.

Place of origin of isolate. Unrecorded, probably the botany garden lilypond. A single-spore culture was made on 10th June 1947, from which a single-hypha culture was made in April 1948, as some doubt was felt as to the purity of the culture, on account of the remarkable variety shown in the size of the oogonium, the degree of pitting of the oogonial wall, and the number of oospores. The new culture again showed this variety.

Discussion. A. imperfecta was erected by Coker as a new species forming, together with a number of closely similar species, "the Prolifera-de Baryana group." Coker has written:- 'In attempting to put into order the members of the Prolifera-de Baryana group we meet with some of the most perplexing problems in the entire family." These problems are discussed by Coker in pp. 96 - 7 of his monograph. The Royal Holloway College isolate resembles the fungus described by Coker as A. imperfecta, except that the oogonia are larger and contain more numerous, and slightly larger oospores, of which a considerable proportion reach maturity. These differences are shown in the following lists:

	R.H.C. isolate	A. imperfecta Coker
Oogonia	44 μ - 100 μ mostly 58-63 μ	37 μ - 60 μ, mostly 40 μ -45 μ
Oospores	3 - 30, usually 6 - 12	2 - 8, usually 4 - 6
	21 μ - 23.5 μ, mostly 22 μ - 23 μ	17 μ - 23 μ, mostly 19.5 μ - 20 μ.
	mostly reach maturity	mostly "go to pieces before maturity"

The failure to reach maturity, of a larger proportion of the oospores formed by Coker's fungus than by the Royal Holloway College isolate, together with the generally smaller dimensions of the oogonia and oospores, may possibly be the result merely of differences in the vigour of the cultures. In spite of this feature influencing Coker's specific epithet, he has not rated it very highly, as he has written:- "Among such a confusion of forms, or descriptions of forms, it is a pleasant relief to find one described that is apparently just like ours. This is Minden's A. de Baryana var. intermedia, which seems identical except that he does not mention the early dissolution of most of the eggs."

Conclusion. The Royal Holloway College isolate is A. imperfecta Coker, but the failure of the oospores to reach maturity is a less pronounced characteristic than in the fungus described by Coker.

A. imperfecta has been found in Glamorgan by Ivimey-Cook (1936).

Fungus 5: identified as Achlya apiculata de Bary
(Plates 5)
and 5a

Outstanding features

Mycelium   Coarse and rather extensive, hyphae becoming segmented.

Oogonia   sporadic; frequently apiculate, formed on thick lateral branches which are often conspicuously curved.

Hyphae stout, forming mycelium which is extensive - though less so than in A. oblongata.

Gemmae formed especially near the surface of the water, where the hyphae become segmented into long cylindrical portions which are usually abstricted, but occasionally discharge zoospores by a lateral opening, while still in position. (fig. A 4)

Sporangia (figs. A 1 - 4; B 1, 2) fusiform; of a width, in the middle, considerably greater than that of the hypha; tapering, often to a point (fig. A 1) Germination of the spores while still within the sporangium quite often observed, even in quite vigorous cultures.

Oogonia (figs. C 1 - 4) usually on a thick lateral branch, which, frequently, is curved (fig. C 4) sometimes making a complete turn. 51μ - 92μ usually about 68μ; spherical or ovoid, with a short neck; frequently with an apiculus, which may be short (fig. C 3) or long, sometimes curved (fig. C 1); wall smooth and unpitted.

Antheridia one or several to each oogonium, on branches arising as often from the oogonial stalk as from the main hypha, and sometimes from the main hypha, antheridial tubes not observed.

Oospores 1 - 8, usually 3 or 4; extremely variable in size - 20μ - 40μ, often about 30μ; their size is apparently unrelated to the size of the oogonium; mature oospores centric (fig. C 4).

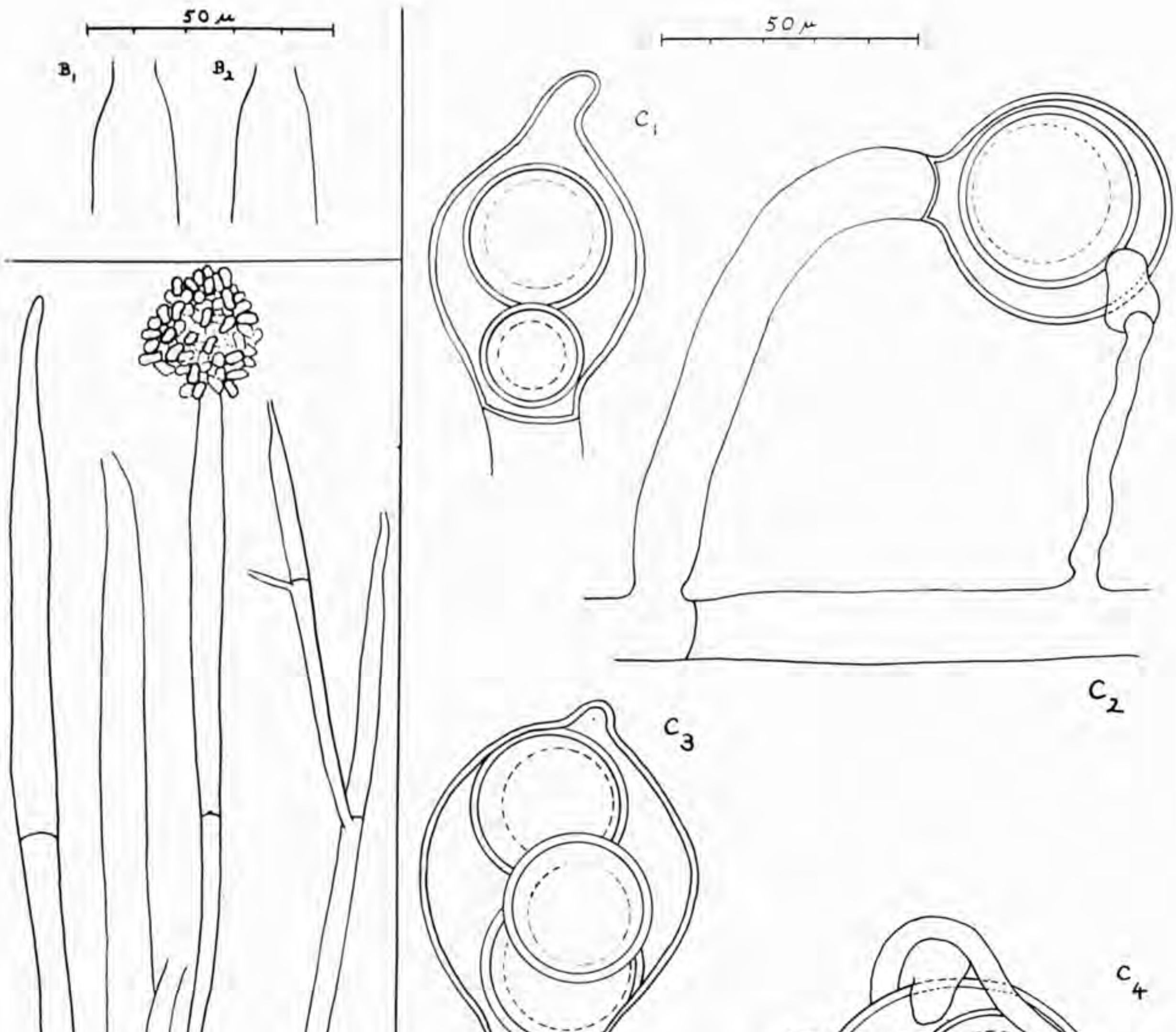


PLATE 5

X 74

X 32

X 32

PLATE 5a

Oogonia recorded directly on sensitive paper.

Achlya apiculata (continued)

Occurrence in the grounds. A tentative record of its collection from the southeast pond was given in 1938. It was collected from the southwest pond on 6th March 1947 and from the southeast pond on 6th May 1947. (It had been collected some time during the autumn 1946, but the origin of the culture had not been recorded.)

Place of origin of isolate. Southwest pond (collected 6th March 1947). This fungus was not identified until 22nd May, when oogonia were observed in the culture. A single-spore culture was made on 25th May 1947.

Discussion on the formation of oogonia. As with A. oblongata, the sporadic somewhat rare production of oogonia in culture has probably prevented its identification more often in the collections, though apparently sterile species with the features of the hyphae and sporangia of A. apiculata have been cultured over long periods, e.g. a culture collected from the botany garden pond on 21st October 1947 was abandoned on 21st November, as no oogonia had appeared, though the segmentation of the hyphae, and the sporangia of a shape characteristic of A. apiculata were recorded for this culture. Oogonia appeared in the cultures intermittently up to December 1947; none have been observed since, probably owing to a decline in vigour due to long periods of culture in water, on the same substratum. It was observed on 9th July 1947, that while oogonia had been formed abundantly by growths on tiny pieces of hemp seed, growths on larger pieces in the same dish had remained sterile. The contrast was very striking, and recalled the generally-accepted view that oogonial production follows depletion in the foodsupply.

Discussion on the identity of the species. The Royal Holloway College isolate agrees well with the description of Achlya apiculata de Bary by Coker, who has commented that the oogonia are "not formed regularly or abundantly except at low temperature" and are produced on branches "which are usually bent and sometimes make a complete turn."

A. apiculata var. prolifica Coker and Couch is said to be distinguished from the species by the following features:-

Regular and abundant production of oogonia at room temperature; smaller size of the oogonia (55μ - 65μ, as against 70μ - 80μ); smaller number of eggs in an oogonium (1 or 2) as against 3 - 5, occasionally 10); greater frequency of apiculate oogonia.

The Royal Holloway College isolate has shown a considerable amount of variation; thus, in a culture examined on 27th June 1947, very few oogonia were apiculate, while a comment was made on 1st November, "apiculus common and pronounced;" in the November culture, a large proportion of the oogonia contained only a single oospore, very few had five, none had more; when the culture was examined again ten days later, the proportion of oogonia with more than two oospores was exceedingly small.

However, although the Royal Holloway College isolate has appeared to present some of the features of both the species and the variety, at different times, oogonium production cannot be described as regular, the characteristic of the variety which is given first place by Coker and Couch.

A. apiculata is included in Ramsbottom's list (1916), and has been collected from the soil of Glamorgan, by Ivimey-Cook and Morgan (1934). Forbes has found it at Bristol (1935a) and Manchester (1935b), and has recorded also A. apiculata var. prolifica in the Bristol list.

Summary 1. There is no doubt as to the identity of the Royal Holloway College fungus as Achlya apiculata de Bary.

2. Single-spore cultures of this fungus have at certain times shown a number of the features of A. apiculata var. prolifica, but has never shown the regularity in oogonial production which is specially characteristic of this variety.

..us 6: identified as Achlya oblongata de Bary
(Plate 6)

Outstanding features

Mycelium	particularly stout and extensive
oogonia	sporadic, large, ovoid or spherical, with thin unpitted walls.
oospores	numerous, not filling the oogonium.
Antheridia	numerous, tuberous, or delicate diclinous branches.

Hyphae stout, forming a mycelium which is extensive, more so than in A. apiculata.

Gemmae formed especially near the surface of the water, by the segmentation of the hyphae into long cylindrical portions; these are not usually abstricted actively, but can be disconnected by movement of the water or other mechanical means.

Sporangia (figs.A 1 - 3; B 1 - 3) characteristically fusiform and pointed (fig.A 2); the spores encyst in a large hollow sphere at the mouth.

Oogonia (figs.A 3; D 1 - 4; E 1 - 2) terminal on thick lateral branches, which may be short (fig. E 1) or long (fig.A 3) Very rarely intercalary. Very large, 74μ - 123μ, often 100μ, measured "the short way" (Coker 1923, p.132); ovoid (fig. E 2) or pyriform (fig.D 4); quite often spherical, with or without a neck (figs. D 1 and E 1 respectively); wall very thin, and unpitted; a solid or partly hollow upgrowth from the septum is quite often present (fig.D 4)

Antheridia numerous; small and tuberous; borne on slender diclinous branches which soon disappear. Antheridial tubes are seen frequently (fig. E 2); penetration of the oogonial wall is effected by a very narrow tube which widens out inside the oogonium (tex-fig.1); a bright, highly refractive band or area where the antheridium makes close contact with the oogonial wall is visible long after all other signs of the antheridium have vanished. (text-fig.2)

Oospores up to 30, usually 10 - 15, but quite often 25 - 30, sometimes 6 - 10; not filling the oogonium. Mostly 25.7μ - 28.6μ, but in a culture in January 1948 there was often a considerable range in the size of the oospores in a single oogonium. Frequently, and quite early, the oospores degenerate; those that reach maturity are subcentric, with a sheath of oil droplets to one side of the globule of protoplasm.

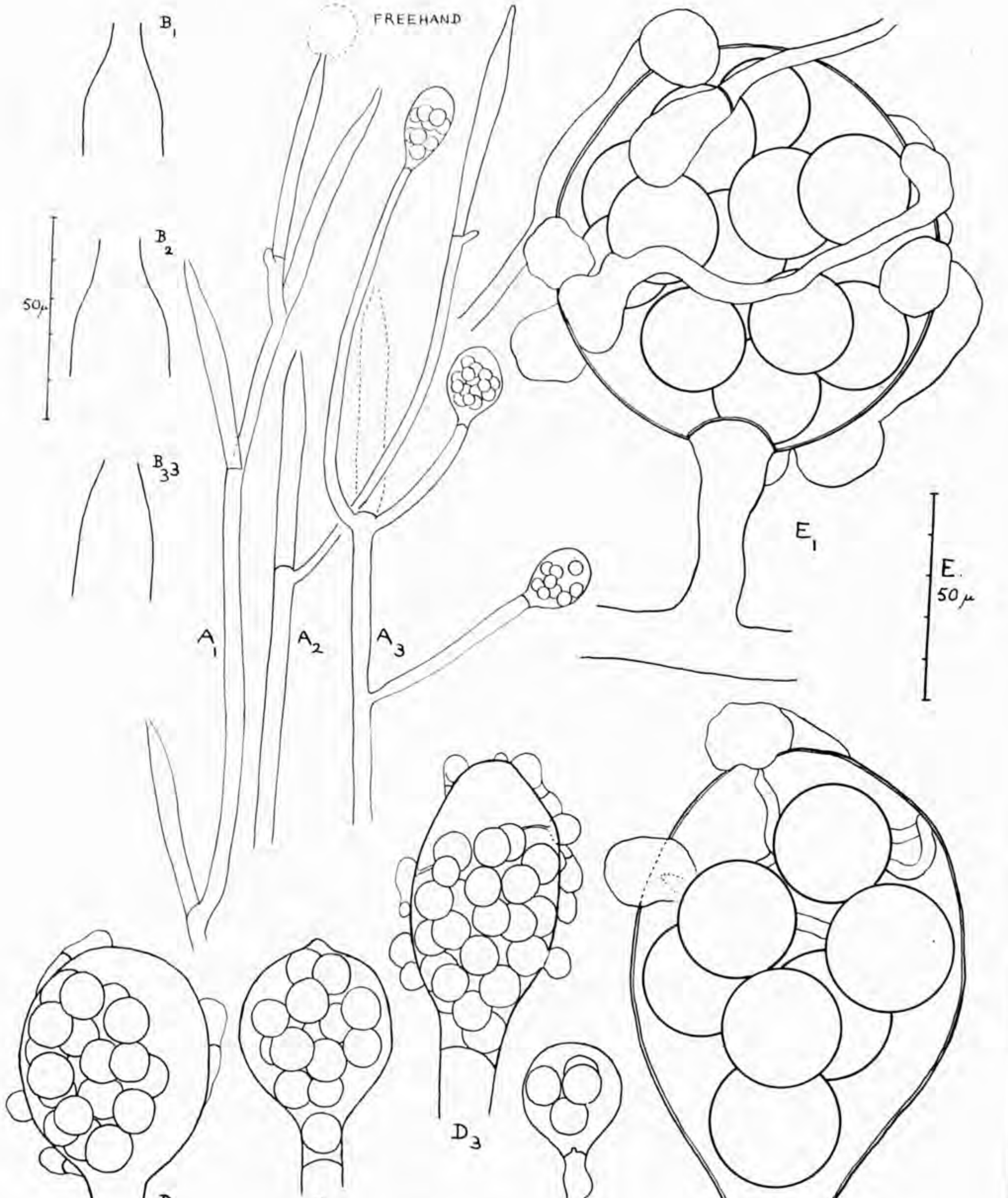


PLATE 6

Achlya oblongata (continued)

Origin of isolate. Probably from southwest pond. Some time during the autumn 1946, a pure culture was made from a single hypha. In November 1947 a single-spore culture was made.

Though a number of cultures have been observed of a fungus with the features of the hyphae and sporangia of A. oblongata, these have remained apparently sterile, and this species has not been identified again.

Oogonia were observed in December, 1946 and at intervals during the spring 1947, but did not appear again until January 1948. The records are based on two sets of drawings and observation, made in December, 1946 (figs. E 1, 2) and in January 1948 (figs. A, B, D)

Discussion. The Royal Holloway College isolate is clearly Achlya oblongata de Bary, agreeing closely in several distinctive features, with Coker's description, though it is surprising that Coker has commented, "no fertilising tubes seen."

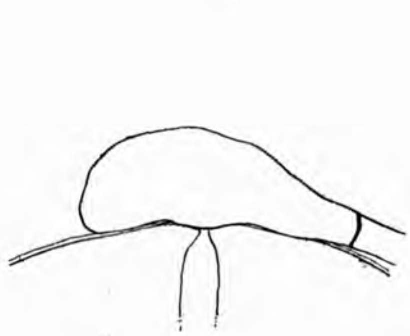
In 1892, A. oblongata var. globosa was distinguished by Humphrey (p.122) on the following features:-

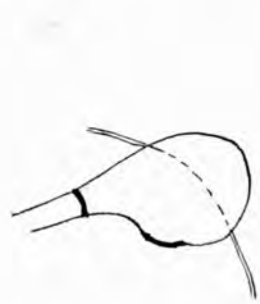
Oogonia "commonly larger than in the type," on very short branches; globular; "oospores reaching twenty-five in number, averaging ten to fifteen;" in the oogonium, "the space unoccupied by spores is much more marked, sometimes amounting to more than half of the cavity."

The Royal Holloway College isolate seems to show the features of the variety as well as of the species; thus, the oogonia, which are often "globular", have been found on both short and long branches; the amount of the oogonial cavity unoccupied by oospores is often large; it was especially so in the January 1948 culture. As the size of the oogonia is so variable, the value of this feature in distinguishing a variety cannot be stressed. It appears, then, that the R.H.C. isolate offers some evidence against the erection of "var. globosa."

Achlya oblongata was found at Bristol by Forbes (1935) who also distinguished A. oblongata var. gigantica on the following features:-

The "outstanding size of the oogonia and oospores;" the "rather cylindrical pointed shape of many oogonia;" and the smaller number of oospores that reach maturity. She gave three figures. No fungus with these characteristics has been found in Royal Holloway College grounds.





Fungus 7: identified as Achlya recurva Cornu
(Plate 7)

This record is doubtful. The culture, collected from the southwest pond on 29th April, 1947, seemed at first to be sterile, but oogonia were observed on June 27th 1947. These oogonia were unhealthy, the contents of many of them having degenerated into a central mass (fig. B 1). Only rough drawings were made, and the scale can only be conjectured. Oogonia have not been observed since.

Discussion on the identity of this fungus. At the time of the publication of Coker's monograph (1923), Achlya recurva had not been found in America. As for Europe, von Minden (1812) termed it "Cornu's inadequately described and therefore doubtful species," and described a fungus which he referred to it. The Royal Holloway College fungus agrees with this description in the features which are listed below:-

The mycelium is coarse, consisting of stout hyphae.
The sporangia are few.
The oogonia are terminal, either on main hyphae (fig.A 2) or on side branches which are slightly curved. According to the conjectured scale, oogonia about 50μ (fig.B 1) or about 60μ (fig.B 2) are formed. These fall within the range given by von Minden, 50μ - 90μ.
The oogonia are covered with blunt, hollow spines.
The antheridia are borne on branches which arise either from the oogonial stalk, or from the main hypha. In von Minden's fungus the number was usually 3.

The Royal Holloway College fungus disagrees with von Minden's description in the following features:-

The oogonia are not numerous.
The oospores are not so numerous as to be "mostly about 10", though this difference may be related to differences in the vigour of the cultures.
According to the conjectured scale, the oospores are over 30μ in diameter; von Minden gives the range as 22μ - 27μ. This difference need not be emphasised, however, on account of the inaccuracy of the drawing.

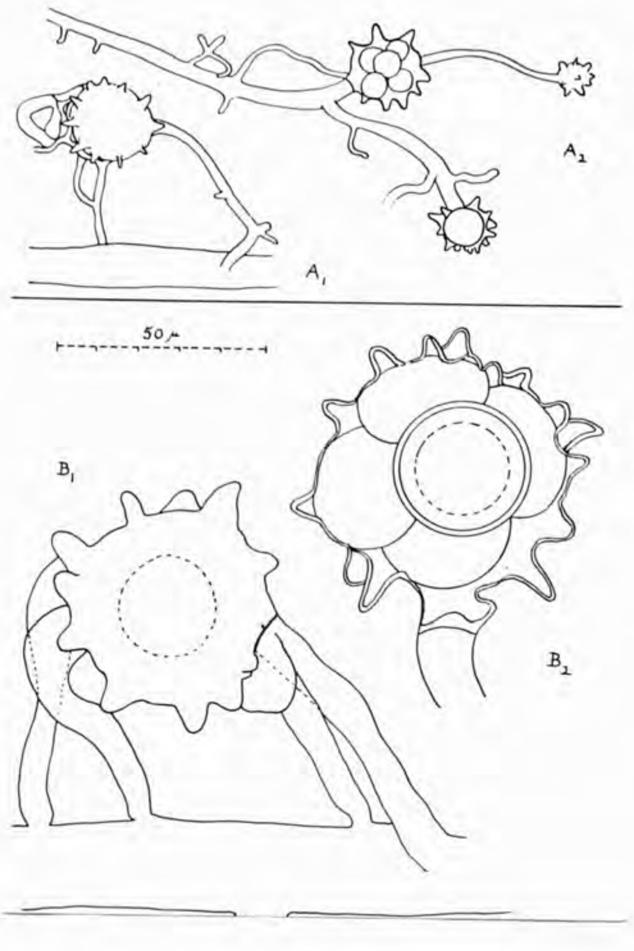


PLATE 7

A. recurva Cornu is included in the list of Saprolegniaceae collected at Bristol by Forbes (1935a), who has given a description which agrees in essential features with that of von Minden. Forbes,has, however, given the usual number of oospores as 5 - 8, and their size as "25μ - 35μ, mostly 27μ - 29μ". In these features, the Royal Holloway College fungus is more nearly similar to Forbes' than it is to von Minden's. The evidence in favour of its identification as A. recurva is thus strengthened.

Conclusion. The Royal Holloway College fungus is probably Achlya recurva Cornu.

us 8: identified as Saprolegnia ferax (Gruith)Thuret
(Plate 8)

Outstanding features

Oogonia abundant, on curved lateral branches, extremely variable in form, but often pyriform

Antheridia present on a very small percentage of oogonia.

Hyphae fairly stout, not usually forming a very extensive mycelium.

Sporangia (figs.A 1 - 5, B 1, 2) plentiful, repeatedly proliferatig; not usually much wider than the hyphae (fig.A 1) but sometimes bulbous where a secondary sporangium exceeds in length the empty sporangium through which it is growing (figs.A 2, 5). A discharged sporangium usually has a well marked collar (figs.B 1, 2)

Gemmae (figs.C 1 - 4) only fairly abundant; irregular; usually elongated.

Oogonia (figs.D 1 - 7; E 1 - 5) exceedingly readily and abundantly formed, sometimes after only 4 or 5 days; first-formed oogonia often terminal on a main hypha, later ones typically on a lateral branch which may be short (fig. D 1) or long (fig. D 5) and is usually curved.
First-formed oogonia usually spherical or pyriform, with a neck (figs. E 4, 1); later ones more variable frequently intercalary (figs. D 2, 6); sometimes, when oospores are formed in the adjacent hypha, with both a spherical part and a "threadlike extension" (cf. Coker 1923 p.40) (figs.D 4, 7); not rarely formed inside empty sporangia (fig.D 3) Up to 86μ, but mostly 60μ - 70μ; wall conspicuously pitted; pits 8.6 - 11.4 , mostly about 10 ; a hollow (fig.E 1) or solid (fig.E 4) upgrowth from the septum is frequently present.

Antheridia (fig.E.5) present on very few oogonia, sometimes apparently absent from a culture; antheridial branch usually arising near the oogonial branch, from a main hypha. Antheridial tubes observed occasionally.

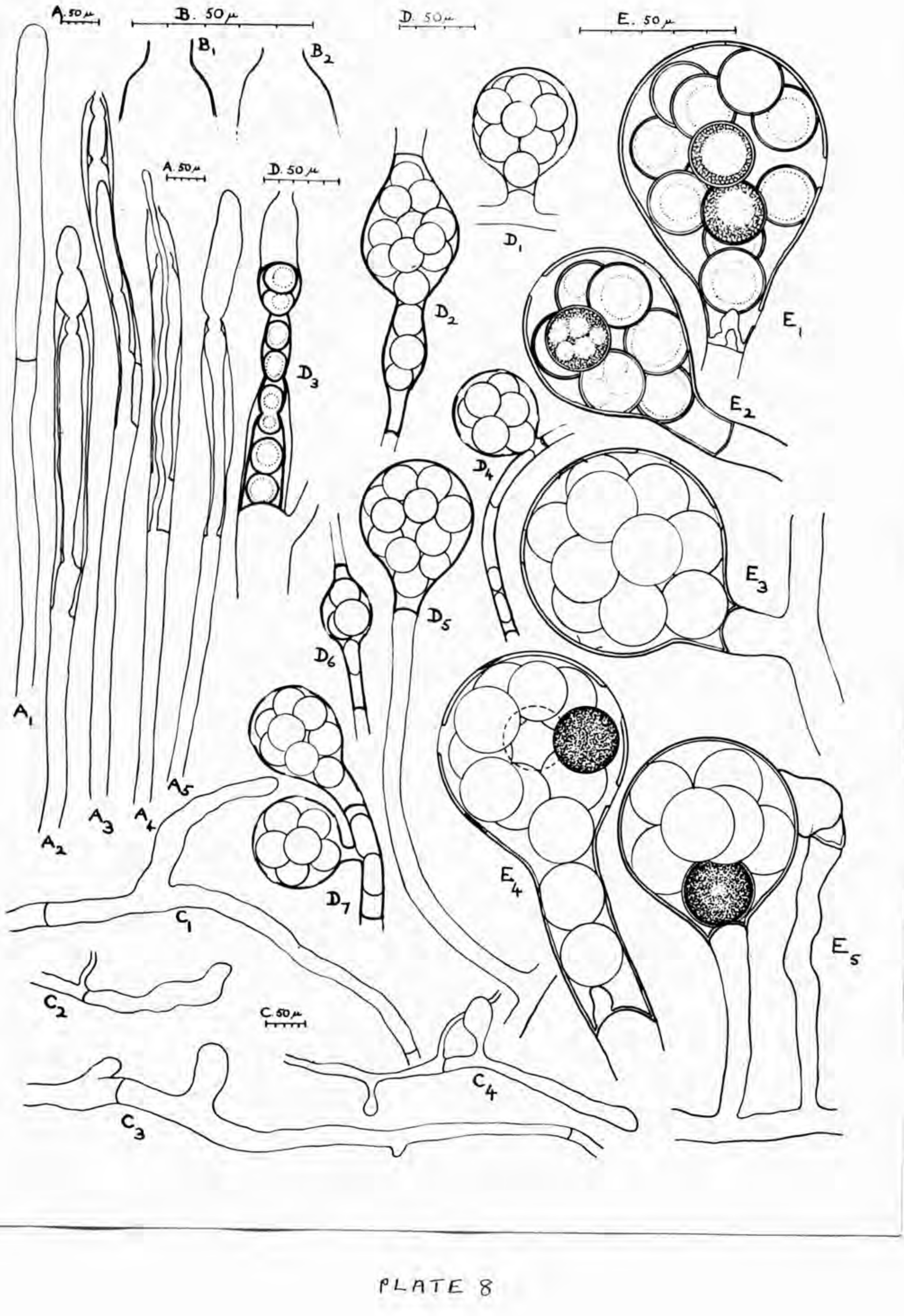

A. 50 μ
B. 50 μ
D. 50 μ
E. 50 μ
B1
B2
A. 50 μ
D. 50 μ
D1
D2
D3
D4
D5
D6
D7
E1
E2
E3
E4
E5
A1
A2
A3
A4
A5
C1
C2
C3
C4
C. 50 μ

PLATE 8

<u>OOspores</u> 2 - 30 or more, often 10 - 15 in vigorous cultures in which there is also a fairly high proportion with 30, not always filling the oogonium; usually spherical but sometimes elliptic through pressure (figs.D 4,6,7) 21µ - 27µ, usually 23µ - 26µ; very dark at first (fig.E 4) developing a conspicuous centrum; becoming paler as the oil droplets separate out from the protoplasm (fig.E 2); centric when mature (fig.E 1)

Saprolegnia ferax (continued)

Occurrence in the grounds.

Botany garden lilypond. S. ferax appears very frequently in collections from the botany garden lilypond, and was found regularly, almost every week, from September 23rd to 25th November 1947, when it was quickly and easily identified in the laboratory, as oogonia were formed abundantly in less than 10 days. It also appeared at intervals in the early months of 1948 - twice in January (once when ¾ inch of ice had been broken through, to collect the pond water) once in February and once in April.

Other ponds

Southwest: Collected in October, 1935; 1938 (slides)
" April, October 1947

Southeast: Collected in November 1938 (slide)

Engineer's: Collected in May 1947

Hazel's: Collected in July 1947

Places of origin of isolates. I. Engineer's pond (Collected 31st May 1947) single-spore culture made at the end of June.

II. Southwest pond (collected 21st October 1947); single spore culture made in November. These two isolates agree closely in all essential features. When compared, after similar treatment, in December 1947 and January 1948, the oogonia of I were fewer, rather smaller, and took longer in appearing than in II; growth was more extensive and less dense, and there was a larger number of gemmae; they were , however, easily recognisable as the same species. The difference in vigour was probably due to the fact that I had been kept in continuous water culture longer than II. In April 1948, when both islates had been kept on agar slopes for some months, I closely approached II in vigour. When I and II were compared in February 1948 with a culture of S. ferax (Gruith) Thuret from Baarn, the Baarn isolate was essentially similar, but appeared to be slightly more vigorous - some oogonia reached 90 in diameter, and there seemed to be fewer small ones; intercalary and "threadlike" oogonia were perhaps more frequent in the Baarn culture.

Discussion. The Royal Holloway College fungus agrees closely with Coker's description of S. ferax, except that the oogonial pits are larger (8.6μ - 11.4μ as against 4.5μ - 5.5μ in Coker's material) and that the oospores seem to be more numerous, on the average, since Coker reports "1 - 20, mostely 4 - 16", while in the Royal Holloway College isolates there are frequently 10 - 15 and in vigorous cultures, a considerable proportion of rat rather large oogonia contain 30 or more oospores. Coker has commented, "neither Humphrey nor we find the large number of eggs, up to 40 - 50, in an oogonium which are recorded by de Bary and other European writers who seem to have copied from him."

As Forbes (1935) has pointed out, it seems probable that the two "forms" described by von Minden "merge into one another," "Form 2" is said to be distinguished by forming .

oogonia typically "on very short side branches," but often in empty sporangia, while "Form 2" forms terminal oogonia, "often on long, bent stalks," and cylindrical oogonia are rare. As in Forbes' material, the Royal Holloway College isolates have seemed to show the features of both of von Minden's "form" in the same culture, the appearance being that of "Form 1" in young cultures, and of "Form 2" in older ones.

S. ferax is included in the lists of Massee (1891) and Ramsbottom (1916), and was collected by Ivimey-Cook (1936) in Glamorgan, Forbes (1935a, b) at Bristol and Manchester, and Brown (1938) at Aberystwyth.

Details of the species are given only by Forbes, who has discussed the taxonomy (1935a), and the seasonal periodicity (1935b), suggesting that S. ferax and S. monoica may be growth forms of the same species.

Ivimey-Cook and Morgan (1934) noted its absence from collections from the soil in Glamorgan, commenting that "it is by far the commonest in water samples."

Summary. 1. Saprolegnia ferax is widely distributed in the Royal Holloway College grounds, and can be collected throughout the year, but it appears to be more abundant, in the botany garden lilypond, in the autumn. (This is in agreement with the autumn peak found by Forbes).

2. Two isolates have been made. The essential features have remained constant over a year of culture in the laboratory, though there has been some decline in vigour.

3. The Royal Holloway College fungus is clearly the same as the one described by Coker as S. ferax (Gruith) Thuret, though it shows the tendency which seems to be characteristic of the species in Europe, to form a greater number of oospores per oogonium, on the average than the American species.
Like that of Forbes, the Royal Holloway College fungus seems to show the features of the two "forms" as distinguished by von Minden.

Fungus 9: identified as *Saprolegnia monoica* var. *glomerata* Tiesenhausen (Plate 9)

Outstanding features

Oogonia abundant, almost as readily formed as in S. ferax characteristically more spherical than in S. ferax; usually on very short lateral branches; often laterally sessile on main hyphae.

Antheridia present on all, or almost all, oogonia; usually several to each oogonium; typically on clustered and contorted branches.

Hyphae rather fine; mycelium not very extensive.

Sporangia (figs. A 1 - 6; B 1 - 3) not usually thicker than the hyphae, but widening slightly, towards the tip (fig. A 3); proliferating; frequently bulbous when a secondary sporangium exceeds in length the empty sporangium through which it is growing (figs. A 4, 5); later-formed ones often rather irregular (fig. A 6); sometimes segmented, discharging by lateral openings (fig. A 2); empty sporangia with a well-marked collar (figs. B 1 - 3)

Gemmae (figs. C 1 - 7) somewhat pearshaped (figs. C 5, 6) or irregular (figs. C 1 - 4, 7); not usually so elongated as in S. ferax.

Oogonia (figs. D 1 - 7; E 1 - 3) abundant, and readily-formed, sometimes within a week. Spherical; generally on short , lateral branches which are usually straight, but occasionally curved. (figs. E 3; D 2, 5, 6); frequently sessile, even including part of the hypha (figs. D, 1, 4, 7); sometimes intercalary (fig. D 3). 35μ - 90μ, mostly 65μ - 75μ; wall conspicuously pitted, pits 5.8μ - 9.8μ, mostly 8.6μ, frequently a hollow (figs. E 2; D 4) or solid (figs. D 3, 5) upgrowth from the septum is present.

Antheridia somewhat tuberous; applied by the apex to the oogonium (figs. D 6, 7); on branches which are typically "clustered and contorted" (cf. Coker 1923, p.51) and arise usually from the main hypha bearing the oogonium (figs. D 2, 5, 7) sometimes from a distant one (fig. E 3), often, the antheridial branches are themselves branched again (fig. D 7) and may occasionally send a branch to another oogonium (fig. E 3). Antheridial tubes often seen.

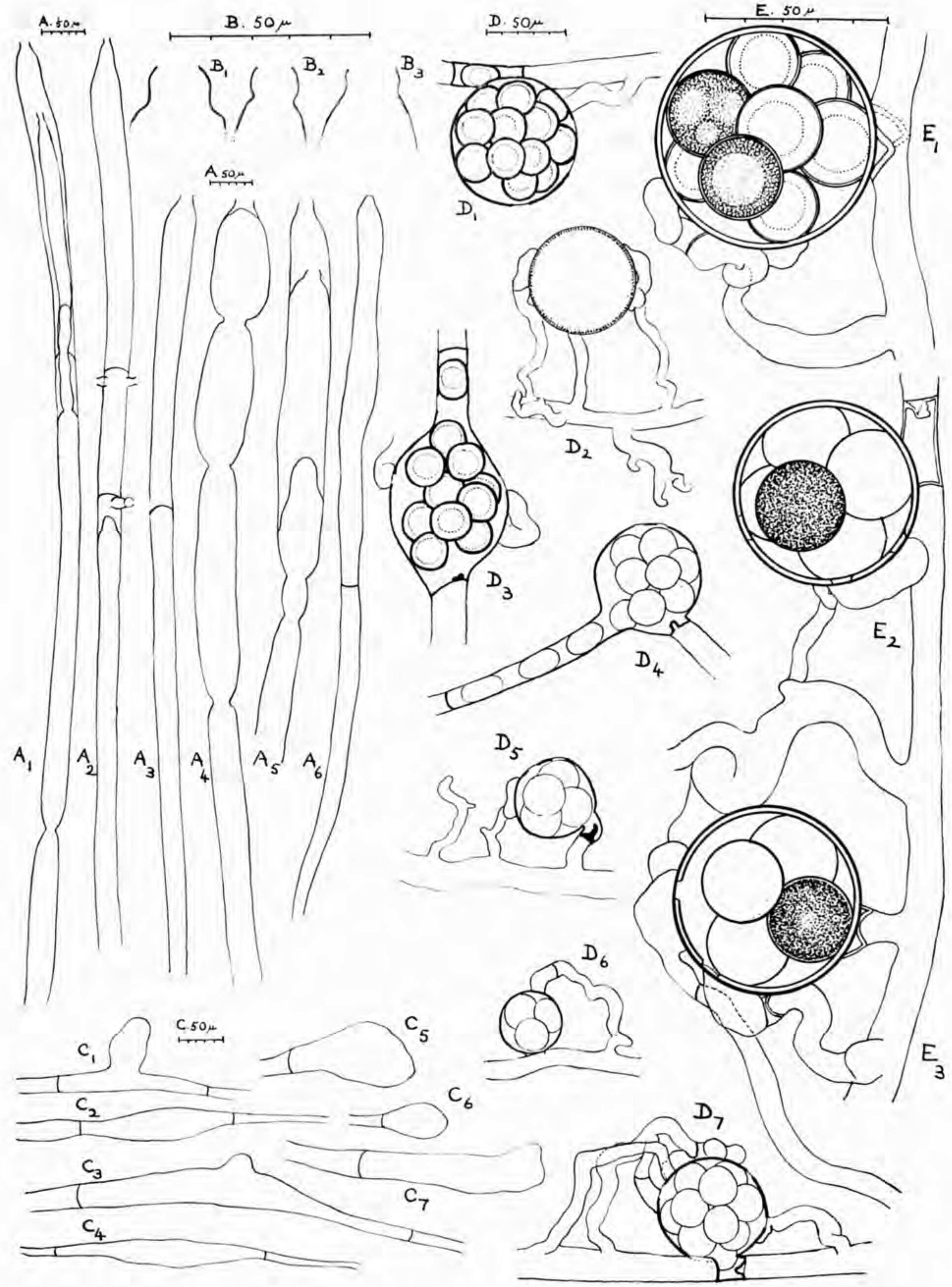

A. 50 μ
B. 50 μ
D. 50 μ
E. 50 μ
B₁
B₂
B₃
D₁
E₁
A 50 μ
D₂
D₃
D₄
E₂
A₁
A₂
A₃
A₄
A₅
A₆
D₅
D₆
C. 50 μ
C₁
C₂
C₃
C₄
C₅
C₆
C₇
D₇
E₃

PLATE 9

Oospores 1 - 30, mostly 6 - 15, not often more than 25; 20 μ - 27 μ, usually 22 μ - 23 μ; generally spherical, but sometimes elliptic through pressure (figs. D 1, 4); dark at first (fig. E 2) developing a conspicuous centrum (fig. E 3), and becoming lighter as the oil drops separate out from the protoplasm, as in S. ferax; mature oospores centric (fig. E 1)

Saprolegnia monoica var. glomerata (continued)

Occurrence in the grounds.

Botany garden lilypond. Like S. ferax, S. monoica var. glomerata appears frequently in collections from the botany garden pond. Though it had been collected twice in November 1946, it was not seen until December 1947 after the autumn at the time when S. ferax was appearing so frequently. It appeared twice in January 1947 (once when ¾ inch of ice had been broken through, to reach the water) and once in Februmry. Between 29th April and 24th June 1947 it was found in almost every collection though often badly parasitised by a species of Olpidiopsis.

Other ponds.

Southwest: May, November (twice) 1947

Engineer's: May, June 1947

Hazel's: May, 1947

Places of origin of isolates.

I Engineer's pond (collected 10th May 1947) single spore culture made in June, 1947.

II Southwest pond (collected 4th November 1947) single-spore culture made in November, 1947.

These two isolates agree closely in all essential features; I, however, appears to have declined in vigour, and cannot be relied upon to produce oogonia e.g. as was shown in March - April 1948, when cultures of I and II had been treated in the same way, II produced numerous oogonia, while I produced none, but formed a more extensive growth with a large number of gemmae; moreover, the oospores of I average slightly smaller than II (22 - 23 μ as against 24 μ). However, during the period of culture, II has itself shown some variation, having an average number of eggs of 6, 8 -10, or 12 - 15, at different times, showing a range in the size of its oospores of 20 - 27 μ. Differences between the two isolates may therefore be regarded as negligible.

Discussion. Coker has described the antheridial branches of his material as "short, typically clustered and contorted, often branched, arising androgynously from the main branches near the oogonia or at times from the oogonial stalks, not rarely reaching also to nearby oogonia on other threads (diclinous), antheridia pear-shaped or tuberous, one or more on every oogonium." This would also serve excellently for the Royal Holloway College isolate. However, the oospores of the Royal Holloway College fungus (usually about 24 μ) are smaller than those of Coker's (usually about 25 - 27 μ) and appear to be more numerous, as Coker reports "one, two, or four, occasionally six or eight, rarely 20 9or more?)". In this latter feature the Royal Holloway College fungus approaches the species S. monoica Pringsheim, although it does not resemble it in other features, and is entirely different from the culture of this species, received from Baarn. It is interesting to note that while Forbes has reported for her isolate of the variety, "oospores 2 - 10 per oogonium, usually 3 - 5, 24 μ - 28 μ diameter, mostly 26 μ - 27 μ", she has also described "forms intermediate in the size of the oogonia and the number and size of the oospores," between the species and variety. (Forbes had collected both.)

S. monoica var. glomerata has previously been recorded in Britain by Ivimey-Cook (1936) in Glamorgan, and by Forbes (1935) at Bristol. It is recorded as S. glomerata (Tiesenhausen) Lund by Ivimey-Cook and Morgan (1934) who had collected it from soil.

Summary. 1. A species of Saprolegnia which has been isolated twice during the present work at Royal Holloway College most nearly resembles S. monoica var. glomerata Tiesenhausen, as described by Coker, though it differs in the average size and number of the oospores.

2. Though some slight variation in the number and size of the oospores has been shown by a single isolate, this fungus has definite and characteristic features which have remained constant during the period of culture of a year, and by which it can readily be distinguished.

3. The fungus is widely distributed in the grounds at Royal Holloway College; it can be collected from the same sources, at the same time of the year, as S. ferax, but appears to be more frequent in the months April to June, while the latter seems to have a maximum frequency in the autumn. (Forbes found a maximum for the species, S. monoica Pringsheim in March).

Fungus 10: identified as "*Saprolegnia paradoxa* Maurizio."
(Plate 10)

Outstanding feature: clustered formation of oogonia on branches arising in a position and manner similar to those of the antheridia; variation in the size of the oospores in a single oogonium.

Sporangia (figs. A 1 - 5; B 1 - 3) relatively slender and delicate; emtpy sporangia usually with a well-marked collar (figs. B 1 - 3).

Oogonia (figs. C 1 - 7; D 1, 2) readily-formed, on lateral branches which may be short (figs. C 1, 2, 4; D 1, 2) or long (figs. C 5 - 7; D 2); very occasionally intercalary (fig. C 3). A lateral branch may divide equally (fig. D 2) or almost equally (fig. C 7) into antheridial and oogonial branches; and secondary branches given off from the oogonial stalk may form antheridia, as one would expect, or oogonia (fig. C 5) or both (figs. C 3, 6); an extreme instance of the latter is shown in fig. D 2.
Spherical, with a short neck; mostly 68 - 86µ, but a proportion of smaller ones, 43 - 63µ, is usually present; pits conspicuous, 5.0 - 8.6µ mostly 7 - 8.6µ; an irregular refractive deposit is sometimes present inside the oogonium around a pit under an antheridium (fig. D 1). An upgrowth from the septum is often present (fig.D 1), unlike *S. monoica*, this is not usually hollow and pointed.

Antheridia on rather thick branches from the oogonial stalk (figs. C 5, 6), the main hypha bearing the oogonial branch (figs. C 1, 2, 4; D 1, 2), or from another hypha (fig. C 1); occasionally arising by the forking of a lateral branch (figs. C 7; D 2) The antheridial branches are themselves sometimes branched (figs. C 1, 2, 4). Extremely delicate branches from the antheridia are often observed; (fig. D 2); these may be related to the frequent blocking of the pit under the antheridium by a refractive deposit. (fig. D 1).

Oospores usually 6 -12; often 15 or more in the larger oogonia, or only 1 - 3 in the smaller ones; extremely variable in size, small ones about 23µ or even 15µ, present among the rest, which are about 30µ. (figs. C 6, 7; D 2) mature oospores centric.

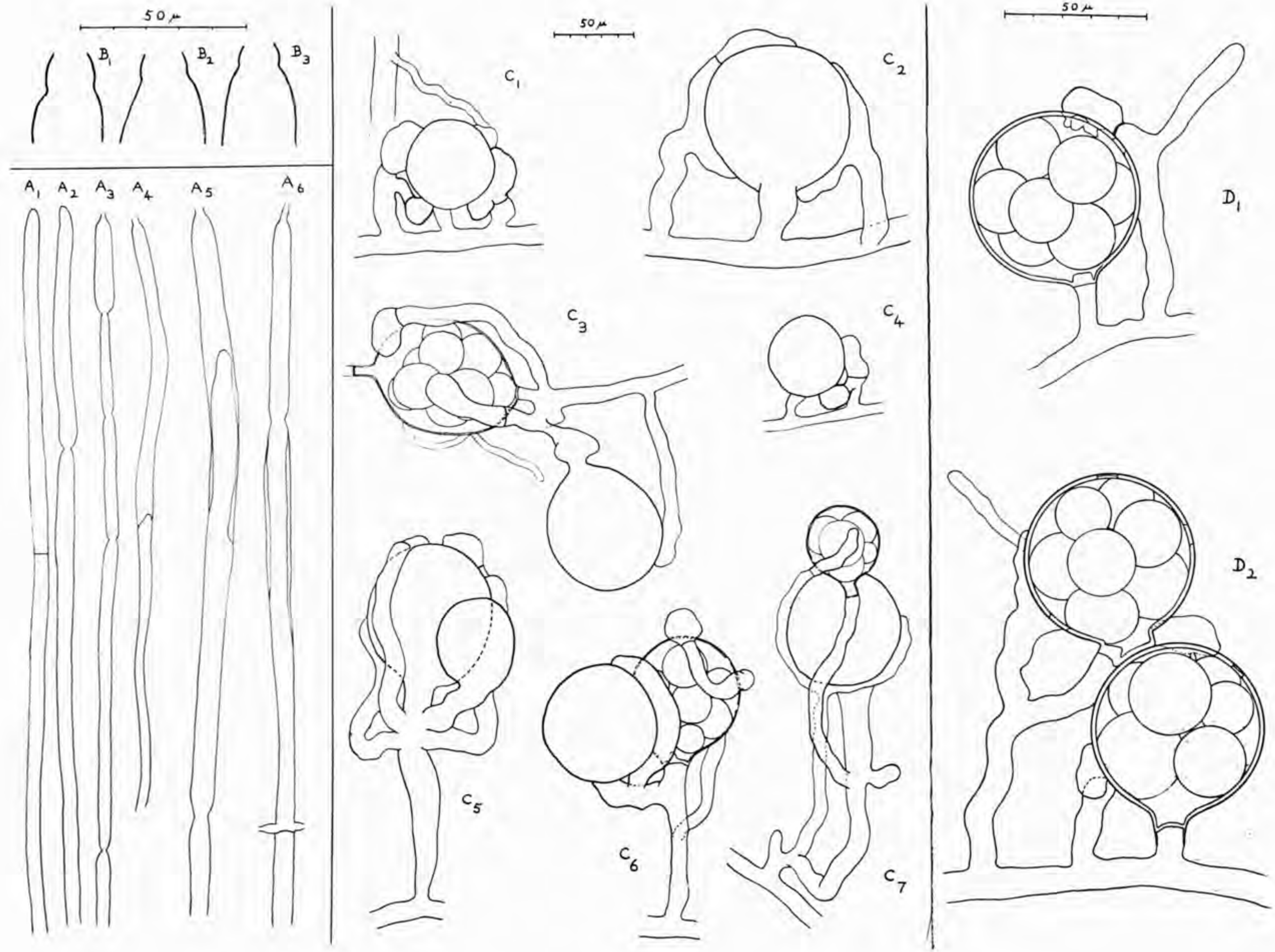


PLATE 10

Saprolegnia paradoxa" (continued)

Occurrence in the grounds.

This species has been observed frequently in collections from the botany garden lilypond, and was collected once from Hazel's pond.

Place of origin of isolate.

Hazel's pond: 1st July 1947. The date on which the single-spore culture was made was not recorded.

Discussion. Particularly in a young culture, this species appears, at first glance, to resemble S. monoica var. glomerata, but it is easily distinguishable from it, later, by the longer oogonial branches, larger oospores, on the average, and thicker antheridial branches, which are much less contorted and branched. These are comparative distinctions. The most outstanding and characteristic features of S. paradoxa are the peculiar relation of the antheridial to the oogonial branches, and the clustered appearance of the oogonia in older cultures.

In her account of the Manchester collection (1935b), Forbes has written: "Sexual organs strongly resembling those described by Maurizio as characteristic of his species, S. paradoxa, have twice been obtained in the present collections, on hyphae which also bear organs characteristic of S. monoica;" The Royal Holloway College isolate agrees well with the description and figures given by Forbes, particularly in two characteristic features on which Forbes has commented:-

"oospores ......, often of very different sizes within the same oogonium."

"The antheridial stalks may themselves bear smaller oogonia." (shown in the fig.1 a.)

Forbes has written also: "The occurrence of these sexual organs, however, on a mycelium which also shows the characters of *S. monoica*, suggests that the so-called "species" *S. paradoxa* is no more than a growth-form of this very variable species. It may be named *S. monoica f. paradoxa.*" However, as the Royal Holloway College isolate has remained constant, and distinct from S. monoica var. glomerata (though showing considerable resemblance to the latter) throughout a period of culture of nine months, it can hardly be regarded as a growth-form of *S. monoica* - particularly as Forbes' reference is presumably to *S. monoica* Pringsheim.

Conclusion. It would seem best to regard the Royal Holloway College isolate as a variety of *S. monoica*, on equal standing with *S. monoica var. glomerata*, while the latter is so named. I would suggest therefore that it be called *Saprolegnia monoica var. paradoxa*.

"*S. paradoxa*" has been found at Manchester by Forbes (1935b) and at Aberystwyth by Brown (1938).

Fungus 11: identified as *Saprolegnia diclina* Humphrey
(Plate 11)

Outstanding features

Gemmae numerous, often in very long chains.

Oogonia extremely variable in size, with thin unpitted walls.

Antheridia numerous, diclinous, antheridial tubes rarely seen.

Oospores numerous.

Hyphae not thick, but quite extensive.

Sporangia (figs. A 1 - 4) wider than the hyphae, broadening towards the tip; relatively short.

Gemmae (figs. B 1 - 8) numerous in most cultures. The first gemmae are formed as terminal swellings, which are usually pyriform (figs. B 4, 5) but sometimes elongated (fig. B 6). Later ones are formed in chains which are often extremely long in old cultures (figs. B 1 - 3, 7). When the culture water is refreshed, they act as sporangia and discharge zoospores through lateral thin-walled exit tubes. (figs. B 7, 8).

Oogonia (figs. C 1 - 10; D) usually terminal on main hyphae, occasionally intercalary (figs. C 1, 8); sometimes two or more in a row (fig. C 8); spherical or pyriform, with a neck (figs. C 1 - 4, 10-, ovid (fig. C. 9) or elongated (fig. C 7) occasionally partly filamentous (fig. C 8); not rarely apiculate (fig. C 6).
Extremely variable in size, 40 - 90 μ in length, possibly longer.

Antheridia numerous, small. The antheridial branches, which are always diclinous (figs. C 1, 5), are delicate and soon disappear. Antheridial tubes are seen occasionally.

Oospores 1 - 20 or more - 23-28 μ, mostly 26 μ, centric.

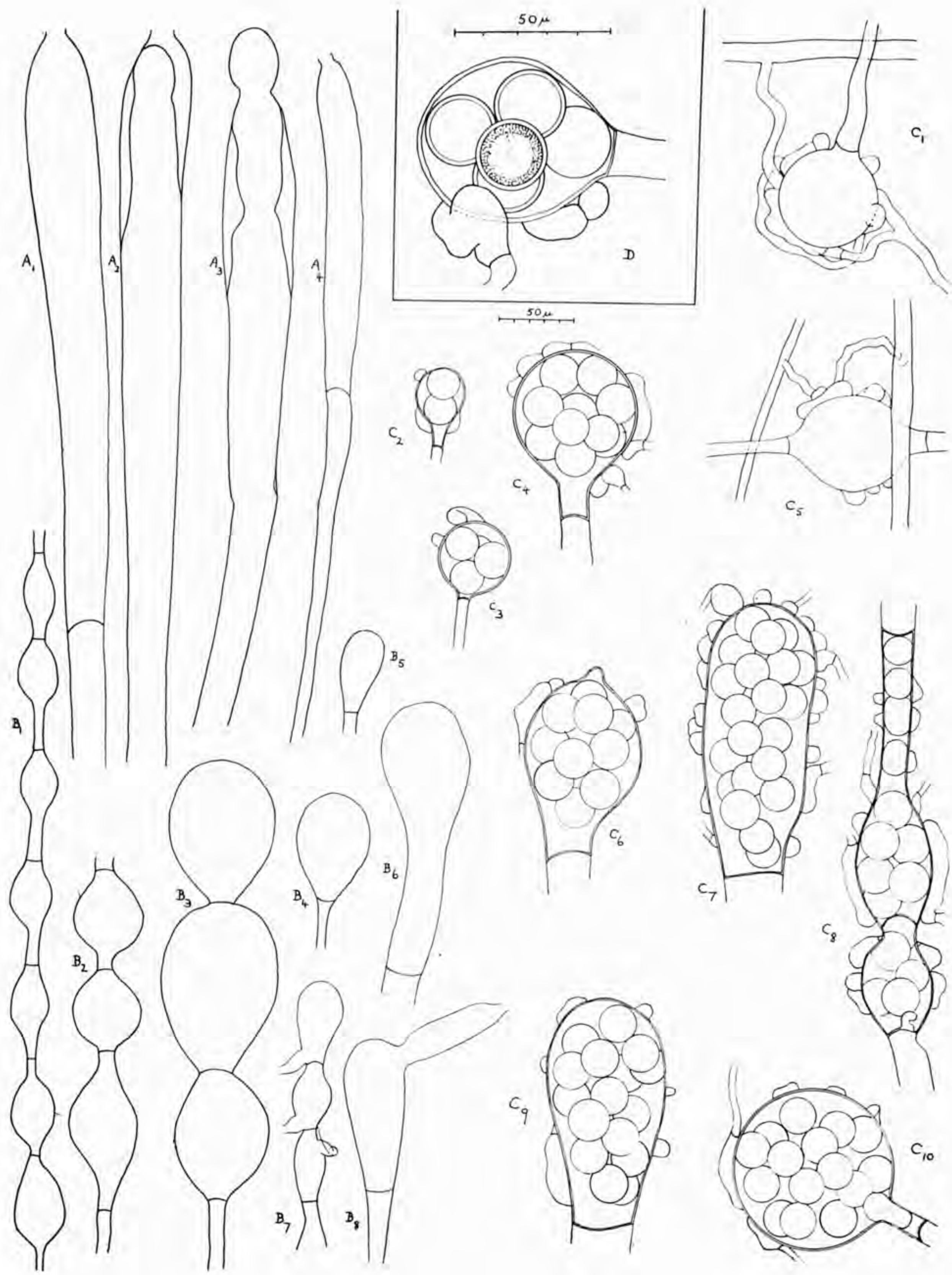

50μ
50μ
A1
A2
A3
A4
B1
B2
B3
B4
B5
B6
B7
B8
C1
C2
C3
C4
C5
C6
C7
C8
C9
C10
D

PLATE 11

Saprolegnia diclina (continued)

Occurrence in the grounds. This fungus was identified in a collection from the botany garden lilypond made on 1st July 1947. The scarcity of oogonia makes it identification difficult, and although fungi producing gemmae with the features of those of S. diclina have been observed in many collections, this species has not again been recorded with certainty.

Place of origin of isolate unrecorded. A single-spore culture was made some time in the autumn of 1947. Cultures have been kept for long periods without the appearance of oogonia. The record is based on two cultures, grown in April and May 1948, in which oogonia were formed abundantly.

Discussion. The oospores of the Royal Holloway College isolate, 23 to 28 μ, mostly 26 μ, are larger on the average than those described by Coker (20 - 26 μ, mostly 23 - 24 μ), and it seems that antheridial tubes may be observed more frequently than in Coker's fungus. Despite these small differences, the Royal Holloway College fungus agrees well with Coker's description. It is clearly Saprolegnia diclina Humphrey.

S. diclina has been found at Manchester by Forbes (1935b) and in Glamorgan by Ivimey-Cook (1936).

Fungus 12: identified as *Saprolegnia anisospora* de Bary
(Plate 12)

Outstanding features

Zoospores of different sizes.

Oogonia sporadic in their appearance in culture.

Antheridia producing antheridial tubes with a characteristic mode of entry into the oogonia

Oospores rarely maturing.

Hyphae delicate.

Sporangia very variable in size, liberating spores of different sizes.

Gemmae numerous in some cultures; spherical or pyriform

Oogonia (figs. 1 - 3) sporadic; usually terminal on slender hypae, sometimes on lateral branches; spherical, with a neck (fig. 1); 40-63 μ, mostly about 45 μ. The wall is unpitted.

Antheridia often numerous (fig. 3); rather small, on slender diclinous branches which soon disappear. Antheridial tubes frequently seen (figs. 1 - 3). A narrow highly-refractive band is usually visible, where the antheridium makes close contact with the oogonium; the antheridial tube usually expands inside the oogonium, to a considerable width (f.g.2)

Oospores 3 - 12, or more; usually 4 - 6. 17.2 - 23.4 μ, mostly 20 - 30 μ; sometimes oospores of very different sizes are present in a single oogonium (fig. 2). The oospores rarely mature, though the proportion which reach maturity is unusually high in some cultures. Mature oospores are centric. Disorganising oospores form numerous oil globules of various sizes, Occasionally a single large globule appears at a certain stage in the process of disorganisation, giving the egg a deceptive appearance of maturity, with an ex-centric structure. In fig. 1, the oogonium contains an oospore which has disorganised and degenerated even before thickening of the wall has occurred.

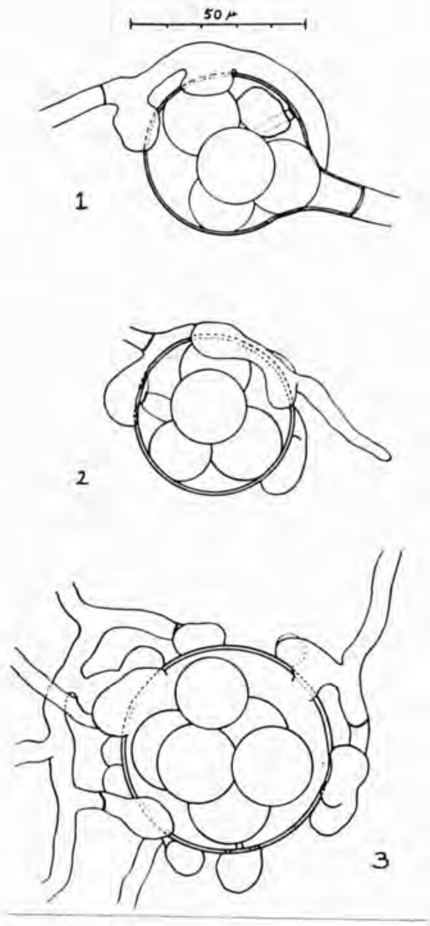


PLATE 12

Saprolegnia anisospora (continued)

Occurrence in Royal Holloway College grounds. This fungus has been collected from south-east and south-west ponds. It seemed to be present in a number of the collections made from these ponds in the autumn, 1947. The dates of its appearances were not recorded, because of some uncertainty as to the identity of the oogonia, which show considerable similarity to those of S. delica.

Places of origin of isolates I. South-east pond (coll. 23rd Sept. 1947). A single-spore culture was made in October, 1947.

II. South-west pond (coll. 2nd October, 1947) A single-spore culture was made in December 1947. The precaution was taken of making a single-hypha culture also, in case growths from single spores should be found to produce zoospores of one size only. From general observations, it seems unlikely that this is so, but the problem has not been investigated in detail.

The production of Oogonia has been sporadic. They were observed only rarely in isolate I during the autumn, 1947, and some cultures remained entirely sterile. Gemmae, however, were produced in large numbers in these cultures. Oogonia were formed abundantly in certain cultures in March, 1948, on which the record is based.

Conclusion. There is no doubt as to the identification of this fungus as Saprolegnia anisospora de Bary. No previous record of the occurrence of this species in Britain has been published.

Fungus 13: identified as *Aphanomyces laevis* de Bary
(Plate 13)

Easily distinguished from Saprolegnia and Achlya included in the present Royal Holloway College list, by its extremely delicate hyphae and sporangia, and small oogonia, and from Aphanomyces stellatus by its smooth-walled oogonia.

Hyphae extremely fine, usually forming a close mat on the substratum.

Sporangia long, delicate; basal septum usually difficult to find, especially as the sporangia often extend right from the substratum; not appreciably wider than the hyphae. Spores formed in a single row, encysting in a loose cluster at the mouth of the sporangium (fig. A)

Gemmae absent.

Oogonia (figs. B 1-5) formed readily in the laboratory and cultures can be kept for months without their appearance, as happened in December 1947 to April 1948; usually formed close to the substratum. 26-30 μ; more or less spherical, though usually distorted by the antheridia; smooth-walled; usually with a long neck (fig. B 2); septum often difficult to see.

Antheridia relatively large; borne by 1, 2, or 3 branches; usually diclinous; repeatedly branched, and wrapping round the oogonium; the delimiting septa sometimes invisible (figs. B 2, 4)

Oospores single; 17 - 25 μ, usually 21 - 22 μ; not nearly filling the oogonium; ex-centric when mature, but it seems that few reach maturity, on hemp seed. In fig. B 1, the oil has begun to separate out from the protoplasm.

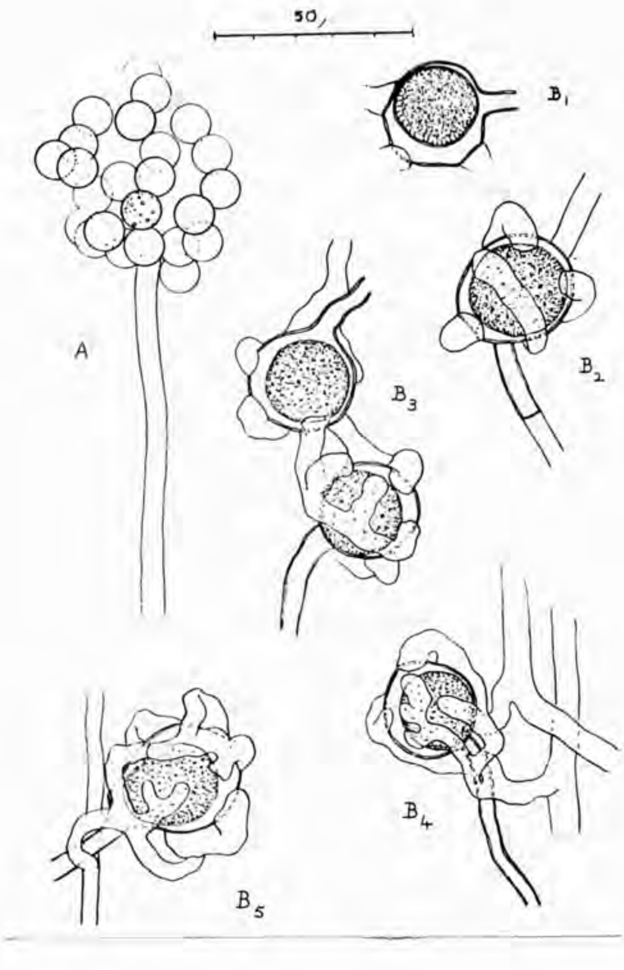


PLATE 13

Aphanomyces laevis (continued)

Occurrence in the grounds. The scarcity of oogonia makes identification difficult. Unidentified species of Aphanomyces collected from south-west pond in 1938 (slide), the botany garden lily-pond in October 1947, and south-east pond in November 1947 (twice) may possibly have been A. laevis.

Other collections are listed as follows:-

Pond	Date of collection	Date when oogonia were observed
South-east	30th Sept. 1947 (with A. stellatus)	19th October 1947
"	21st October 1947	culture(a) 15th November 1947 culture(b) Still no oogonia on 2nd January 1948
"	2nd December 1947	1st January 1948
"	9th December 1947	1st January 1948
South-west	14th October 1947	27th November 1947

(Except between December 17th and January 1st the cultures had been examined at frequent intervals.)

Place of origin of isolate South-east pond (coll. 30th Sept. 1947). This fungus has not been obtained in single-spore culture. The drawings were made in October and November 1947 from a single-hypha culture.

Discussion. The Royal Holloway College isolate is clearly A. laevis de Bary, according to Coker's description; in Coker's monograph the oogonium is figured with a neck (Plate 55, figs. 4, 7) which is especially long in fig. 7; in only one figure (fig. 6) is an antheridial septum shown, despite the fact that in two other figures (figs. 4, 7) the oosphere stage has been reached and antheridial tubes are shown.

A. laevis has been found by Trow (1895), who has described how he obtained it in single-spore culture, and by Ivimey-Cook and Morgan (1934) who have collected it from soil.

Fungus 14; identified as <u>Aphanomyces stellatus</u> de Bary. (plate 14a and b)

Easily distinguished from species of <u>Saprolegnia</u> and <u>Achyla</u> included in the present list, by its extremely delicate hyphae and sporangia, and small oogonia, and from <u>Aphan. laevis</u> by its papillate oogonial walls. Sometimes parasitic in the hyphae of a species of <u>Achlya</u>.

<u>Hyphae</u> extremely fine.

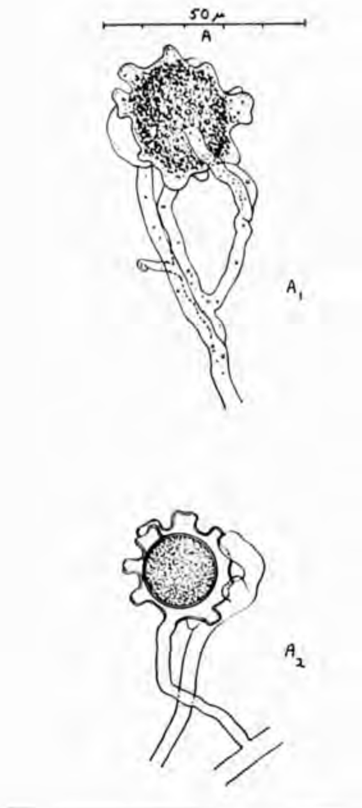
<u>Sporangia</u> apparently much the same as in <u>Aphan. laevis</u>.

<u>Gemmae</u> absent.

<u>Oogonia</u> more or less spherical, with hollow papillate projections; septum usually difficult to see, if present. Intramatrical and extramatrical.

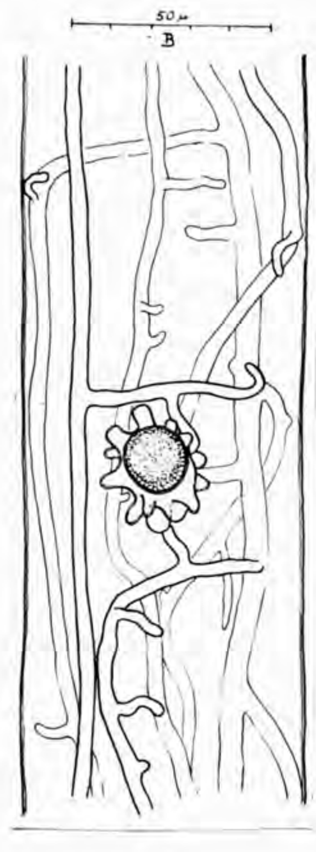
<u>Antheridia</u> relatively large, rather tuberous, on branches from the same hypha as the oogonium (fig. A 1) or from different ones (fig. A 2); branched, but not nearly so numerous as in Aphan. laevis; septa difficult to see, if present. Antheridial tubes not observed.

<u>Oospores</u> 16-21 μ; when mature, ex-centric, according to Coker's definition (1923, p.10), with an inconspicuous lunate series of droplets on one side in optical section (1923, p.164) as in fig. B.



a

Extramatrical oogonia



b

Hyphae and oogonia of Aphan. stellatus inside a hypha of Achlya sp.

PLATE 14

Aphanomyces stellatus (continued)

Occurrence in the grounds. South-east pond, 30th September, 1947, and south-west pond, 14th October 1947. Drawings of oogonia were made in October. Attempts to isolate the fungus were unsuccessful. Continuous culture on hemp seeds was tried, but when the cultures were examined in January 1948, Aphan. stellatus seemed to have been replaced by a species of Achlya, which was unhealthy and deformed, and could not be induced to form oogonia; examination of its hyphae revealed the much narrower ones, and oogonia, of Aphan. stellatus inside them (fig. B). Extramatrical oogonia, also, were found on the mycelium among the bases of the Achlya hyphae. A pure, apparently healthy, culture of the Achlya species was later obtained from this, which formed an extensive, rather coarse mycelium. No oogonia have been observed at any time in this isolate of Achlya.

Discussion. Coker has described this species as "typically saprophytic" but has also commented: "We have found several times in Chapel Hill a form of this species differing from the typical only in being parasitic on species of Achlya. The plentiful oogonia are borne both inside and outside the Achlya threads."

Aphanomyces stellatus de Bary has been recorded in the lists of Massee (1891) and Ramsbottom (1916), and Ivimey-Cook and Morgan (1934) have collected it from soil.

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SECTION II

Pythium undulatum Pet. and its position in the Pythiaceae.

Contents

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SECTION II: Sub-section I

PYTHIUM UNDULATUM Petersen

Contents.

PYTHIUM UNDULATUM PETERSEN

INTRODUCTION

In the autumn of 1946, when collections were being made of pondwater from various sources in the grounds, a fungus with large ellipsoid sporangia borne on strong, more or less undulating sporangiophores, frequently appeared on the bait. The large size of the sporangium and the marked variety of habit immediately drew attention. Thus, while tufts of sporangiophores were sometimes found branched in a cymose manner, the sporangia being renewed by subsporangial branching, as in text-fig.1, more often sporangia were found in which multiple proliferation had occurred, as in text-fig.2. The base of a secondary sporangiophore was usually conspicuously undulated.

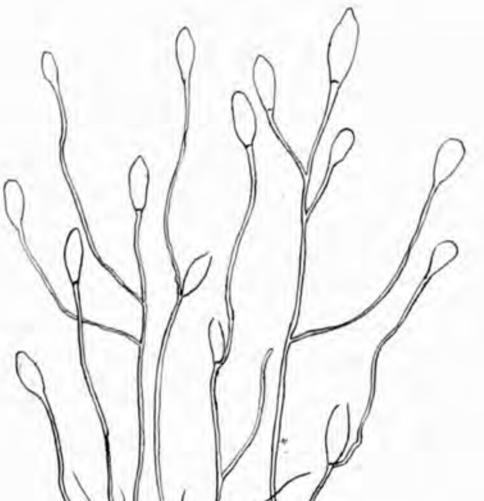

Fig. 1

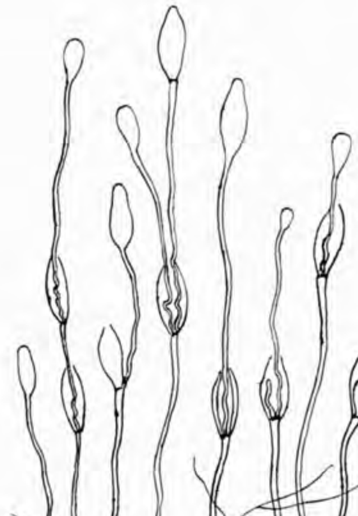

Fig. 2

The size and shape of the sporangia did not encourage the identification of this fungus as a member of the genus _Pythium_. Moreover, the wide hyphae, unbranched for considerable lengths, and filled with coarse, rather dark protoplasm, presented an appearance decidedly suggestive of the Saprolegniaceae. Although many sporangia were watched carefully, sporulation was not observed until the following January, by which time experience had revealed the signs that indicated imminent discharge. The protoplasm was then observed to escape into a vesicle, within which the maturation of the zoospores occurred. Subsequently, the absence of oogonia but abundant formation of thickwalled chlamydospores seemed to confirm the identification of the fungus as _Pythium undulatum_ Petersen.

The apparently considerable degree of differentiation of the protoplasm before its emergence from the sporangium, together with other features, seemed to present _P. undulatum_ as a particularly interesting subject of study, and reference to the literature on it did indeed reveal a number of problems. Consequently, a careful study was carried out on a culture isolated from a single spore.

In March, 1947, a detailed account and discussion of P. undulatum, which had been published by Drechsler in October 1946, was discovered and read. Close agreement was found between the Royal Holloway College isolate and Drechsler's account. However, several points seemed to justify further investigation, and the study was continued.

METHODS OF STUDY

Maintenance of cultures

The single-spore culture, isolated from material collected from the lilypond in the botany garden in September, 1946, has been maintained on a variety of agar slopes, chiefly cornmeal, oatmeal, and malt. Single spore cultures have been made again twice, from subcultures, in January and February, 1947.

Satisfactory growth is obtained on most media. The rate of growth of P. undulatum is remarkably rapid, and cultures fairly free from bacteria have been obtained easily by Raper's method, described in the section on Saprolegniaceae.

Production of Sporangia

Sporangia are not obtained readily, or in any great quantity, when portions of the usual kinds of agar permeated with mycelium, are transferred to sterile distilled water, even when the water is repeatedly refreshed. Abundant production of vigorous sporangia has been induced by the following methods:-

1. Use of hemp seeds of substratum.

Boiled half hemp seeds are placed on an agar culture, usually cornmeal, two or three days after innoculation, and the treated plate is incubated at 24°C for about twenty-four hours; the half seeds are then transferred to sterile distilled water in Petri dishes and left at room temperature for another twenty-four hours or less. On examination, the cut surface of the seed is usually found bristling with erect sporangiophores bearing sporangia which discharge throughout the day. A supply of sporangia for the next day is usually ensured by refreshing the water, but further production cannot be relied upon, the mycelium becoming exhausted.

2. Use of special nutrient agars.

(a) Hemp seed agar can be made from a quantity of boiled hemp seeds removed from the testa and ground to a pulp,

10 gms. dextrose,
15 gms. agar, and
1000 c.c. distilled water.

(b) Waterlily decoction agar has been made by washing well three goodsized healthy waterlily leaves, cutting them into small pieces, and boiling and mashing them. The filtrate from the pulp, and 15 gms. agar, are made up to 1000 c.c. with distilled water.

Portions of a culture on a thinly-poured plate of either of these agars, transferred to sterile distilled water two or three days after innoculation, usually yield numerous active sporangia in one or two days. Good production of sporangia may be encouraged by changing the water once or twice on the first day.

These methods have not been found equally successful at all times of the year, as production of sporangia is depressed by extremes of temperature.

Examination of Zoospore Germination

Zoospores have been watched under different conditions:-

1. In sterile distilled water or dilute sugar solution under a coverslip on a plain or hollowed slide, and in hanging-drop culture.
2. On a film of agar, usually cornmeal, poured thinly over a slide or coverslip, and placed in a damp chamber, or covered with sterile distilled water.

The zoospores have been collected for observation in various ways:-

1. By drawing out water containing large numbers of swimming spores by means of a pipette.
2. By placing coverslips, plain or covered with a thin film of agar, in the bottom of a Petri dish containing active sporangia, and leaving them until a sufficient number of zoospores have settled on them.

3. By nipping off sporangiophores bearing sporangia which have discharged zoospores into a vesicle, and drawing them off by means of a pipette. This method usually permits of quite rigorous washing of the discharged sporangium and vesicle, as the latter is relatively tough; certainty of the origin of all spores from a particular sporangium is thus ensured. If the sporangia are nipped off before they discharge, and transferred to fresh water, they are liable to show abnormal behaviour as a result of the disturbance.

Examination of Chlamydospores

Chlamydospores are formed abundantly after a few days on waterlily decoction agar; then early formation on other agars such as cornmeal has been induced by placing boiled half hemp seeds on the culture.

Attempts to induce the Formation of Oogonia

Cultures have been made on a large variety of media, under different conditions.

Agars.

Cornmeal, oatmeal, malt, potato dextrose, bean, dung, tomato juice, hemp seed and waterlily decoction agars have been used both at full strength and diluted.

Solutions.

Knop's, Petri's, and other salt solutions have been used, usually containing a trace of ferric chloride.

The addition of small amounts of soil, grated carrot, and macerated portions of plants, especially waterlily, has also been tried.

Conditions.

Cultures have been kept at various temperatures - on the laboratory bench, in the incubator at 24°C, and out of doors.

They have also been subjected to changes, by alternate periods at low and high temperatures. For example, during the frosts of the winter of 1946-7, some of the diluted agar cultures buried in saw dust in an outside shed were brought at intervals into the laboratory and kept there for a few days. Cultures kept on the laboratory roof in milder weather were, of course, exposed to considerable diurnal changes in temperature.

Despite the recommendation of a number of these methods for inducing the formation of oogonia, no success has been achieved with P. undulatum. Middleton (1943, p. 9) has found cornmeal agar the most satisfactory medium "for studying sexual reproduction in Pythium," and has suggested the addition of "a small amount of soil," or of "whole or macerated portions of the host plant from which the fungus was obtained," for species which do not readily form oogonia. The use of a grated carrot agar for oogonium-production in *Pythium* had been suggested in 1928 by Johann, and that of humus extract in 1933 by Rands and Dopp. Although the latter workers claimed to disprove the hypothesis that the value of humus extract is in its iron content, traces of iron have been added to the solutions used in the investigation, with this possibility in mind.

Pythium undulatum. Sporangia, recorded directly on sensitive paper. X30.

A STUDY OF PYTHIUM UNDULATUM PETERSEN OCCURRING IN THE GROUNDS OF ROYAL HOLLOWAY COLLEGE

The Sporangium

Form and Development.

In a vigorous culture, the sporangium is ellipsoid, often up to 130 μ in length under favourable conditions, occasionally reaching 170 μ; it is borne on a sporangiophore which is 6 - 8 μ in width for the greater part of its length but may widen to 12 - 15 μ immediately under the sporangium. This widening forms a very distinctive feature (fig.I). The largest sporangia are obtained on natural media such as decaying waterlily leaf, or on boiled rose hips, and a good size is reached on hemp seeds. Large vigorous sporangia are formed when portions of a culture on waterlily decoction agar, or hemp seed agar, are put into distilled water which is repeatedly refreshed. If less favourable media are used, or when the culture is becoming stale and generally declining in vigour, smaller, fatter sporangia are produced, of such dimensions as 30 x 50 μ (fig.II). Under very unfavourable conditions, the sporangia are of various, often irregular, shapes. (fig.III).

Whatever the ultimate shape of the sporangium, it originates as a spherical swelling, terminal on a strong erect hypha, the sporangiophore; it remains spherical until it has reached a considerable size, after which it develops into an elongated shape with a blunt apex. When the swelling has attained its maximum size, it is cut off from the sporangiophore by a septum whose plane is usually flat at first, but becomes arched up into the sporangium in domelike fashion; after its formation, the apex of the sporangium loses its blunt outline and becomes more pointed, and the sporangium assumes its characteristic ellipsoid shape, with its greatest width about one-third of its length behind the apex. The protoplasm of the mature sporangium has a coarse, dark appearance owing to the presence of numerous, relatively large oil drops. (Plate 15 p.123 figs. 1-6 ; text-fig.3)

Fig.3

Fig. I
Normal

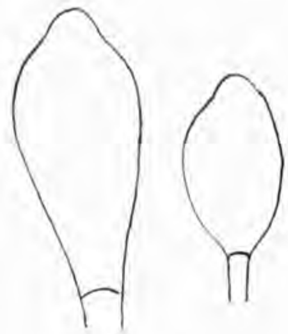

Fig. II
Subnormal

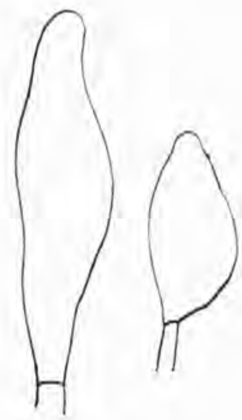

Fig. III
Abnormal

THE SHAPE OF THE SPORANGIUM

Fig. IV
By a sessile papilla

Fig. V
By a short exit tube

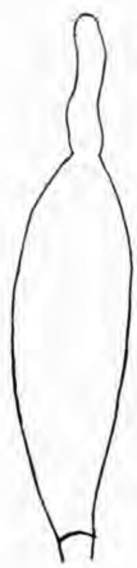

Fig. VI
By a germ hypha

THE GERMINATION OF THE SPORANGIUM

Sporulation.

The first sign that a sporangium is preparing for discharge is in the apex, which gradually becomes pale as the granular contents withdraw from it until there is a narrow completely hyaline zone separating the granular protoplasm from the tip of the sporangium, where the wall is slightly more refractive than that of the rest of the sporangium, (text-fig. 4a). The brightness of this region increases, as the refractive part of the wall thickens and its area extends, forming a gleaming cap over the more or less pointed apex, with a slightly convex thickening directed inwards from the very tip, (text-fig.4b). Viewed in optical section, the zone between this cap and the protoplasm is completely clear, while at high and low levels of focus some granules may be seen, extending right up to the cap; thus it appears that the clear zone is an almost spherical region lying in a cuplike depression in the protoplasm, (text-fig.4c)

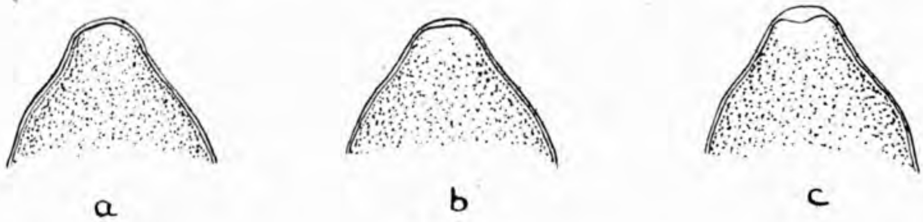

Fig. 4

At this time, numerous small spherical hyaline regions or bodies can be clearly seen scattered throughout the protoplasm of the sporangium; if these are vacuoles, they are not contractile, and are fairly constant in position. A sporangium at this stage presents a remarkable and characteristic spongy appearance which is due to the presence of these hyaline regions and the "lumping" (cf. Butler, 1907) of the protoplasm around them; the limits of any particular mass cannot be exactly distinguished, and the effect is most striking when the sporangium is seen under low rather than high magnification. (Plate 15 p.n3, figs. 7, 8)

Soon, the cap begins to lose its hard glistening appearance and becomes grey and translucent, though still remaining rather refractive; the subapicular region gradually becomes re-occupied by the granular protoplasm, which then extends again up to the hyaline cap, (text-fig. 5a).

Narrow indentations of the protoplasm become visible at the apex and around the periphery of the sporangium, as the separation of the spore masses becomes more distinct, and it is sometimes possible to see definite lines of demarcation. Occasionally, one seems to be able to distinguish a mass which is decidedly delimited from those round about. (Plate 15 p.n3 fig8; text-fig.5b)

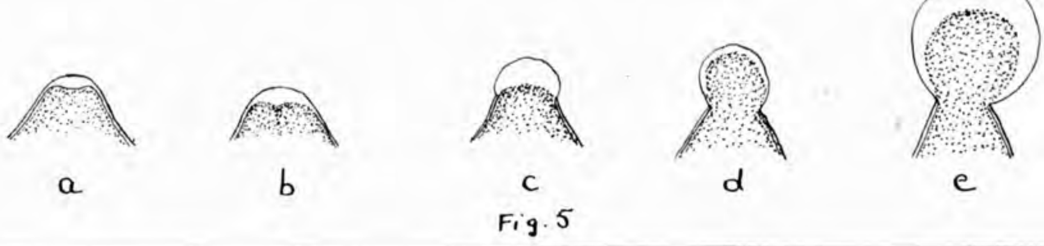

Fig. 5

When the sporangium has reached this stage, immediate discharge is to be expected. The hyaline cap becomes "softer" and greyer, no longer appearing to be separated from the underlying protoplasm by a sharp line. Slowly the cap swells into a thick hyaline drop; momentarily, the swelling is halted, (Plate 15 p.123 fig. 9 ; text-fig.5c) apparently by the thin outer membrane of the wall; this suddenly gives way, releasing the protoplasm, which rushes out pushing in front of it the grey material, which soon disappears, and one can then see a delicate spherical expanding membrane around the rapidly increasing mass of protoplasm which is pouring out of the mouth of the sporangium, (Plate 15 p.123 fig. 10 ; text-figs. 5d, e). This mass appears at first to be almost spherical and homogeneous, until about a third of the sporangial contents have escaped, after which its outline becomes irregular and delimitation of the spore units can be clearly seen. The second half, at least, of the outflowing protoplasm is always divided up into distinct units, which are, however, very "soft," and become elongated as they emerge closely pressed together in a mass in the middle of the sporangium, and somewhat constricted as they pass through the opening, (Plate 15 p.123 fig. 11).

Short flagella are often visible on the distal periphery of the mass in the vesicle before discharge is complete; these soon elongate into long lashes which can be seen undulating in the clear region between the protoplasm and the membrane of the vesicle. Each spore unit is early seen to possess a single vacuole of a size comparable with that of the hyaline regions visible in the protoplasm before its discharge, (Plate 15 p.133 figs. 11,12).

The irregular mass in the centre of the vesicle exhibits at first a slight oscillatory or rocking motion probably due to the action of the flagella. Soon the separating masses of protoplasm begin to show individual writhing movements, and to assume a characteristic shape, with their somewhat flattened side, against which the vacuole lies, and from which the flagella arise, directed to the outside of the mass. As the spores become separate and move apart, their movements become more and more pronounced, until, at about fifteen minutes or less after the beginning of the outflow of the protoplasm, they are milling round in a frenzied manner, battering against the membrane of the vesicle. Sometimes, with the first rupture, this membrane appears to disintegrate, allowing the spores to escape in all directions; but often, when it is fairly tough, most of the spores follow the first in the path of its escape, and some may remain swimming within the vesicle for a number of seconds, apparently seeking a means of escape. The torn remains of the vesicle are sometimes visible for a short time after the escape of the zoospores, but eventually disappear, though a minute fragment may remain visible for longer at the mouth of the sporangium, which is sometimes slightly lipped, (Plate 15 p.133 figs. 13,14)

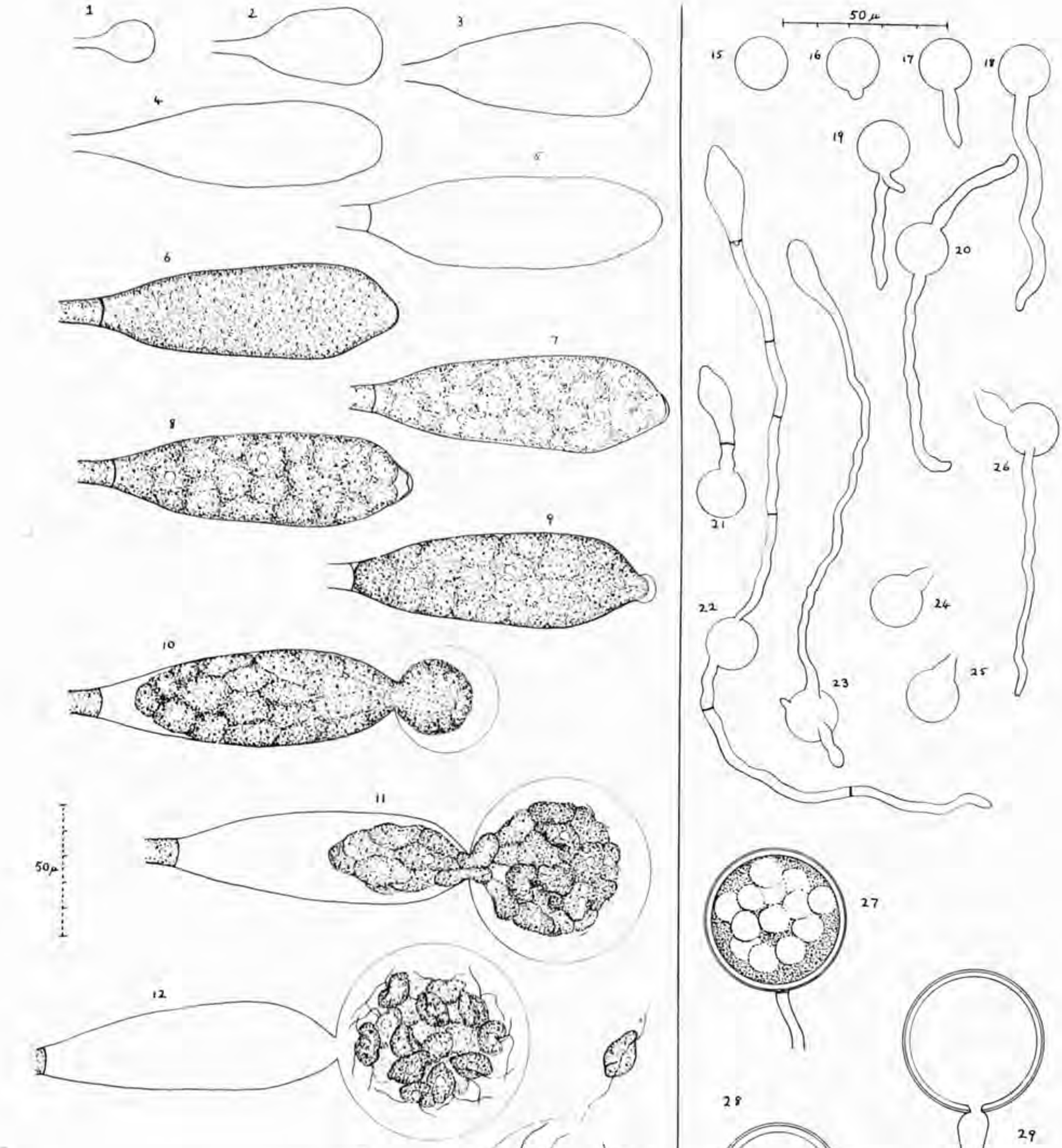
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PLATE 15

The production of secondary sporangia.

In a vigorous culture, proliferation through the empty sporangium soon occurs, the new sporangium being produced within, or more usually beyond the previous one. The proliferating hypha may branch soon after its emergence through the septum, or several separate ones may develop; as many as six sporangiophores have been seen emerging from the mouth of a single empty sporangium. (Text-fig. 2 p. 108)

In less vigorous cultures, particularly when discharge of the first-formed sporangia is delayed or prevented, renewal takes place by subsporangial branching, when one or more secondary sporangiophores of variable length arise immediately below the septum of the primary one. (Text-fig. 1 p. 108)

In either event, the base of the secondary sporangiophore is usually narrow and conspicuously undulated; the undulations may continue, to a lesser degree, throughout most of the length of the sporangiophore, but usually die out towards its upper part where it begins to show the characteristic widening under the new sporangium.

Although the sporangiophore is usually unbranched, it may occasionally branch in a manner not apparently related to any order in the formation of the sporangia. (Text-fig. 1 p. 108)

Less characteristic modes of behaviour of the sporangium.

Under unfavourable conditions, when immediate discharge is prevented, the sporangium often rests for some time with a hemispherical region at the apex entirely occupied by a clear translucent substance apparently in a static condition; the spherical hyaline regions scattered through the protoplasm remain, with a hard, more refringent appearance than at first; and eventually a single large central "vacuole of senescence" (cf. Drechsler, 1941) may develop.

If the water of a culture containing such a sporangium is refreshed, discharge may occur in the characteristic manner; the wall of the region previously containing the hyaline substance usually remains afterwards as a short exit tube. The presence of such a tube on an empty sporangium, therefore, usually indicates that the sporangium has not matured under conditions that favour prompt discharge.

More often, however, especially if the sporangium has been allowed to remain dormant for a considerable time, one or more hyphae grow out. The extending hypha carries with it at its tip the refractive material of the papilla, unless hardening of the latter has set in, when, it seems, a path is dissolved through it by the hypha.

Under more abnormal conditions, certain less frequent irregularities in sporangial behaviour occur. Sometimes, for example, the sporangiophore swells terminally, and then continues its growth as a hypha, without cutting off a sporangium. It is to be expected that protoplasm undergoing the differentiation prior to its discharge, should be particularly susceptible to changes in the environment; thus, a variety of irregularities in behaviour can be induced merely by adding fresh cool water to a slide culture bearing sporangia at different stages. In this way, premature discharge can be brought about, when the protoplasm emerges into the vesicle in an entirely homogeneous mass; or the protoplasm may pour out without the formation of a vesicle, and segment into incompletely-separated, irregularly-shaped spores. Sometimes a type of subnormal behaviour occurs, in which the spore-units emerge sluggishly, becoming unusually elongated during their passage through the mouth of the sporangium; a discharged spore unit is then often seen to be drawn out behind into a fine thread by which it is attached to the spore-unit following.

These abnormalities in behaviour are significant only in so far as they indicate the importance of observing the sporangia under optimum conditions.

The Zoospore

Form.

The zoospore is a somewhat dorsi-ventrally compressed body, slightly pointed at both ends, but especially at the anterior end. There is a ventral groove, which runs antero-posteriorly, twisting from the left to the right. It is this groove which causes the pointed appearance, as it is especially pronounced at one side of the anterior end and flattens out somewhat on the opposite side at the posterior end.

An "end-on" view shows the spore to be convex dorsally and concave with a definite indentation on the ventral side. This indentation results from a view of the longitudinal groove in profile; owing to the twist of the groove, its position varies according to the depth of focus. It is presumably on this view that the generally-accepted but decidedly-limited description of the zoospore of members of the Pythiaceae as reniform or kidney-shaped, or bean-shaped, has been based. This is not the view usually obtained of a swimming spore, but it is frequently seen in the spores on the periphery of the mass maturing in the vesicle, when the groove is always directed towards the outside of the mass, and the two flagella arising out of it, in a median position, can be seen undulating in the region between the protoplasm and the membrane of the vesicle. At this time, the spores may appear to have a less definite shape than they do actually possess, owing to their twisting and writhing motion, and to their irregularity of outline due to the presence of the groove. Although they are somewhat "soft" and compressible, a fact which may be readily noted as they squeeze through the narrow opening of a tough vesicle, the shape of a freeswimming zoospore is a definite and characteristic one.

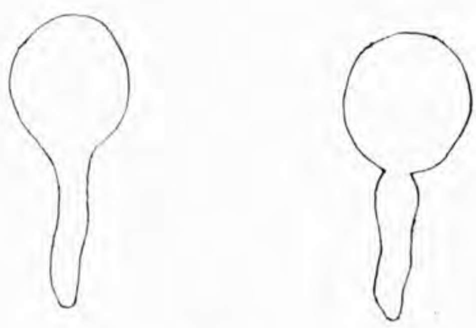
Movement.

The zoospore swims in a clockwise spiral path, probably due to the twisted groove, rotating on its long axis at the same time. The longer flagellum is directed posteriorly, the shorter one anteriorly.

Zoospores swim for a variable time; in a drop of distilled water on a slide, they may settle down almost at once, or they may swim for half an hour or more. Just before settling, a spore sometimes makes faster, jerky movements along short paths in various directions, and then suddenly stops; it settles on its pointed anterior end, presenting an "end-on" view, and vibrating slightly as it becomes spherical, until finally it is quite motionless within a delicate wall. One or two slightly thickened twisted threads may be visible at one side for a short time afterwards; these may represent the remains of the flagella. The newly encysted zoospore is 13 - 15 μ in diameter.

Behaviour of the Zoospore after settling down.

Germination. In a nutritious medium, the encysted zoospore produces a germ hypha, which arises as a blunt, rounded, hyaline swelling; soon a few granules and droplets appear in the extending hypha, and when it has reached some length the protoplasm has the usual coarse granular appearance, except at the tip, which is somewhat finely granular or hyaline. When the cyst wall is very thin, it appears to extend with the growing hypha ~~(fig.)~~, but when some thickening has occurred, the germ hypha is constricted at the base, where it passes through the thickening (text-fig. 6a, b).



a b

Fig. 6

While the hypha is extending, a large central vacuole develops (different from the contractile vacuole of the swimming spore), but it seems that when the sporeling is well established, as for example in an agar film slide culture, protoplasm may pass back into the cyst, which loses the vacuole and may possibly serve as a reservoir for the protoplasm. From the germ hypha the extensive branched mycelium is usually developed.

The sensitivity of germ hyphae to aeration and food concentration gradients has been observed; spores encysted under a coverslip, near the edge, have often been found with their germ hyphae lying more or less parallel, in a row, directed towards the edge of the coverslip; and scattered oil drops liberated from the hemp seed substratum are sometimes reached by a number of germ hyphae from all directions, from encysted spores round about.

Under less favourable conditions, a miniature sporangium is produced at the tip of a short or long hypha. This behaviour has been observed once, in material received from Miss van Beverwijk of Baarn, when sterile distilled water had been added to a slide culture of sporelings on a cornmeal agar film. The hypha and terminal sporangium are developed at the expense of the contents of the spore, which becomes vacuolated, and eventually empty, and as the protoplasm draws out of the basal part of the hypha, behind the growing apex, the empty regions are cut off successively by septa. The sporangium developed in this way possesses an apical papilla like that of a sporangium formed on the ordinary mycelium, and probably discharges in a similar way. The discharge has not been observed, but several empty sporangia have been seen. (Plate 15 p.123 figs. 21-23).

Repeated emergence. Spores in sterile distilled water on a slide have been observed to liberate zoospores like themselves, through a short exit tube. The empty cysts with their exit tubes are frequently seen. (Plate 15 p.123, figs. 24,25)

The addition of fresh water to a slide bearing zoospores which have begun to germinate by means of a hypha will sometimes cause them to retract the protoplasm from the hypha and discharge a zoospore through an exit tube. (Plate 15 p.123 fig. 26)

The Mycelium

Habit.

The hyphae are of variable thickness. On a solid medium such as cornmeal agar or waterlily leaf decoction agar strong main hyphae 7-8 μ wide are formed, which extend to the edge of the plate, giving off much narrower laterals which may be repeatedly branched and are sometimes very irregular. In a recently-inoculated culture, there is a tendency for the main hyphae to be found on the surface of the medium while the laterals penetrate into it.

The degree of undulation is very variable; while it may not be obvious in the vegetative mycelium it is usually noticeable in the sporangiophore, especially at the base of a secondary sporangiophore, which is frequently convoluted in a series of narrow twists; the rest of the sporangiophore may be smooth and sinuous, or the undulations may be of such small amplitude as to be distinguishable only under magnification with the high power objective. In June, 1947, several cultures were observed in which the sporangiophores were not merely undulating, but made a series of wide turns in the manner of a corkscrew; in addition, some spores collected at the time, and germinated on agar films on slides, produced germ hyphae which were remarkably undulated immediately on emergence, though they soon straightened out. This extraodinary behaviour may have been induced by the warm temperature and poor aeration, as the cultures were grown in rather small vessels; it has not been observed since.

Structure.

Vigorous hyphae are full of granular protoplasm with a decidedly coarse appearance due to the presence of numerous oil droplets; this coarseness, in the relatively straight, unbranched sporangiophores, gives them an appearance which has a striking resemblance to that of members of the Saprolegniaceae. In the older, less vigorous parts of the mycelium, the droplets tend to run together, so that considerable lengths of hypha may be filled with oil. The empty portions of the hyphae are cut off by septa, as the protoplasm withdraws and moves on behind the active growing tip.

The hyphae often have a distinctly brownish colour; when chlor-zinc iodide is added, the walls go a characteristic reddish-pink colour.

Intrahyphal hyphae.

On some media, such as cornmeal agar, there is a frequent occurrence of a curious phenomenon. A main hypha apparently bursts at the apex, allowing a small quantity of protoplasm to flow out; the evacuated region may or may not be cut off by a septum; in either case, the living protoplasm behind forms a new narrower hypha which undulates and may branch within the walls of the old one. Such intrahyphal hyphae may be seen passing through considerable lengths of empty hypha. It seems as though the escaped and degenerated protoplasm stimulates the production of innumerable minute branches around it, which coil and twist over it so as to form a "knot" of some size at the broken end of the hypha. Sometimes numerous hyphae break out through the walls of the old hypha for some distance behind the tip, and twist themselves around it. The possibility had been considered, for some weeks, that this behaviour showed the presence in the hyphae, of a phycomycetous parasite which might be the cause of the undulation. After attempts to isolate the parasite had been found completely unsuccessful, reference to Butler's "Researches on Fungi," (vol.V, p.134) showed the formation of intrahyphal hyphae to be of fairly frequent occurrence among Ascomycetes. They are defined by Butler as "younger more slender hyphae running through the cavities of older thicker dead parts of hyphae." It is interesting to note that Dodge, in 1912, recorded his belief that the intrahyphal hyphae of Ascobolus magnificus were those of a parasite, and did not correct his misconception until 1920.

The Chlamydospore

Formation and structure.

The chlamydospores are mostly spherical or sub-spherical bodies, but they are sometimes rather elongated, or pyriform. They usually arise terminally on short lateral branches, but frequently become lateral by the continuance of the growth of the branch, (Plate 15 p.123, figs. 27,28), and are quite often intercalary.

Chlamydospores are formed on various media, though they have not been found on bean agar, and occur rarely in potato citric agar. In solid media, they are formed at all depths as well as on the surface; they are not often found in liquid media, though they do sometimes occur on the mycelium immediately surrounding hemp seeds in water.

In hemp seed agar and waterlily decoction agar, they are formed readily in four days or less, and are 30-45 μ in diameter, mostly about 40 μ in the latter medium. On cornmeal agar they may take three weeks or more to develop, but appear after a few days in large numbers around halved hemp seeds placed on the agar; the addition of drops of olive oil also encourages their formation, but to a much less degree.

As the chlamydospore matures, the wall thickens, and after a week or two, a thin hyaline outer wall (that of the parent hypha) can be distinguished from a thick dark inner one. The spore itself is extremely dense and dark, and contains a number of rather large, spherical, oily bodies, (Plate 15 p.123, fig. 29)

Germination.

Results which were at first puzzling have been obtained when portions of agar plate cultures containing chlamydospores have been transferred to distilled water. Sometimes the chlamydospores germinated by means of a vegetative hypha, which was usually constricted at its base (Plate 15, fig. 29). More often a sporangium was produced terminally on a hypha of variable length; such a sporangium did not apparently differ from those formed on sporangiophores from the mycelium except, perhaps, in a tendency to a small size, and discharged in the characteristic manner. Sometimes, however, the chlamydospores could not be induced to germinate at all, though they seemed to be perfectly healthy. These results are explained by the observation of Drechsler (1946) that chlamydospores which have been formed for some time require a resting period before germination (see p. 150).

Absence of Oogonia.

The fungus has been grown on a variety of media, under various conditions of temperature; some casual examinations of waterlily leaves and petioles have been made, for possible intramatrical oogonia; at no time, however, has there been any sign of the formation of oogonia by the Royal Holloway College isolate of P. undulatum.

Notes on the Occurrence of P. undulatum in Royal Holloway College Grounds.

General distribution.

Pythium undulatum is easily recognised in a mixed culture, by its large ellipsoid sporangia and other distinctive features, so that confidence has been felt in its identification without isolation.

It was abundant in collections from the botany garden lilypond throughout the autumn of 1946, and the present study has been based on a culture isolated in September of that year. It also appeared frequently in collections from the southwest pond. At this time, a varied assortment of bait was in use, and P. undulatum was found at various times on grapes, haws, grape pips, orange pips, and the peel of a pear, but most frequently on hips and their stalks; subsequently, the bait being limited, as a rule, to hemp seeds, the records of P. undulatum are intermittent, as it very soon becomes outgrown by members of the Saprolegniaceae in mixed cultures on hemp seeds, although in pure culture it shows satisfactory growth on this substratum.

It has been collected from the lilypond again, in 1947, once in January, once in March, and twice in December; on December 2nd, two inches of ice were broken through in order to reach the pondwater, and on December 9th, the water was greatly discoloured as a result of the effect of the frost on the last remains of the waterlily leaves. In these collections, saprolegniaceous growth was evidently suppressed, though hyphae of a Saprolegnia badly parasitised by Olpidiopsis were present in the December 9th cultures.

P. undulatum has also been collected twice more from the southwest pond, in July 1947; a few chlamydospores were found on the mycelium in the water around the grape which had been used as bait.

It was collected from the Engineer's pond at the end of May, 1947, and from Hazel's pond in January, 1948. Also in January, 1948, it appeared in a collection from the soft damp soil beside the tiny stream running into the Engineer's pond.

The relation of the R.H.C. isolate to waterlily.

Petersen, Dissmann, and Drechsler have all collected P. undulatum from various species of waterlily, and Drechsler has definitely related the premature discolouring of the leaves to attack by this fungus.

There is a good growth of Nymphaea tuberosa var. rosea in the pond in the botany garden. In April and early May, the leaves are crisp and pinkish as they grow up towards the surface of the water and unroll, later becoming a deep green as they lie flat on the surface of the water. Early in June, however, they begin to turn yellow in patches, become waterlogged, and eventually become black and soft.

These discoloured leaves are usually rather slimy. When they have been washed, and portions are placed on nutrient agar, mycelium of a number of pythiaceous species almost always grows out; if portions of this agar, permeated with mycelium, or pieces of the discoloured leaves, are placed in distilled water, a mixture of vegetative mycelia and sporangia appears. Large spherical, proliferating sporangia of a species of Pythium on strong, branched sporangiophores, are usually among the first to be formed, and are decidedly predominant; this species has been isolated, and is referred to as "IXA." The more slender hyphae of another *Pythium* species showing abundant production of filamentous sporangia are also of frequent occurrence. *P. undulatum* is sometimes found, especially in the region of the leaf where discolouration has proceeded further. Among the others that appear is a species of *Pythium* with rather small spherical spoangia, and less extensive growth than "IXA," and a *Phytophthora* with proliferating sporangia. In the mixed fungus "flora" of this waterlily, therefore, *P. undulatum* is not predominant; its presence, recognised by means of the sporangia can hardly have been missed, since sporangia are readily formed by the mycelium in waterlily leaves or waterlily decoction agar, the medium most frequently used for these experiments.

Repeated attempts at reproducing in the laboratory the discolouration of the leaves, by inoculating them with a pure culture of *P. undulatum*, have so far met with no success, though a blackening of the leaf around an innoculum of "IXA" occurs in twenty-four hours or less.

These observations, and the general occurrence of *P. undulatum* in a number of ponds where there are no water-lilies, together with its ready saprophytic growth on a variety of substrata, suggest that it is at any rate not a vigorous parasite of the waterlily in these grounds.

AN ACCOUNT OF THE LITERATURE ON PYTHIUM UNDULATUM PETERSEN AND "PYTHIOMORPHA UNDULATA" (PET.) APINIS.

Pythium undulatum was described as a new species by Petersen in 1909, who defined it as follows:- "Mycelium intramatricale difficile distingendum. Mycelium extramatricale e hyphis non ramosis, longis, plus minus indulatis, diam.c.3-6μ, constat. Zoosporangia terminalia, ellipsoidea, interdum parva papilla apicali instructa, diam. c.130 x 50μ. Zoosporae duobus ciliis lateralibus munitae diam. long. 15 - 20μ, in vesica ut in Pythio, exeunt. Hyphae in et per zoosporangia evacuata penetrant et zoosporangia nova formant. Color membranae mycelii et zoosporangiorum leviter fuscus. Organa sexualia non observata. Frequens in laminis et petiolis (rarius gemmis) Nymphaeae albae et Nuphar lutei."

In 1927, Dissmann gave a detailed account of the morphology and physiology of *P. undulatum*, and described the formation by it of chlamydospores.

In 1929, Apinis described a fungus which he believed to be the same as Petersen's, transferring it, without stating his reason, to the genus "*Pythiomorpha*." He gave the following description of "*Pythiomorpha undulata* (Petersen) n. comb.":- "Hyphen 3-8 μ dick, ohne Einschnürungen, meist schwach verzweigt, in vom Sporangium entfernteren Teilen mehr oder weniger unduliert. Membran der Hyphen zeimlich dick, hyalin, zuweilen schwach gelblich, oder schwach bräunlich gefärbt, mit Chlorzinkjod sich nicht bläuend. Gemmen fehlen. Sporangien länglich oval, 20-52 x 50-167 μ gross. Oft junge Sporangien in die alten ein- oder durchwachsend so dass die durchwachsenen Sporangien zu 2 - 3 in einer Reihe angeordnet sind Sporen 10 - 18 μ gross, mit zwei seitlichen Cilien. Nach dem verlassen des Sporangiums incistieren sich die Sporen, häuten darauf und bilden nachdem dem Keimschlauch. Unter Umständen incistieren sie sich im Sporangium und keimen daselbst. Organe der geschlechtilichen Fotpflanzung nicht mit Sicherheit beobachtet."

While Apinis believed Pythium undulatum to be synonymous with "Pythiomorpha undulata," in subsequent records the same specific epithet has been applied to two genera. Thus, Pythium undulmatum has been recorded by de Wildeman (1931), Matthews (1931), Sparrow (1932), Lund (1934), and Drechsler (1941, 1946); "Pythiomorpha undulata" has been recorded by Cejp (1932), and Lund (1934). Lund has thus claimed to have observed both.

The chief points of interest in these records are given below:-

Investigator	Type of Account	Size of Sporangium	Vesicle	Comments
		(a) Pythium undulatum		
1909 Petersen Denmark	not detailed	130 x 50 μ	present	vesicle is only mentioned not described, but discharge is said to be "as in Pythium."
1927 Dissmann Bohemia	very detailed	40 - 140 μ	present	variability of sporangial size reported. chlamydospores found, 6 - 92 μ. often 30μ, 59μ, or 69μ.
1931 de Wildeman France	brief	not stated	not mentioned	not isolated. a doubtful record.
1931 Matthews U.S.A.	not detailed	22-55 x 11-18 μ	figured	presence of vesicle may be assumed. chlamydospores found, 14-24 μ.
1932 Sparrow	critical, but not detailed	40-45 x 12-15 μ	present	variability of sporangial size reported. chlamydospores found, 10-50 μ.
1934 Lund Denmark	a record only	-	present	only one sporangium observed; not stated to differ from "Pythiomorpha undulata," except in the vesicle.
1941 and 1946 Drechsler U.S.A.	very detailed	30-90 x 20-40 μ	present	stated to be similar to Dissmann's fungus. chlamydospores found, 15-75 μ.

Investigator	Type of Account	Size of Sporangium	Vesicle	Comments
(b) "Pythiomorpha undulata"				
1929 Apinis Latvia	not detailed	50-167 x 20-52 μ	not mentioned	believed to be the same fungus as P.undulatum Petersen. no reason given for the transfer.
1933 Cejp Bohemia	brief	up to 100-150 μ long	absent	reference is made to it only, in describing the characteristics of the genus "Pythiomorpha."
1934 Lund Denmark	not detailed	45-117 x 35-43 μ, often 70 x 40 μ.	absent	believed to be distinct from P. undulatum Petersen.

General Discussion of the Problems arising out of the Records.

There is apparent disagreement among the various records of the size of the sporangium of P. undulatum. However, Dissmann has shown by experiments on a pure culture, that the sporangia are remarkably variable in size; Sparrow, also, has commented on their variability. This feature, therefore, cannot be used as a criterion in identification, although the ability to attain a large size under certain conditions may be regarded as a characteristic of the sporangia of P. undulatum.

The regular formation of a vesicle has been described as characteristic in the material observed by Petersen, Dissmann, Sparrow, and Drechsler, and is implied in Matthews' record in which a sporangium is figured with the zoospores in a vesicle at its mouth; Lund's record of P. undulatum is based on a single sporangium, of which he has written:- "I myself have once seen a sporangium at the mouth of which the zoospores were lying in a vesicle, this fungus was probably P. undulatum. But most of the sporangia observed were of the Pythiomorpha type." Lund therefore believed in the occurrence of Pythium undulatum and "Pythiomorpha undulata" as two distinct fungi. On the other hand, Sparrow has considered "Pythiomorpha undulata" to be synonymous with Pythium undulatum, and has expressed the opinion that "there seems little justification for changing the original binomial."

It has been shown by Blackwell, Waterhouse and Thompson that "Pythiomorpha" is invalid as a genus, and that species which had been classed under that name may be identified as aquatic species of Phytophthora or Pythium. There is a possibility, therefore, that an aquatic Phytophthora closely resembling Pythium undulatum, (except in the maturation of its zoospores within the sporangium) has been described under the name "Pythiomorpha undulata;" this possibility has been recognised by Drechsler. The sole evidence in its support is given by the good accounts of Apinis and Lund, and the more meagre one of Cejp.

Drechsler has made the statement:- "If ellipsoidal sporangia approximately of the extraodinary dimensions given in the original diagnosis of P. undulatum were to be found usual and characteristic for some one particular species - preferably for a species in which undifferentiated protoplasmic contents are delivered into a vesicle and then are fashioned into motile zoospores - no serious misgivings could be entertained with respect to the correct application of the binomial." Accordingly, Drechsler has shown how closely Dissmann's record approximates to that of Petersen, and has described his own isolate under the heading "Pythium undulatum Petersen sensu Dissmann." The following account of Pythium undulatum is based chiefly on Petersen's diagnosis and the detailed description of Dissmann and Drechsler. The salient points of the two latter important papers are listed later. (p. 153)

An Account of the Features of Pythium undulatum as described in the Literature.

(A summary is given at the beginning of each section; the details are in single spacing.)

The Sporangium: (a) Form and Conditions of Formation

The sporangium is usually described as ellipsoid, (e.g. Petersen, Drechsler) or "narrowly ovoid" (Sparrow), and may reach a length of 140 µ. Dissmann has shown experimentally that the size of the sporangia varies greatly according to the medium, and Drechsler has considered the sporangia of his material to be closely similar to those of Dissmann, despite their limited size. Dissmann considered that, while the size varied, the form of the sporangia remained fairly constant; according to Sparrow, however, "under foul environmental conditions" the sporangium of his material "may assume various shapes." The time required for the formation of sporangia depends on the medium and the state of vigour of the mycelium. (Dissmann).

Size and Form. Petersen described the sporangia as terminal, "seldom lateral," ellipsoid, opening at the apex "sometimes with a papilla;" he gave their measurements as 130 x 50 µ.

Drechsler has pointed out that the sporangia figured by Petersen are not in agreement with these measurements, one of them, according to the scale given, measuring approximately 240 x 70 µ; even if this is ignored, however, and only the measurements stated in the diagnosis are considered, the large size of the ellipsoid sporangia is regarded by Drechsler as "the most distinctive character" in the original description. With regard to his own isolate, he has written:- "The extraordinarily large sporangial measurements given by Petersen, which might be adequate for determing the application of his binomial if they were found usual for some fungus properly referable to the genus, have assuredly not been found usual in the Massachusetts waterlily fungus, whether it was grown on artificial media or on its natural substratum, though its sporangia have regularly been of generous dimensions." Despite their apparently limited size, the sporangia are considered by Drechsler to agree closely with those described by Dissmann.

Dissmann had shown experimentally that the size of the sporangia of his isolate varied according to the medium in which the mycelium had been grown before being transferred to distilled water or some solution of low nutritive value. In distilled water, after growth in 0.5 - 1.0% peptone solution, only a small number of sporangia were formed, and they measured only 30-40 μ; in pondwater, after growth in 5% maltose, vigorous sporangia up to 80 μ long were formed. The largest sizes were attained in nonsynthetic media; thus, in pondwater to which a very little waterlily leaf decoction had been added, the sporangia reached 120 - 140 μ, thereby resembling most closely the sporangia occurring naturally. While there was such variation in the size of the sporangia, their form remained fairly constant.

Conditions of formation. Dissmann had found also that the time required for the formation of sporangia, after the transfer of the mycelium, varied, according to the nature of the nutrient medium, from a few hours to ten days, and that mycelium which had shown a good growth in a natural medium such as oatmeal, formed sporangia earlier than mycelium from a synthetic medium. The "age" of the mycelium was also important; mycelium which had been growing in waterlily leaf decoction for three days formed sporangia two days after transfer to distilled water, while mycelium which had been growing in the same medium for fourteen days took eight days in distilled water to produce sporangia.

(b) The various modes of behaviour of the sporangium.

No writer has described sporulation in detail; the typical mode of discharge is recorded by Dissmann and Drechsler simply as the emptying of the undifferentiated contents into a vesicle; the formation of a short exit tube normally occurs in the material of Drechsler, who has stated his opinion that its absence from some of the sporangia figured by Dissmann is evidence of their identity as sporangia of a species of *Phytophthora*. Renewal of the sporangium takes place by proliferation, both within and beyond the empty one, or by subsporangial branching, the later method, according to Sparrow, being "usual under excellent environmental conditions." Some irregularities in sporangial behaviour have been described by Dissmann.

Discharge. The protoplasm that pours into the vesicle is described by both Dissmann and Drechsler as "undifferentiated;" no details of sporulation are given by either writer. Drechsler remarked that the vesicle is clearly visible in every instance, and commented that Petersen's statement, "Zoosporae ....., in vesica ut in Pythio, exeunt," does not make it absolutely clear whether the zoospores were fashioned in the vesicle, or were only surrounded by an evanescent vesicle," as sometimes occurs in Phytophthora." This criticism does not seem necessary.

Drechsler has commented also that Petersen did not explain how dehiscence of the sporangium occurred, if not by a papilla; it seems probable, however, that different conceptions of the papilla are involved, and that Petersen recognised it only when it was situated at the apex of a beak-like outgrowth. Some of the empty sporangia figured by Petersen show a well-marked exit tube, while the form of the opening of others suggests that their discharge had been by means of a sessile papilla.

Describing the "prolate ellipsoidal sporangia" produced on waterlily leaves, Drechsler has written:- "These sporangia on attaining a definitive size were often found provided with an apical papilla, which sometimes, after renewal of the water, would form a virtually sessile cap of dehiscence. More often, however, the papilla grew out into a short evacuation tube before the hyaline cap was formed; so that after the undifferentiated protoplasmic contents had migrated into the inflated vesicle, and had been converted into zoospores, the empty sporangial envelope was usually found bearing distally a recognisable tubular prolongation much like the similar envelopes drawn by Dissmann from material referable to his pure culture." In Drechsler's material, "usually the empty tube either terminates abruptly with a plain rim or is minutely lipped at the orifice, but in scattered examples it is found reflexed...."

Believing, therefore, that an "evacuation tube" is normally produced by the sporangia of P. undulatum, Drechsler has pointed out that its absence from certain of Dissmann's figures, particularly those showing multiple nesting in which "some of the inner envelopes terminate so far within the outer envelope that formation of a globose vesicle would have been obstructed," suggest that Dissmann included a Phytophthora among the sporangia which he ascribed to P. undulatum, occurring naturally in a mixture on waterlily leaves. "Among proliferous species of Pythium such obstruction does not ordinarily occur..." The "generous width" of the openings of these sporangia provide further evidence in support of their identification as Phytophthora, according to Drechsler. If this is so, it is interesting to note that they show an ellipsoid shape and dimensions entirely similar to those of the sporangia of P. undulatum. Drechsler's comment continues:- "In contrast, both of the correctly (sic) evacuated zoosporangial envelopes drawn by Dissmann from material derived from the pure culture that he treated as P. undulatum show a recognisable evacuation tube at the apex."

Abnormalities. Dissmann has described "certain frequent irregularities" in the behaviour of sporangia observed in hanging drop cultures. Sometimes, the plasma content began to extrude in the form of a small bubble, as in the first stages of normal discharge, but did not extrude completely, growing out, instead into a thick hypha. Occasionally, as for example in 1% peptone, and in cultures full of bacteria, a sporangium sprouted into hyphae at the tip. Rarely, the zoospores emerged without the formation of a vesicle, but the slow rate of their emergence and the irregularity of their form, suggested that they were of doubtful vitality.

The Mycelium.

The growth form of the mycelium is well expressed as a "robust mycelial habit," with hyphae of a "generally flexuous appearance." (Drechsler). The hyphae are described as "more or less undulating;" "frequent undulations" are recorded by Sparrow, but no comment on this feature appears in Matthews' account.

In most media, the main hyphae are 6 -8 μ wide, and sparingly branched; Dissmann has shown experimentally that both the width of the hyphae and their whole growth forms vary according to the medium, and has commented on their resemblance in certain media, to the hyphae of the Saprolegniaceae. Dissmann has observed, also the accumulation of fat in the older hyphae, and the pinkish red colour given by the walls when chlor-zinc iodide is added. Although Drechsler has described the penetration of the hyphae of P. undulatum by those of other fungi, no writer has commented on the formation of "intrahyphal hyphae" in P. undulatum, by its own mycelium. (see p. 132)

Habit. In the material described by Sparrow, the hyphae of the "sparingly branched, extensive mycelium" are "somewhat variable in diamter, but usually measure about 5 - 7.4 μ." Growing in maizemeal agar, the main hyphae of Drechsler's isolate often attain a width of 8 μ within 100 μ or 150 μ of the tip, though if the filaments are followed backward hardly any further widening is to be noted; lateral branches are produced "in moderate quantity" which "are of lesser thickness, and show more or less irregular secondary ramifications."

Dissmann has shown that both the width of the hyphae and the whole growth form vary with the medium. Thus, while the width is 6 - 8 μ in most media, in pea decoction it may be only 3 - 4 μ; very regular dichotomous branching is described as charactersitc in liquid media, but in pea decoction the branching is irregular, and in 1% peptone the hyphae are very straight and unbranched for greater lengths, presenting an appearance resembling that of members of the Saprolegniaceae. Dissmann has described also undulated lateral branches which he has called "hunger hyphae;" there are developed in solution of low nutritive value.

Petersen's description seems to refer to the sporangiophores only, not to the vegetative mycelium:- "It is difficult to trace the intramatrical mycelium; the extramatrical consists of long (often several millimetres) unbranched, more or less undulating hyphae;" these are 3 - 6 μ in diameter.

The contents of the hypha. Vigorous hyphae are densely filled with protoplasm, which is described by Petersen as refractive. According to Dissmann, there are very many fat globules, which are also stored in larger quantities in the older parts of the mycelium; here, fatty degeneration of the plasma often occurs so that the fat globules run together and fill large portions of the hyphae. The hyphae then soon die, and the fat is released into the surrounding medium. Dissmann has observed that this fat is coloured by Sudan III, and goes red with Nile blue: with osmic acid it goes black, though not very intensely. The "hunger hyphae" are very refractive; they are often much vacuolated, and their plasma is filled with small fat globules.

The walls. The pinkish red colour shown by the walls of the hyphae when chlor-zinc iodide is added, reported by Dissmann, is not mentioned by Petersen, although he was aware of the reaction, as a characteristic of the "Pythiomorpha" genus. Petersen recorded, however, that "the membranes of the hyphae and sporangia are more or less brownish coloured."

Antagonism and parasitism. Drechsler has described the extraordinary phenomenon of parasitism among congeneric and less closely related fungi, and has given the following account of the attack on hyphae of P. undulatum by Plectospira myriandra:- "When the waterlily parasite is grown on maizemeal-agar plate cultures in opposition to P. myriandra, its mycelial advance is halted along the zone of encounter as its individual hyphae become enveloped by short branches extended from filaments of the saprolegniaceous form; envelopment in all instances being followed by darkish degeneration of the protoplasm within the Pythium hyphae." Similar injury is caused by the co-generic species P. oligandrum, P. acanthicum and P. periplocum.

The chlamydospore.

Chlamydospores are not mentioned in Petersen's description, but have been described in detail by Dissmann and Drechsler, and are included in the accounts of Matthews and Sparrow. Drechsler's closely resemble those of Dissmann, and Sparrow's are probably the same; although Matthews has stated that those formed by her isolate are similar to Dissmann's, the chlamydospore which she has figured suggests dissimilarity.

According to Dissmann and Drechsler, the chlamydospores are usually spherical or subspherical; their size is variable, and determined by the richness of the medium and the vigour of the mycelium. The mature chlamydospore is surrounded by a thick double wall, and contains much fat. Drechsler has shown that a resting period is necessary before germination takes place, when the germ hypha produces terminally a sporangium which is entirely similar to those formed by the mycelium.

In structure and germination, the chlamydospore of P. undulatum is considered by Drechsler to resemble closely the oospore of P. helicoides.

Form and structure. Chlamydospores are usually spherical or subspherical, but sometimes, according to Drechsler, cylindrical or transversely constricted. Dissmann has described them as partly intercalary, partly terminal swellings, which are cut off by crosswalls from the neighbouring regions of the hyphae, which are poorer in protoplasm; each newly-formed swelling surrounds itself with a thin wall immediately within the wall of the parent hypha. After a few weeks, the doubleness of the wall surrounding the chlamydospore is clearly visible, the outer membrane having remained thin and colourless, while the inner one has thickened considerably, and assumed a yellow colour. Older chlamydospores are often rough, through the flaking off of this outer wall. The different composition of the two membranes is indicated by their different reactions with a number of stains, according to Dissmann. The maturation of the chlamydospores involves the accumulation of fat, and microtome sections of chlamydospores a month old show them to contain little protoplasm and much fat. Drechsler has written of the resting spores formed by his isolate:- "Their identity with the chlamydospores described by Dissmann becomes clearly manifest at maturity, when they are found crowded internally with an abundance of globules varying commonly from 4 to 10 in diameter;" evidently, the number of these globules in a single spore varies from four or five to two hundred; refringent "orbicular bodies," mostly 2.5 to 3 in diameter, are also present in Drechsler's spores.

Conditions of formation, and variation in size. Dissmann has stated that the chlamydospores are always found on solid media such as waterlily decoction agar, maizemeal and oatmeal agars, after four or five days, and are sometimes found, though rarely, in liquid media. He had found them in mud, in the spring, but believed their occurrence in nature to be rare. In the laboratory, experiments with different culture media showed the size of the chlamydospores to be extremely variable, and to be directly related both to the richness of the medium, and the state of vigour of the mycelium. Dissmann found the commonest sizes, within a range of 6 - 92μ, to be 36μ, 59μ and 69 μ. The range given by Drechsler is 15 - 75 μ.

Germination. The results of experiments carried out by Dissmann in an attempt to bring about the germination of chlamydospores of different ages were very variable. Chlamydospores which were only a few days old, formed in maizemeal agar, behaved like sporangia and released zoospores after one or two days in distilled water. When older chlamydospores were put into distilled water, they produced one or more hyphae, but those which had been formed for a month or more could not be induced by any means to germinate.

Drechsler has explained this behaviour as follows:- Newly-formed chlamydospores are able to act as sporangia since they are indeed little different from such organs; in older chlamydospores, however, the thickening of the wall and the accumulation of fat have begun, and after twenty days, the time stated by Drechsler to elapse during the maturation of the spore, a long resting period is necessary before germination can take place. Drechsler obtained the following results with spores transferred to water after being allowed to rest for longer periods than any stated by Dissmann:-

Age of spores	Per cent. germination	Time required
65 days	10 - 25	7 days
100 days	50	2 days
180 days	almost all	1 day

Sometimes a mycelium is developed from the germtube of a resting spore, but in the oldest material a sporangium, which is similar in all respects to those formed on the mycelium, and releases zoospores in the characteristic manner is produced in almost every instance.

The resemblance of the chlamydospore to the oospore of P. helicoides. The structure of the mature resting spore and the changes which take place during germination, have led Drechsler to draw a comparison between it and the oospores of other species of Pythium. As a result, he has avoided the use of the term chlamydospore, preferring to regard the resting body of P. undulatum as a parthenospore, homologous with the oospore of P. helicoides. He has written:- "One might be inclined to dismiss the morphological parallelism as being perhaps of fortuitous character if it were not so strongly corroborated by the physiological similarity manifest in the prolonged dormancy of the reproductive bodies under discussion; such dormancy being familiar among oospores and parthenospores, but wholly unknown among the subspherical conidia and chlamydospores formed by numerous species of Pythium, including, for example, P. de Baryanum and P. ultimum."

The absence of oogonia.

Oogonia have not been reported for P. undulatum, although it has been grown in a number of different media, and Dissmann explored variousparts of the waterlily plant.

The relation of P. undulatum to waterlilies.

Petersen, Dissmann and Drechsler have all collected P. undulatum from waterlilies. While Petersen seems to have regarded the fungus as a saprophyte, Drechsler has referred to it definitely as "the waterlily parasite," concluding from the results of innoculation experiments on waterlily leaves that it is responsible for their premature discolouring. The source of Sparrow's material is not stated; Matthews' was collected from soil.

Petersen had observed that this "species seems to live especially on decaying leaves and stems of Nuphar and Nymphaea," but had collected it also from "old fruits of Iris" and branches of trees; as Drechsler has pointed out, it appears from these statements and from his general observations on the biology of aquatic fungi, that Petersen did not attribute any pathological relationship to the fungus.

Dissmann's attention had been drawn to the leaves of Nymphaea candida by their early yellowing in May or June instead of in the autumn, when he examined them and found them to be permeated by the hyphae of several members of the Pythiaceae. P. undulatum and P. proliferum were predominant, but, in addition, two other species could sometimes be recognised, and Achlya americana was found occasionally. It is not clear from Dissmann's account to which fungus he attributed the primary attack on the leaves, and which he regarded as weak parasites or saprophytes.

Drechsler wrote in 1941:- "In our Northern States the foliage of some white waterlilies, including Nymphaea odorata Ait, and Nymphaea tuberosa Paine, often undergo premature decline from multiplication and extension of water-soaked or discoloured areas. When portions of affected tissue are excised from such areas, pressed between sheets of absorbent paper to remove excessive water, and planted on agar media, Pythium mycelium almost always grows out very promptly." "Among several hundred cultures derived through such procedure from material collected in Massachusetts, New York, and Wisconsin, a species closely similar to P. helicoides, probably identical with Dissmann's P. proliferum, was abundantly represented; as was also a species that, judging from the large globose reproductive bodies produced by it in maizemeal agar, must almost certainly be the same as Dissmann's P. undulatum."

Again, in 1946, Drechsler wrote:- "In July and August, 1944, occasion was taken to try out the fungus on young unblemished waterlily (Nymphaea sp.) leaves .... Soon after their removal from the pond, the leaves were placed in large glass damp-chambers, planted with slabs excised from a maizemeal-agar plate culture of the phycomycete, and stored at a temperature of 18°C. In two or three days the leaf areas under the slabs took on a dark brown discolouration. This discolouration continued to spread steadily, with the result that in ten days it had come to extend over irregularly circular patches forty to sixty millimetres in width, though the leaves that had not been planted with the fungus still retained then their fresh green colour throughout. When pieces of discoloured tissue near the periphery of the brown waterlogged patches were removed to a shallow layer of distilled water in a Petri dish, and then stored at a temperature near 18°C, extramtrical hyphae 3 to 7 μ wide grew out into the liquid." These hyphae produced terminally the characteristic sporangia described above in the section on sporangia, under the sub-heading, "The various modes of behaviour of the sporangium." Drechsler's account continues:- "That the extramatrical hyphae and the sporangia really derived from the material planted on the leaves, rather than from some adventitious parasite, could hardly be doubted in view of their close resemblance to the extramatrical hyphae and sporangia produced in moderate quantity following migration of slabs excised from young maizemeal-agar plate cultures permeated exclusively with vigorous mycelium of the Massachusetts fungus." From this it is clear that the discolouration of the leaves is attributed by Drechsler to P. undulatum.

Summaries of the main Items in the Accounts of Dissmann and Drechsler.

Pythium undulatum Petersen (Dissmann, 1927)

1. The isolation of P. undulatum from the leaves of Nymphaea candida, where it grows usually in association with P. proliferum, often with two other species of Pythium as well, and occasionally, in the older parts of the leaves, with Achlya americana.

2. The effect of varying the content and concentration of the culture medium, on the width and branching of the hyphae, and the size of the sporangia.

3. The various modes of behaviour of the sporangium, the normal behaviour being the emptying of the "undifferentiated contents" into a vesicle.

4. The structure and germination of the chlamydospores, and their variation in size according to the nature of the culture medium.

Pythium undulatum Petersen sensu Dissmann (Drechsler, 1946).

1. The taxonomic position of P. undulatum, viewed in the light of previous records, (without reference to de Wildeman or Cejp).

2. The features of the mycelium and sporangia of Drechsler's isolate.

3. The structure and germination of the chlamydospores, and their apparent homology with the oospores of P. helicoides.

4. The antagonism and parasitism occurring among *P. undulatum*, other species of *Pythium*, and species of *Plectospira*.
5. The agreement of the features of Drechsler's isolate with Dissmann's description, of which an account is given, and the conclusion that the two fungi are the same species, *P. undulatum* Petersen.

Discussion on the Identity of the Fungi recorded as *Pythium undulatum* Petersen and "*Pythiomorpha undulata*" (Petersen) Apinis

The identity of the fungi isolated and described in *pure* culture by Dissmann (1927), and Drechsler (1946), as *P. undulatum* Petersen can hardly be doubted. (Drechsler has pointed out that some of the sporangia attributed to *P. undulatum* by Dissmann in figures drawn from a *mixed* culture may possibly be those of a species of Phytophthora.)

Matthews (1931) does not seem to have doubted the identity of her material as *P. undulatum* Petersen, despite the small dimensions of both the sporangia and the chlamydospores formed in her cultures, which were grown on hemp seeds, and has stated that the chlamydospores "were similar to those described by Dissmann." Sparrow (1932), however, has made the criticism that the chlamydospores in her figures "appear to have smooth, hyaline walls rather than the rough, brown ones, as found by Dissmann ....." On the same grounds, Drechsler has expressed the opinion that the identity of his own material with Matthews' is "quite improbable," and that thickwalled bodies like those described by Dissmann "may be formed by several members of the genus and more particularly, perhaps, by aquatic members intimately akin to the waterlily parasite," so that these bodies cannot therefore be used as a basis for identification.

On the other hand, according to Drechsler, there is considerable similarity between his material and that described by Sparrow (1932), in which chlamydospores, closely similar to those of Dissmann, are formed.

The account of de Wildeman (1931) is too incomplete to justify confidence in his record of P. undulatum, particularly as he has himself expressed uncertainty as to the purity of his culture. Marked undulations of the hyphae are described, and a figure is given of narrow ellipsoid nested sporangia, terminal on an undulating sporangiophore which widens conspicuously under the sporangium. No scale is given, however, and it appears that the record must remain a doubtful one. No reference is made to it in Drechsler's discussion of P. undulatum.

As Lund's (1934) record of P. undulatum is founded solely on the formation of a vesicle by one sporangium, which was distinguished by this single feature from the sporangia of the fungus which he has described as "P. undulata," it seems inadequate for serious consideration.

On the contrary, Lund's description of "Pythiomorpha undulata" is fairly detailed and shows great similarity to that of Apinis (1929). The hyphae are "often undulated, slightly branched, when old often yellowish," and become "a reddish violet colour" with chlor-zinc iodide. Apinis had not mentioned the latter feature in connection with "P. undulata," but recognised the reaction as a characteristic of the genus "Pythiomorpha." The definite statement is made by Lund that the zoospores are "formed in the sporangium," and escape through a terminal opening. Abnormalities occur, the spores sometimes encysting and germinating within the sporangium (as described by Apinis), or, occasionally, the sporangium germinating by means of hyphae. Proliferation and subsporangial branching occur.

Cejp's (1933) references to "P. undulata," are made in order to illustrate the characters of the genus Pythiomorpha; the undulation of the hyphae, and their reddish colour with chlor-zinc iodide, the tendency to irregularity in the shape of the sporangium, and proliferation, are mentioned, and abnormalities in sporangial behaviour similar to those in the material of Apinis and Lund are described.

The comments made by Cejp on the influence of environmental conditions on sporangial behaviour are interesting. He has written:- "We obtain a better development of the sporangia when we add to the water a trace of some acid, for inst. HCl so that pH is about five." "*P. undulata*" has been found by Lund in "highly acid" (pH 3.5 - 4.5) and "neutrally acid" (pH 5.2 - 7.5) situations. The sporangium *P. undulatum* was identified by Lund in material from the "highly acid" one. Cejp has also pointed out that Minden's (1915) observation of the formation of a vesicle in "*Pythiomorpha gonapodyides*" may have been a mistake "due to the fact that under special outside conditions of environment some sporangia instead of normal emptying, discharge the whole plasmatic contents" without dividing; it seems equally possible that the converse may be true, and that abnormal behaviour of the sporangia of *P. undulatum* may have caused it to be recorded as "*Pythiomorpha undulata*".

Until a detailed morphological and physiological investigation has been made of a fungus resembling those described as "*P. undulata*" by Apinis, Cejp and Lund, no decision can yet be reached as to whether the same specific epithet has been applied in the past to two different fungi, one a *Pythium* and one a *Phytophthora*, or to two modes of behaviour of a single species, *Pythium undulatum*.

During the present study, contact has been made with Lund, Cejp and Middleton, and a number of other investigators whose work brings them in touch with water moulds, including Johannes in Germany; a culture of *P. undulatum*, indistinguishable from the material collected at Royal Holloway

College, was received from Miss van Beverwijk, Baarn, in February, 1947.

Note: In 1891, an interesting record was given by Massee under the name "Saprolegnia elongata." (British Fungi, p.217) As Coke (p.76) has pointed out, this is apparently "based on mixed material, in part a _Pythium_ and in part a _Saprolegnia_." The oogonia, with 3 - 4 oospheres, and antheridia, may have probably been those of a species of _Saprolegnia_; but the sporangia in the figure (Plate 3, fig.47) are clearly those of a member of the Pythiaceae. The sporangium is described by Massee as "terminal, elliptical, 50-60 x 18-21 μ, filled with zoospores which escape from an opening at the apex." Proliferation is shown, and the sporangiophores, which are described as "slender, 3 - 4 μ thick, often flexuous," with undulations, and a conspicuous widening under the sporangium.

CONCLUSIONS

The identity of the Royal Holloway College isolate, and that of Miss van Beverwijk, seems beyond question, and these isolates therefore provide two additions to the records of Pythium undulatum Petersen.

Close agreement has been found with the description by Dissmann and Drechsler except in the following points:-

1. Greater variation in the shape of the sporangium has been observed in the R.H.C. fungus than can be inferred to have occurred in Dissmann's, from his statement that while the size of the sporangium varied greatly, the shape remained fairly constant. Sparrow, however, has commented on the considerable changes in shape in the fungus observed by him.
2. Discharge of vigorous sporangia of the R.H.C. fungus usually takes place by means of a sessile papilla; only when discharge has been delayed is a short exit tube formed. Drechsler has found the latter mode of dehiscence to be the more usual one in his isolate.
3. While Drechsler has referred to his fungus as "the waterlily parasite," convincing evidence of the strongly parasitic nature of the R.H.C. isolate has not been found.

No previous writer has made any detailed observations on sporulation, or commented on the unusually short time (fifteen minutes or less) that elapses during the whole process, from the first rush of protoplasm out of the sporangium to the escape of the zoospores from the vesicle. As it seemed at first that P. undulatum shows a greater degree of differentiation of its protoplasm before discharge than commonly occurs among members of the genus Pythium, and in general presents an unusually gross appearance, the need was recognised of a proper understanding of the genus as a whole.

The following account of the vegetative features and asexual reproduction of members of the genus Pythium, in which emphasis is laid on the details of sporulation, shows that no departure is made by P. undulatum from the limits of this wide and varied group.

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SECTION II: Sub-section II

An Account of the Genus *Pythium*

Contents.

AN ACCOUNT OF THE GENUS PYTHIUM

I. THE MYCELIUM

Characteristics of the Hypha

Hyphae are usually 2-5μ wide, but vary from 1-8μ, according to Middleton. The branching is abundant and irregular. It is essentially monopodial, but, according to Butler, dichotomy occurs in P. intermedium. Drechsler (1946) has compared the "flexuous appearance" of Pythium hyphae with the "stiffly branching aspect" of those of Phytophthora. Middleton observed that the mycelium may be intramatrical or extramatrical, usually both on solid substrata, and that when a host is invaded, the hyphae are both inter- and intracellular. Butler recorded that there is not "any formation of rhizoids, such as occurs in the Saprolegniaceae." Höhnk (1932) showed that the number of lateral branches formed by P. epigynum in a water culture depends on the available food and not on the amount of water.

Butler observed that in the living protoplasm of the hypha there is abundant fat which blackens in Flemming's solution, and that glycogen occurs in highly refractive droplets. Kanouse and Humphrey reported a strongly lyophilic nature as a striking characteristic of

P. afertile.

Trow found difficulty in obtaining the cellulose reaction on the wall of P. ultimum, only a faint blueing resulting from the addition of chlor-zinc iodide. The work of Thomas, described under the heading "Discussion on the relation between Pythium and Phytophthora," reveals that the cellulose is not only masked by an outer covering of pectic material in active growing regions of the hyphae, but it is also strongly impregnated with fatty acids which prevent the fixation of dyes.

Vigorous growing hyphae are hyaline and coenocytic, but as the available food material becomes scanty, or conditions become otherwise unfavourable for growth, the protoplasm draws out of the older regions and moves on into the active parts, and the empty parts behind are cut off by septa. Butler described the formation of a septum as follows:- "As the protoplasm grew scanty, an accumulation of granules was observed a little behind that portion of the hypha that was still well filled," and the septum appeared in the midst of the granules.

Appressoria

Appressoria are formed when hyphae make a surface contact; these are extremely variable, even within a single species, and are described by Middleton as "simple or complex, straight, curved or globular in shape, becoming more or less flattened when applied to a firm surface." In P. adhaerens they are "slightly arched and clavate, or slender and somewhat sickle-shaped, occurring singly or in clumps of great complexity." (Sparrow, 1931.b.)

"Budlike lateral processes"

The hyphae are usually regular, but irregularities, to which the noncommittal term "hyphal swellings" may be applied, are of frequent occurrence both in species with filamentous sporangia and in species with spheroid sporangia.

Both Marshall Ward and Butler remarked on the irregularities occurring in the subgenus Aphragmium. Butler has described "bud-like lateral processes," also seen by Marshall Ward, "which become crowded with dense protoplasm and are probably capable of preserving their vitality under conditions which would be fatal to the more slenderly-nourished hyphae;" they often continue growth by putting out a hypha. Whereas Butler regarded these structures as "buds," serving for perennation, Marshall Ward considered them to be reservoirs of protoplasm either for the immediate needs of the mycelium or for the formation of oogonia. Both investigators ignored the possibility that they might be sporangia. Edson, however, realised that in the fungus which he called Rheosporangium aphanidermatus, (re-named Pythium aphanidermatum by Fitzpatrick), the irregular, lobulate swellings discharge the zoospores, and he used his term "presporangia" for them. Sideris (1931) regarded such structures as "morphologically identical with and indistinguishable from" sporangia until the latter discharge zoospores. He called them "plasmatoögoses", (plasmatic outgrowths), and considered them "of great importance in the characterisation of species," according to their occurrence singly or in groups. Swollen irregularities of the mycelium distinct from sporangia have been recognised also by Sparrow, and by Subramaniam who regarded them as reservoirs.

Chlamydospores

Although thickwalled chlamydospores are formed abundantly by certain species, e.g. P. undulatum and P. afertile, it seems that neither Matthews nor Middleton has regarded the formation of chlamydospores as of any importance in the genus Pythium. They do not appear in the section of Middleton's monograph headed, "Morphological observations," and are even omitted from the description of P. undulatum.

Kanouse and Humphrey reported the formation by P. afertile of chlamydospores, which they described as "spherical or elliptical, 9-27μ ; sometimes irregular. Usually formed terminally but become lateral by the formation of hyphae from one or more places." A septum, which might be as much as 30μ away from the swollen part, is formed on either side of an intercalary chlamydospore. Kanouse and Humphrey observed that these bodies are formed in large numbers on flies, but, owing to the unusual lyophilic nature of the fungus, they do not appear on oil agar. They have never been seen to produce zoospores. Matthews has observed, "We have been unable to see a reason for not considering them as conidia."

Gemmae

At various times, structures have been described to which the name "gemmae" has been given. However, no member of the genus Pythium forms any organs which are comparable with the gemmae of the Saprolegniaceae.

Butler observed in P. rostratum certain bodies which he called "gemmae." They were "produced on one occasion on an old fly culture, and, inoculated on a fresh fly, gave a typical culture. They were pear-shaped buds, arranged in a zig-zag manner, each succeeding one arising from the side of the previous." Butler remarked, "I have not observed them elsewhere, though the vegetative buds ...... are probably allied bodies." "They resemble the gemmae of Saprolegniaceae but have not the same morphological value." That is, Butler accepted the view expressed by Maurizio that in Saprolegniaceae the gemma represents a modified sporangium. He defined the Pythium gemma as a "vegetative bud representing a portion of mycelium containing within itself a sufficient store of reserve nutriment to enable it to withstand isolation from the plant without losing its power of renewed growth."

These organs described by Butler have not been observed since, and they are altogether omitted from the description of P. rostratum in Middleton's monograph.

Bodies "of irregular form" appearing in P. epigynum "at the end of the growth in length of the hyphae or later on" have been called "gemmae" by Höhnk (1932). As these bodies are distinguished from resting sporangia only by the fact that the latter are formed earlier, "during the vegetative period of the mycelium," and are more regular in shape, there seems insufficient reason for giving them a different name.

The so-called gemmae described by Sideris (1932) in P. polycladon (= P. vexans) are "represented by extrusions of protoplasm through diametrical breaks produced near the tips of hyphae," and germinate giving rise to many hyphae simultaneously. As they are produced only under slightly adverse conditions, when the organism is grown in culture media not suitable for the production of "conidia" or zoosporangia, they appear to be an abnormal growth phenomenon.

II. THE SPORANGIUM

Features of the Mature Sporangium

An outline of the chief features

There are three main types of sporangium;-

1. An undifferentiated portion of the mycelium set off by crosswalls and bearing an exit tube.
2. A complex of irregular digitate or lobulate intercommunicating elements set off by crosswalls from the rest of the mycelium and having an exit tube.
3. A bursiform, utriform, spherical or somewhat ovoid, terminal or intercalary organ set off by septa from the more of less differentiated hypha, with or without an exit tube.

Sporangia of the first two types have been grouped together by Middleton and called "filamentous," to distinguish them from the rest, which he has called "spheroidal." According to Sparrow (1943), "intergrades between some of these types" may occur.

A wide range of size, as well as of shape, may be found in a single species.

The occurrence is indicated of two kinds of vacuole, possibly differing greatly in composition and function, in the sporangia of species of Pythium. The first kind is associated with sporulation, the sporangia of most species possessing one or more vacuoles previous to discharge; the second kind is a vacuole of senescence; though it seems that in the sporangia of some species which can liberate zoospores after a period of dormancy, rejuvenescence occurs.

Dehiscence of the sporangium takes place by means of a papilla which may be sessile or situated at the end of an exit tube of variable length (alternatively called "discharge tube," "evacuation tube," "emission tube," or "emission collar.")

Vacuoles.

"A ripe sporangium ready to discharge is always vacuolated," according to Butler, whose observations refer especially to P. proliferum, P. rostratum, P. intermedium, and P. gracile. While the sporangium of P. gracile is of type (1), those of the other three species are of type (3). "P. proliferum invariably possesses one or more large vacuoles, usually of an irregular shape," but, in this species, "the eventual condition is one in which the sporangium contains a single irregular vacuole."

Marshall Ward, also, had mentioned the vacuoles of P. proliferum. Sparrow (1931b) has described them in P. adhaerens, and Edson in P. aphanidermatum, species which have sporangia of types (1) and (2) respectively.

Drechsler (1941) has stated that, unlike other species, a vacuole is not formed prior to sporulation, by a vigorous sporangium of P. marsipium, but that the "sporangia here, like those of other oomycetes, sooner or later show a vacuole of senescence, which often increases in size until the granular protoplasm is crowded into a thin parietal layer."

The exit tube.

Butler described a "beak," which he believed to be "of constant occurrence." It is not found in a definite position, but is "of constant length relative to the diameter of the sporangium." Thus in P. proliferum it is always rather less than one quarter of the diameter of the sporangium. In filamentous sporangia, Butler considered the "beak" to be represented by the modified apex of the hypha: "It is always occupied at its upper part by the subapicular vacuole." He observed "granules of a bright oily appearance move up and disappear in the vacuole" of P. gracile.

Matthews has pointed out that the length of the exit tube is variable, and determined by environmental conditions. Indeed, there are various reports of its presence or absence. Thus it is stated on p.3 of Middleton's monograph that the sporangium, "whether filamentous or spheroidal, gives rise to a delicate emission tube which is usually from 2 to 5 μ in diameter and which varies from 5 to 500 μ in length," while on the same page the papilla of five species is described as "sessile." Elsewhere Middleton has written of P. undulatum, "regardless of the position of the sporangium, the vesicle is sessile, arising from the papilla."
Drechsler (1941) has reported that a discharge tube is usually formed in P. undulatum and in P. oedochilum, but that the sporangium of the latter sometimes possesses only the "small, apical protrusion" which serves in dehiscence. Similarly, according to Drechsler, in P. palingens, the "dome-shaped apical papilla, usually present on a sporangium when it attains a definitive size, may function directly in dehiscence."

The Resting Sporangium

A summary.

Sporangia sometimes remain quiescent for a considerable time before releasing zoospores. It appears that the dormancy of sporangia of P. proliferum may last for several months. A large vacuole is present in the resting sporangium of most species, but not in P. marsipium (Drechsler, 1941); in this species, when water is added, "discharge of the sporangial contents ensues more promptly than in most allied species."

A resting sporangium sometimes germinates by means of a hypha. Its behaviour is determined largely by environmental conditions, the addition of fresh water encouraging the liberation of zoospores. Light and aeration have also been shown to influence the behaviour; obviously, the state of vigour of the protoplasm at the time of formation of the sporangium, the length of the quiescent period, and the conditions under which the dormancy has been maintained, must also be important factors.

As a sporangium which produces a germ hypha does not apparently differ from one which liberates zoospores in any other feature, there is no reason for distinguishing it by another name. The term "conidium" is entirely inappropriate. It is clear that a classification such as that proposed by Sideris in 1929, based on the production either of zoosporangia or of "conidia" is quite impracticable.

Details.

Marshall Ward kept sporangia of P. proliferum "dormant many months in a cool cellar," and came to the conclusion that they "retain their power of forming zoospores for long periods." Butler also noted this power, after keeping them for a month, and observed that there is no alteration in structure, but that the striking feature is still the large vacuoles. Marshall Ward considered a "thick outer wall, short beak-like process, and large central vacuole" to be characteristic of the dormant sporangia observed by him. This difference may be due to the fact that these sporangia had undergone a longer, and possibly cooler, period of quiescence.

The dormancy of a resting sporangium is terminated either by the liberation of zoospores or by the formation of a germ hypha. In P. epigynum, according to Höhnk, (1932), only a few of the first-formed sporangia liberate zoospores at once, most of them becoming resting sporangia, and the proportion of later-formed sporangia which sporulate immediately is even smaller. The older the resting sporangium of this species, the less likely is it to produce zoospores, and after six weeks "only rarely are zoospores formed." Among the first-formed sporangia, however, "the percentage of those which germinate immediately may be influenced by the addition of fresh water ..." The influence of environmental factors in determining the behaviour of sporangia has been appreciated by various investigators. Thus, Butler induced the discharge of zoospores by supplying the sporangia with a large amount of well-aerated water, in the light. Drechsler (1930) has also stressed the value of aeration and illumination.

Sporangia and other "spherical to pyriform resting bodies" (Matthews) which germinate by producing a hypha have sometimes been erroneously referred to as "conidia," although their close relationship to sporangia has been well appreciated, as the following statement by Butler shows:- "There is abundant evidence that conidia and sporangia are of common origin, the conidium in Pythium representing merely a sporangium which has failed to form zoospores and which germinates directly. Thus in P. intermedium, where both conidia and sporangia are ordinarily freely produced, they not alone present no difference to the eye, but their behaviour is wholly a matter of environment. A given spore may, if appropriately treated, empty its contents as zoospores, or put out a vegetative hypha from which a new plant arises; and even after one or the other of these two methods of propagation has commenced, some change in the conditions may cause the other to be the final result of germination." "Indeed, the change from sporangia into conidia is probably exceedingly common."

Thus, while the production of zoospores is the natural, and, according to Middleton, in "filamentous strains" the only function of a sporangium, the development of a germ hypha may be regarded as the result of its suppression by environmental forces. Since the various structures termed "conidium" and "gemmae" are usually capable of liberating zoospores under favourable conditions (except P. ultimum), it seems desirable to include them under the term "sporangium," "inasmuch as morphologically and phylogenetically only one structure is under consideration." (Middleton).

Sporulation: (1) A summary

Introduction.

According to Matthews and Middleton, sporulation is characterised by the passage of the protoplast in an undifferentiated state through a short or long exit tube into an evanescent vesicle in which the zoospores are formed. In founding the genus *Pythium* on *P. monospermum* (and *P. entophytum*) in 1858, Pringsheim wrote:- "Die Schwärmsporen bilden sich nicht, wie bei *Saprolegnia* und *Achlya*, noch innerhalb der Sporangien selbst, sondern diese öffnen sich noch vor Entstehung der Schwärmsporen an ihrer Spitze, und ihr noch völligformloser Inhalt tutt in jenem unveränderten Zustande, in welchem er gewöhnlich Schlauch und Sporangium erfüllt, aus der Oeffnung hervor und sammelt sich vor derselben zu einer Protoplasmakugel an welch noch von einer aüssert zarten Membran umhüllt erscheint."

However, detailed descriptions by a number of investigators show that different stages in differentiation are reached before discharge occurs, in different species, and even in different sporangia of the same species, and that this variation is due only in part to differences in environmental conditions. It seems best, therefore, to describe the emerging protoplasm as "relatively undifferentiated." Details of this differentiation, as compared with that of species of *Phytophthora*, are given in Appendix II.

The membrane within which the zoospores develop until their escape as mature freeswimming bodies has been regarded by some investigators as the sporangium proper. Thus the papillate structure which pours its contents into it has been called a "presporangium" by Edson, and a "prosporangium" by Sideris (1930). However, comparison of this papillate structure with the sporangium of a related genus such as Phytophthora indicates the homology of the two organs. It is therefore generally regarded as the sporangium, and the membrane within which the zoospores mature is called the vesicle.

A summary of the process.

The changes undergone by a sporangium, whether filamentous or spheroid, prior to its discharge follow a certain sequence which is characteristic throughout the genus. The following summary is based on the outline given by Butler:-

1. Changes occur in the appearance of the hyaline cap of the "beak". The apex becomes very bright owing to the presence of a highly refractive substance which appears to be derived from the apical wall; Sparrow (1931b) has observed also, in P. dictyosporum, two further subapicular regions, a slightly less hyaline zone and a pale finely granular one. As the granular protoplasm retracts, the whole "beak" becomes clear.

At this time, the protoplasm, which is evidently in motion, develops an appearance which Butler has described as "lumpy," due to some differentiation of the protoplasm which foreshadows its division into zoospores. There is considerable disagreement between the accounts which have been given by different investigators of the degree to which this differentiation may proceed, due, perhaps, to some extent, to differences in the conditons under which the observations have been made. Thus, while Drechsler (1941) has described a considerable degree of differentiations of the protoplasm before its discharge as a regular feature of P. marsipium, different sporangia of P. proliferum (and of P. aphanidermatum) have been observed to reach very different stages before discharge. It seems, however, that the complete delimitation of the zoospores into separate units is of rare occurrence in Pythium (see Appendix II, pp. 217-220)

2. The large central vacuole disappears, and the sporangium decreases in size by an appreciable amount. Although Büsgen noted the occurrence of a large vacuole in P. proliferum, it seems to have been overlooked by most of the early writers. Butler has attached to it great importance in the process of sporulation, and Drechsler (1941) has described its absence from the sporangium of P. marsipium as an aberrant feature.

3. The apex of the sporangium swells, the protoplasm flows up the beak and pours out of the sporangium, pushing the refractive substance in front of it, into a spherical

mass surrounded at some distance by the delicate membrane of the expanding vesicle.

4. The maturation of the zoospores takes place in the vesicle. Four stages may be distinguished:- (1) cleavage areas appear in the gently rocking mass. (2) Short flagella can be seen waving on the periphery of the mass, and the separate spores begin to show individual movement. (3) The spores are clearly vacuolated, and the flagella can be seen to have elongated. (4) The oscillatory motion of the zoospores changes to a rapid swimming movement, and the vesicle finally bursts, liberating the mature spores.

Various lengths of time have been alleged by different investigators to elapse during the whole process of zoospore formation and maturation. The speed of the process is probably determined chiefly by the conditions of the environment and the state of vigour of the protoplasm, although it appears that maturation tends to take longer in species with filamentous sporangia than in those with spheroid ones.

Sporulation: (2) Details

1a. Changes in the "beak."

"The first indication that zoospore formation is about to occur appears in the apex of the beak," according to Butler. Observing P. rostratum, he noted that the whole "beak" became clear, and that the apex was very bright.

De Bary (1860) and Hesse had commented on the gradual thickening of the originally delicate apical wall, of P. proliferum and P. de Baryanum respectively, and the replacement of the protoplasm in the "beak" by a colourless gelatinous substance which passes over at its base into the lateral walls. This substance entirely fills the "beak" of P. proliferum, according to de Bary and Butler, and "almost half" fills that of P. de Baryanum, according to Hesse. All three observers commented on the definite contour of the protoplasm, separating it from the refractive substance. The statements of de Bary and Hesse are quoted in full as follow:-

De Bary: "Die nächste in dem Sporangium wahrnehmbare Veränderung besteht in einer Verdickung seiner Anfangs sehr zarten Membran, in dem Zurücktreten des Plasmainhaltes aus der Endpapille und seinem Ersetztwerden durch eine homogene, gläizende, farblose Substanz. Angangs in geringer Menge vorhanden, fullt diese zuletzt die ganze Papille aus und geht an der Basis dieser in die ziemlich derbe Seitenwand über, so dass sie wie eine kegelförmige Verdickung dieser erscheint. Sie besteht, ihrer Aehnlichkeit mit zweifellosen Gallertablagerungen und ihrem späteren Verhalten zufolge, aus einer der Cellulose nahe verwandten Gallerte. Die Plasmamasse, welche durch sie verdrängt wird, bleibt stets an ihrer Aussenseite von einem scharfen, wenngleich sehr zarten Contour umzogen, der sich in die Umrisslinie des die Seitenwand berührenden Plasma fortsetzt, und den Primordialschlauch anzeigt."

Hesse: "Gleich nach vollendetem Längenwachsthum des kurzen Fortsatzes nämlich sieht man, wie sich die anfangs zarte Membran der Zoosporangien etwas verdickt und wie allmählig der protoplasmatische Inhalt aus der Endpartie des kurzen Fortsatzes zurücktritt und ersetzt wird durch eine farblose, gallertartige Substanz, zuerst ist diese gallertartige etwas glänzende Masse nur in der alleräussersten Spitze des Fortzatzes eines solchen Zoosporangium's sichtbar, aber später vermehrt sich dieselbe derartig, dass sie nicht nur den engen Kanal des Fortsatzes bis bald zur Hälfte ausfüllt, sondern dass sie auch noch von da aus ein Stück weit in die Wandpartie der anderen basalen Hälfte des Fortsatzes übergeht. Die jenige des protoplasmatischen Inhaltes des Zoosporangien, welche durch diese gallertartige Masse aus dem Ende des Fortsatzes zurückgedrängt, zeigt sich stets an ihrer Aussenseite begrenzt durch einen zarten aber deutlich sichtbaren Contour, welcher in die äusserste Umrisslinie des die Wand des fortsatzes berührenden Protoplasmas übergeht, und den Primordialschlauch andeutet und die gallertartige farblose

Substanz ist auf zufassen als ein Sekretionsproduct des sich allmählig aus dem Fortstazende des Zoosporangium's zurückziehenden Primordialschlauchs."

In P. adhaerens, Sparrow (1931b) has reported the presence of a "narrow zone of hyaline, highly refractive material ....., which appears in optical section as a slender crescent." "The dome of glistening substance appears to possess, shortly before the discharge of the content, a double contour when viewed in optical section." In P. dictyosporum, (1931a) Sparrow distinguishes "three definite regions: an upper, very refractive, crescent-shaped one; immediately below this, a slightly less hyaline zone; beneath this, a pale, slightly refractive, very finely granular region. The distal end of the latter zone is crateriform, and bears at its apex a highly refractive bit of material which suggests a pore. The protoplasm becomes more densely granular a short distance below the tip." The second zone distinguished by Sparrow apparently corresponds with the vacuolar region which he has described in P. adhaerens (1931b). Sparrow made the comment in 1931 that "little information is available regarding the rather critical changes occurring in what has been termed the "sub-apical" region of the sporangium, immediately preceding the formation of the vesicle. This has possibly been due either to the rapidity with which these stages occur, or to the fact that attention has been fixed on the more obvious enlargement of the brightly glistening apical material."

1b. The "lumping" of the protoplasm.

According to Büsgen, the protoplasm of the sporangium of P. proliferum appears at this stage to be divided up by a network of groups of granules arranged in rows. The network soon disappears, and equidistant vacuoles and bright spots can be seen (frequently grouped around a specially large vacuole). Light spots separated by darker, dense granular lines which seem to indicate division have been observed by de Bary also. According to Hesse, the "circular areas" in the sporangium of P. de Baryanum appear to be separated by light streaks which seem to foreshadow a separation but disappear again as quickly as they came. The full quotations are as follows:-

Büsgen: "Bei genauer Beobachtung lassen sich als Beginn der Sonderung in den runden Sporangien dieser

Pflanze Spuren eines Körnernetzes wahrnehemen. In einigen Fällen waren Maschen sichtbar, welche die Sporen zunachst an Grösse übertrafen, aber rasch weiter getheitt wurden. Meist jedoch wurde ein Netz nur angedeutet durch untereinander nicht mehr zusammenhängende Gruppen geradlinig aneinander gereihter Körnchen. Charakteristisch ist das Erscheinen von siemlich äquidistanten Vacuolen und hellen Stellen, die hautig um eine besonders grosse Vacuole regelurässig gruppirt sind. Nach dem Auftreten derselben ist gewöhnlich jede Spur des Kornernetzes verschwunden. Die hellen Stellen entsprechen etwa ihrer Zahl nach den späteren Sporen."

de Bary: "In dem Plasma des so vorbereiteren Sporangium tritt nun bald ein Anzahl hellerer, durch dunklere, dichte körnige Streifen getrenner Flecke auf, welche eine bevorstehende Theilung anzudenten scheinen."

Hesse: "Nach vollendetem Zurückzug des Primordialschlauchs aus der Endpartie des Fortsatzes sicht man plötzlich im Centrum des protoplasmatischen Inhaltes des Zoosporangium's einige durch helle Grenzstreifen von einander getrennte rundliche Flecke auftreten, die eine Theilung anzumelden scheinen, die aber eben so schnell wieder verschwinden, als sie entstanden sind."

These accounts have been criticised by Butler, who observed only a lumping of the protoplasm. In a general account of sporulation in Pythium he wrote:- "While the beak is forming, it" (the protoplasm) "becomes lumped into heaps around the periphery of the sporangium. These are sometimes very apparent, as in P. intermedium, sometimes difficult to see. They are separated from one another by thinnings in the protoplasm. I believe they are constant in position, though, owing to the rotation of the protoplasm, they appear to be continually changing both shape and position. In number they appear fewer than the number of spores to be produced, though this may result from difficulties of observation due to the spherical shape of the sporangium. They remain visible up to just previous to the discharge of the vacuole, after which they are no longer to be seen. I have never been able to detect any relationship between them and the small secondary vacuoles that are sometimes present; and there is certainly never anything like what occurs in Peronospora, where each spore origin is marked by a distinct vacuole occupying its centre." "The equidistant vacuoles mentioned by Büsgen are certainly of rare formation, if, indeed they ever occur once the large sporangial vacuole is formed." Butler did not see anything which was comparable with Büsgen's "Spuren eines Körnernetzes," or with Hesse's "helle Grenzstreifen." He did, however, observe "sharp dark dots surrounded by a clear area," which remain "distinct to the end," and considered that they might be nuclei.

In *P. marsipium*, according to Drechsler (1941), a lumping of the protoplasm occurs similar to that described by Butler. There are "numerous, more or less evenly distributed, subspherical hyaline bodies, approximately 3μ in diameter" present in the sporangium for some time before its discharge. "Perhaps more because of some lumping of protoplasm about these bodies than because of any definite demarcation, the sporangial contents present an appearance vaguely suggestive of cleavage, a little like that presented, for example, in sporangia of *Phytophthora* ready to discharge their zoospores." Drechsler has expressed his view that the stage of differentiation reached before discharge in *P. marsipium* is more advanced than in most allied species.

Variation in the behaviour of different sporangia of the same species has been described, in *P. aphanidermatum* by Edson, and several times in *P. proliferum* (see appendix, p.218) and it appears that the apparent disagreement among different investigators may be due to the different conditions under which the observations have been made.

2. The disappearance of the vacuole.

In *P. rostratum*, according to Butler, the large vacuole, previously "more than half" the size of the sporangium, gradually becomes rounded, and discharges. In the material which he was observing, "while the vacuole was discharging, the beak collapsed somewhat, and a portion of its contents was drawn back into the sporangium, forming a vacuole near the base of the beak." "The beak soon filled out again." After the discharge, the sporangium is much diminished in size - by one-tenth of its original size, in *P. intermedium* - its turgescence is lost, and the protoplasm appears "more finely granular and homogeneous than before." The disappearance of the larger granules has also been observed by Höhnk, in *P. proliferum*. According to Butler, the protoplasm also changes "a little in colour and transparency, showing a paler tint of the faint blue under artificial light which it previously had."

Butler observed also that a "few small contractile vacuoles are sometimes visible at this period, ... but their constant formation, if it occurs, has escaped my attention." In *P. proliferum*, Höhnk has recorded that at this stage each "plasma part ("spore origin") "now contains "one or a few small vacuoles" which, however, are not permanent and disappear immediately before discharge. Marshall Ward also noted that in this species, by the time that the tip of the "beak" has become pale and diffluent, and has begun to protrude like a gelatinous drop, the vacuoles have almost disappeared, "a number of minute bright points, slowly playing in the granular contents probably representing them." It is possible that the dots seen by Butler at this stage may correspond with these "bright points."

P. marsipium stands apart from most other species. Drechsler has written: "During the period immediately preceding discharge, the sporangium here does not reveal any vacuole comparable with the large lacuna-like vacuole that, in most congeneric forms, increases in size until it abruptly disappears very shortly before the protoplasmic materials are expelled." The protoplasmic reorganisation associated with such vacuolisation may be accomplished here much less obtrusively just before the sporangium enters on its prolonged condition of readiness for discharge."

3. The outflow of the protoplasm into the vesicle.

The initial stages in P. dictyosporum have been vividly described by Sparrow (1931a) as follows: the refractive dome loses its double contour and begins to enlarge, while the expansion of the finely granular, crateriform protoplasm distally is initiated. (See the section headed "1a. Changes in the beak.") No change in the position of the lower, more densely granular material has yet occurred. This stage lasts "only an instant" and is followed immediately by the next stage. The hyaline cap continues to expand, the cone of pale protoplasm becomes even more distended, and its refractive "pore" apparently bursts or deliquesces, "resulting in the liberation into the vesicle of a very finely granular material." The lower, more granular protoplasm starts "to move toward the tip of the sporangium, where it will be discharged into the enlarging vesicle." Sparrow has commented, "thus it may be seen that the finely granular, somewhat hyaline protoplasm with its crateriform apex appears to play an intimate part in the initiation of the ejection of the protoplasm from the tip of the sporangium. That it is of a somewhat different material from the underlying coarse substance filling the rest of the sporangium is apparent not only from its distinctive physical properties, such as refractivity and expansive power, but from its greater affinity for such stains as muchaematin, ruthenium red, and Bismarck brown." Sporangia of P. adhaerens and P. angustatum, which are also of the filamentous type, have been observed by Sparrow,(1931b), to go through the same stages.

Marshall Ward has described how, in material of P. proliferum, which he observed, "a large clear vacuole appeared in the protoplasm at the end of the sporangium opposite the beak, and the pale swelling at the apex of the beak suddenly began to be inflated like a blown up bladder." He has described also how the "hyaline dome" of P. gracile "commenced to swell up gradually," and has compared the subsequent outflow of the protoplasm with "the rush of endoplasm often noticed in the protrusion of a large pseudopodium by a vigorous amoeba; the flow of granules was most rapid in the axial portion, and the last particles followed more slowly."

Butler has compared the discharge in P. rostratum with the flow of porridge forced through a hole, emphasising an essential difference, as follows:- "The granule directly opposite the opening was not always the first to escape. It was sometimes shoved aside by one to the right or left. From this I have been led to suppose that even at this stage the spore-origins are definitely formed, and though fused into a mass in which individual spores cannot be made out, each nucleus has a hold on a mass of cytoplasm which passes out with that nucleus. Hence, in passing out, when the nucleus engages in the tube it draws its cytoplasm with it, whether this be directly in the centre or to one side of the opening."

Butler's account continues: "As the protoplasm passed out, it rotated slightly except that part actually in the tube. The unescaped part remained in a diminishing, more or less spherical, mass, pressed against the opening of the tube until the last portion passed out." Marshall Ward wrote, of P. proliferum: "Even as the last granules passed slowly up the axis of the beak, the slowly writhing mass of protoplasm began to divide up into separate blocks."

4. The "final fashioning" of the zoospores within the vesicle.

According to de Bary, Büsgen and Hesse, the light spots which were visible in the protoplasm before its escape from the sporangium, but apparently disappeared during its passage through the exit tube, reappear, and the cleavage lines or areas again become visible. De Bary has written:- "Unmittelbar nach dem Austritt in die Blase tritt dagegen eine der obigen gleiche Sonderung in helle Flecke und dunklere Interstitien wieder ein, um bleibend und sofort von der Theilung gefolgt zu werden."

There is close agreement in the accounts, by these and other investigators, of the cleavage into zoospores and their maturation. Marshall Ward, Butler, Edson and Sparrow have given detailed descriptions which are essentially similar, except that the process in P. aphanidermatum, as described by Edson, appears to require a longer time than in other species.

The process may be considered in four stages:-

Stage 1: In P. adhaerens, according to Sparrow, immediately after discharge "a slight surging movement of the minute particles of the protoplasm is perceptible. After a few seconds slight contractions in certain regions lend a lumpy, irregular appearance to the mass. Faint peripheral lines of cleavage, which demarcate irregularly polygonal areas, soon become visible in the now more contracted content." "About three minutes after discharge a rocking movement of the mass is noticeable." Butler's description of P. gracile is similar: "Two to three minutes after the outflow, the mass begins to move as a whole, and cleavage areas appear. Vacuoles may appear in the mass ..... probably due to a too rapid leakage of water into the vesicle. This penetrates the protoplasm and is expelled again by contractile vacuoles." "Cleavage lines like indistinct grooves" are not visible in P. aphanidermatum until seven minutes after the outflow of protoplasm, according to Edson.

Stage 2: Butler has stated that the flagella "appear rather suddenly five to ten minutes after the outflow," but he has expressed the opinion that they are probably formed some time before they become visible. Sparrow has seen short, hyaline flagella after six minutes in P. adhaerens, and Edson after nine minutes in P. aphanidermatum. Edson has described how the flagella "elongate during the progress of cleavage so as to give the appearance of being pushed out slowly from within." "The growth is sufficiently rapid almost to be seen at a magnification of two thousand diamters - that is, one readily notes that they have increased in length in the course of a few seconds ...." Edson calculated that the combined length of the two flagella is approximately equivalent to twice the circumference of the spore. He believed them to be put forth from the blepharoplast by a process of "gradual elongation."

Edson has also recorded the occasional occurrence of a phenomenon not mentioned by other observers: "About the time the first indications of cilia" (sic) "are observable small bubble-like protuberances suddenly puff out here and there and then instantly disappear as if they had burst. Thereafter cleavage indentiations appear at the points of rupture...."

The movement of the mass has been described by Butler as a shaking or rolling, at first, but he noted a "short amoeboid stage" in which "pseudopodia" were put out, and retracted. Sparrow has observed that the movement of the spore initials gradually becomes more pronounced, "assuming a twisting or writhing character" as they slowly become separated from each other. Six minutes after discharge the initials, which are by this time definitely delimited, can be seen to "exhibit a slight individual movement." In P. aphanidermatum, according to Edson, the definite outline of the spores cannot be made out until thirteen minutes, and an individual motion seventeen minutes after discharge.

Stage 3: One minute later, as Edson observed, a "large vacuole" ~~vacuole~~" appears in each spore. In P. adhaerens, according to Sparrow, a small vacuole can be seen in "each of the spore masses" " at about eight minutes," by which time the short, hyaline flagella have increased in length, and appear as "dark, flexible lashes."

Stage 4: The "motion becomes oscillatory and vigorous," according to Butler. In P. adhaerens, according to Sparrow, after eleven minutes, "the spores have become nearly mature individuals. They still continue, however, to oscillate somewhat until they are perfectly formed. About fourteen minutes after egress this motion is gradually superseded by a frenzied milling around of the zoospores within the confines of the vesicle. Ultimately, the vesicle is ruptured by one or more zoospores, usually in the upper portion, and the immured swarmers escape." The zoospores of P. aphanidermatum, according to Edson, require twenty-six minutes to attain their "adult shape," and at twenty-nine minutes they escape "through a puncture produced in the membrane by the force of their impact on it."

The time required for the maturation of the zoospores.

Various reports have been given of the time which elapses during the maturation of the zoospores. Examples are listed below:

	Investigator	Species	Comments
1915	Edson	P. aphanidermatum	Usually about 30 minutes; sometimes only 17 mins.
1927	Kanouse and Humphrey	P. afertile	Less than 30 minutes, which they considered to be "an unusually short time."
1883	Marshall Ward	Un-named	29 minutes
1872	Cornu	P. proliferum	20-30 minutes
1941	Drechsler	P. oedochilum	20-25 minutes
1936	Saksena	P. deliense	20-25 minutes "between the formation of the vesicle and the liberation of the spores."
1883	Marshall Ward	P. gracile	About 23 minutes, but the time is not made clear in the description.

Investigator	Species	Comments
1883 Marshall Ward	P. proliferum	16 minutes. One sporangium took only 13 minutes, but appears to have been abnormal.
1860 De Bary	P. proliferum	16 minutes
1931 Sideris	Nematosporangium species	15-20 minutes
1932 Sideris	Pythium species	10-15 minutes
1943 Middleton	Various species	10-50 minutes, usually 15-20 minutes.

Taking into account the fact that seven minutes elapse before "cleavage lines" are visible in P. aphanidermatum, according to Edson, the times given by him for the subsequent stages in differentiation are not so dissimilar from those given by other observers as at first appears. No exact comparison can be made, however, as it is not always clear from what stage the time is measured; according to Butler, four minutes may elapse during the actual process of discharge in P. proliferum, from the first rush of protoplasm to the passage of the last remnants into the vesicle.

The influence of environmental conditions on the sporulation of P. proliferum is implied in the statement by Höhnk, (1933), that the time required for the process is "about twenty minutes, sometimes more than two hours." Clearly, the state of vigour of the protoplasm must also be an important factor.

Some theories on the cause of the outflow of the protoplasm.

Whereas Marshall Ward believed the outflowing protoplasm to be "impelled from behind by the pressure of fluid in the vacuoles" which "contained some soluble material, excreted by the protoplasm," Butler thought that the protoplasm is "attracted out by the formation of a chemotactic substance in the subapicular vacuole."

The force of positive aerotaxy which Hartog believed to operate in Saprolegnia, and possibly in Phytophthora, seems unlikely in Pythium, in view of the confining effect of the vesicle. His alternative theory of negative pneumotaxy, however, seems more acceptable, for the protoplasm may well be considered to be expelled by a tendency to escape from the products of its own metabolism which had accumulated within the crowded confines of the sporangium.

Two further possibilities seem worth consideration: the protoplasm may be pushed out by the swelling of the wall of the sporangium, or of some substance derived from the wall; or the outflow may be a natural result of the decrease in pressure at the mouth of the sporangium, caused by the expansion of the vesicle.

An Account of the Vesicle

Summary

The nature of the vesicle has in the past been the subject of much conjecture, both as to the seat of its origin, and as to the substance of which it is composed. Evidence in support of the modern view that it is formed from the mucilaginous papilla has been given by the results of microchemical tests.

It appears that the various reports on the mode of escape of the zoospores may be accounted for by differen

in the extensibility of the vesicle in different species, or even in the same species: for whether the spores escape simultaneously or successively probably depends on its strength and elasticity. Probably, the same characteristics determine its durability.

In most species, the ejected protoplasm does not occupy the entire volume of the vesicle. *P. adhaerens* appears to be an exception (Sparrow, 1931b). The distance between the protoplasm and the membrane of the vesicle, and the increase in size of the whole organ, have been considered by Edson and Saksena to give evidence of the function of the wall as a semipermeable membrane.

Butler has described the function of the vesicle as that of keeping a chemotactic substance in close proximity to the mouth of the sporangium. Its value as a protective covering during the maturation of the zoospores is evident; and it also permits that this process may be carried out in a medium in which the metabolic products of the protoplasm are less concentrated than they had been in the sporangium, before its formation.

An historical account of some theories on the nature of the vesicle.

In 1858, Pringsheim called the vesicle "a delicate membrane," and stated as follows his uncertainty as to its origin from the innermost lamella of the sporangium or as a new formation on the periphery of the extruded protoplasm:-

"Von dieser bleib es mir jedoch ungewiss, ob sie von der undurchrissenen innersten Lamelle der Sporangium-Membran herrühst, welche wachsend über die Oeffnung des Sporangium hinaus sich ausgedehnt hat, oder ob sie in Folge einer Neubildung im Augenblick des Horvortretens der Protoplasmamasse an deren Umfange entstand."

In 1872, Cornu first used the name "vesicle," and stated his belief that it is derived from the apex of the sporangium. He described its formation as follows:- "Dans le genre Pythium, (P. monospermum) le plasma s'épanche au dehors en refoulant devant lui l'extrémité du sporange et la couche situeé au-dessous, qui se gonflent sous son influence comme une bulle de savon et l'entourent d'une membrane mince que j'appelle la vésicule."

In 1860, de Bary described the origin of the membrane of the vesicle from the "gelatinously thickened wall" (1887). He considered the colourless slimy substance of the swollen apex of the sporangium to consist of jelly-like material closely related to cellulose, secreted by the protoplasm which at first occupies the papilla:- "Sie besteht, ihrer Aehnlichkeit mit zweifellosen Gallertablagerungen und ihrem späteren verhalten zufolge, aus einer der Cellulose nahe verwanditen Gallerte." "Die Gallertmasse in der Endpapille ist sonach, gleich der Verdickungsmasse der Seitenwand, deutlich als ein Secretionsproduct des scharf umschrieben bleibenden, sich aus der Endpapille zuruck ziehenden Primordialschlauches zu erkennen."

In 1874, Hesse gave a similar account to that of de Bary, also believing the "gelatinous" substance to be a product of secretion.

Marshall Ward (1883) considered that the membrane is probably cellulose, and contains mucilage. He used the terms "mucous globe" and "gelatinous vesicle" in describing P. gracile, and "jelly-like envelope" in P. proliferum. He described how the "softened apex of the beak became rapidly distended into a vesicle," in P. proliferum, and wrote of the "distension of the pale (cellulose?) apex into a larger and larger vesicle." He mentioned also the "hyaline dome" of P. gracile, which he described as a "cap of diffluent cellulose" which "commenced to swell up gradually."

Butler, in 1907, expressed his opinion that the membrane probably consists of modified cellulose, and is "formed from the wall of the apex of the beak, altered probably by the action of a cellulose-softening ferment." It seemed to him that "the bright appearance "of the apex" closely resembles that of a cellwall at the point where a fungus hypha is boring through it."

The views on the nature of the vesicle that Edson (1915) and Sideris (1930) appear to have held differ considerably from those of other writers. Edson has not used the term "vesicle" at all. It seems that he thought that the membrane is, or arises from, a lining of the sporangium which "may be seen advancing along the presporangium," (now called sporangium) "cavity, drawing out with it the last portions of the contents." After egress, "this body......remains at the mouth of the presporangium wall during cleavage, although it does not appear to be attached to it by any visible means."

In a general account of the process in Pythium, Sideris has stated his belief that the wall of the vesicle constitutes "a part of the so-called protoplasmic membrance of the prosporangium," and that it is identical with this membrane. "It emerges almost simultaneously with the flowing protoplasmic contents" of the sporangium.

Experimental evidence of the pectic nature of the vesicle.

The results of tests carried out in an attempt to determine the nature of the vesicle have been given by Sparrow (1931b) as follows:-

"In microchemical tests designed by various investigators to indicate the presence of cellulose, hemicellulose, callose, protein, and pectic materials, positive results were obtained only with those which have been emphasised as specific for the last-named substance. The dome is also stained slightly by muchaematin, a mucilage stain. It was noticed that when the dome was stained, the dye coloured even more deeply the protoplasm immediately below this structure. This suggests that the contents in this region of the sporangium is modified for the production of the vesicular material."

Observations on the physical nature of the vesicle.

The "space" between the protoplasm and the surrounding membrane: Butler has observed that in P. rostratum, in the early stages of discharge, the vesicle is "blown up" faster than the protoplasm flows in, so that there is a "considerable space between the wall of the vesicle and the contained protoplasm." Later, the flow of the protoplasm increases in rate, and the "space" diminishes. Both Cornu and Hesse have commented on the distance between the protoplasm and the surrounding membrane. P. adhaerens seems to be exceptional, as Sparrow (1931b) has written: "The ejected protoplasm appears as a smoothly granular, homogeneous mass which completely fills the vesicle."

Edson has interpreted the increase in the distance between the protoplasm and the membrane as evidence of the semipermeable nature of the vesicle:- "During the progress of cleavage, the wall seems to function as a semi-permeable membrane, since the distance between it and the cytoplasm increases somewhat and the size of the entire body increases by a few microns." Describing P. deliense, Saksena has written:- "During the internal cleavage, the membrane of the vesicle seems to function as a semipermeable membrane; the organ, in effect, does not stop enlarging."

Strength and durability. The strength and elasticity of the vesicle evidently determine the mode of escape of the zoospores. In P. aphanidermatum, according to Edson, the vesicle usually seems "to burst and then contract so as to allow the zoospores to escape almost simultaneously in all directions." In P. afertile, it is evidently relatively tough, for it is sometimes stretched by the impact of the zoospores upon it, according to Kanouse and Humphrey, who have observed also the "dumb-bell shape or pinched appearance that the spores have when leaving the vesicle. The spores usually leave by one exit, but frequently their behaviour indicates two or more openings. At other times a few spores leave by an opening and the remainder swim away in any direction, showing that the vesicle has already disappeared."

Edson found that in P. aphanidermatum it is usually impossible to discover any trace of the membrane afterwards. Marshall Ward, however, reported for P. proliferum that a "distinct remnant of the lower third of the vesicle remained attached to the apex of the widely open beak. The upper parts appeared to become completely dissolved in the water." According to Drechsler (1941), portions of the vesicle of P. marsipium can be seen for an hour or two after the escape of the spores," but this is to be considered as "more than ordinary durability." The following non-committal statement about P. adhaerens has been made by Sparrow (1931b):- "The vesicle is ultimately dissolved into the surrounding medium."

The Fate of the Emptied Sporangium

After the discharge of the sporangium, a new sporangiophore may, in some species with spheroid sporangia, grow through the septum and form a new sporangium within or beyond the old one. This has been called "uniaxial replication" by Drechsler (1941) but is usually referred to as "internal proliferation" or merely "proliferation." Eight proliferous species are described in Middleton's monograph. In these species, as well as in others, a new sporangiophore may arise outside the empty sporangium, immediately below the septum. This may be referred to simply as "sympodial" or "subsporangial branching."

After the discharge of a sporangium of P.proliferum observed by Marshall Ward, a few minute granules were left in the cavity, and a slight residue on the inner walls, "no doubt representing in part excreted material." "The walls of the emptied sporangium collapsed a very little, and a large number of minute bacteria could be observed attached to the outer surface in all cases." The presence of a number of bacteria around the sporangium, at the time of the discharge of the vacuole, has also been observed by Butler.

Both Marshall Ward and Butler noticed that immediately after discharge the septum can be seen bulging up into the sporangium. Butler described it as follows:- "After discharge the septum is seen to project into the sporangium in dome-like fashion. It has acquired a highly refractive clear appearance, such as may be noted when a hyphal end is engaged in boring through a cellulose wall. It may probably be interpreted as indicating the action of a cellulose dissolving ferment. Beneath the bright band of the septum is a clear space without granules, probably of hyaloplasm. As the hypha grows on, it does not appear to bore right through the septum but rather to soften the latter so that it is carried on by the growing apex and gradually merges in the new sporangium wall." Eight proliferous aquatic species were described by Middleton (1943).

Butler has written also that often in proliferous species, "the power of renewing groth across an emptied sporangium is lost. This especially occurs in culture, where a form distinctly proliferous when first obtained may be renewed for several generations without any of the sporangia appearing proliferous. In this case the innovation invariably arises from just beneath the sporangium and grows out more or less latterally." This branching takes the place of proliferation, and, according to Butler, "is specially to be expected where growth is vigorous, but the conditions are not such as favour zoospore formation." "Even when a sporangium has discharged, the renewal of growth may be subsporangial, owing probably to some condition which causes the apex to lose its power of penetrating the septum."

III. THE ZOOSPORE

Features of the Mature Zoospore

The usual description of the zoospore as reniform or kidney-shaped, or bean-shaped, with two flagella (sometimes called cilia) emerging from the "hilum," seems to have been accepted without criticism by all writers on the subject. The limitations of such a description are discussed here in connection with the zoospores of P. undulatum.

The nature of the zoospore as a "soft" body of which the shape is subject, to a considerable extent, to environmental forces, was appreciated by Marshall Ward when he described the zoospore of P. gracile as a "reniform amoeboid body."

Within the zoospore a single conspicuous contractile vacuole is always present; Höhnk (1932) has observed that the time between contraction and expansion of the contractile vacuole of P. epigynum increases as the zoospore matures. Refractive granules, also, are usually present.

According to Middleton, two to a hundred and twenty five or more zoospores may be released into a single vesicle. The range in size, measured at encystment, is 4 - 18 μ but the zoospores of most species are 8 - 12 μ in diameter.

The duration of motility, in a single swimming period, is determined largely by environmental conditions. The usual comment which has been made by Drechsler, in describing a large number of species, is that the zoospores swim "for some time."

Examples of the times which have been recorded by various other investigators, for different species, are listed below:-

	Investigator	Species	Comment
1883	Marshall Ward	*P. gracile*	25 minutes
1932	Höhnk	*P. epigynum*	10-25 minutes
1931	Sideris	*Nematosporangium* species	2-30 minutes or longer, depending on temperature
1932	---	*Pythium* species	5-60 minutes or longer, "depending on environmental conditions."
1931a	Sparrow	*P. dictyosporum*	depends on "the conditions of the surrounding medium."
1931b	---	*P. adhaerens*	5 hours, at 21°C.

The Encystment and Subsequent Behaviour of the Zoospore

A summary.

After coming to rest, the zoospore assumes a spherical shape, loses its flagella and contractile vacuole, and encysts. Its subsequent behaviour is determined largely by the conditions of the environment, chiefly those of food and water.

The various types of behaviour exhibited by zoospores of Pythium may be summarised as follows:

Germination by a germ hypha. (a) The zoospore enlarges and a central vacuole appears; the refractive granules disappear as they are used up, and the vacuole increases in size, as a germ hypha is put out, from which the mycelium soon develops. This is the usual, ultimate behaviour of a zoospore, under conditions favourable for growth. Other types of behaviour help to tide over unfavourable conditions.

(b) A germ hypha is put out as before, but instead of developing into an extensive mycelium, a miniature sporangium is formed precociously at the apex of a long or short hypha, and usually a single zoospore which matures within a vesicle, is liberated.

Repeated emergence of morphologically similar zoospores. The encysted zoospore itself behaves as a sporangium. It enlarges, and develops a large central vacuole which, according to Butler, is comparable with that of the ordinary full-sized sporangium prior to its discharge. An exit tube is developed, with an apical papilla, and the protoplasm is discharged into a vesicle, from which a single zoospore escapes.

This process may be repeated many times, but a resting period is necessary between successive emergences. Several names, of which "repeated emergence" seems the best, have been applied to it; these are discussed under a separate heading.

Details

Germination. The germination of the zoospores of P. aphanidermatum has been described by Edson as follows:- they "come to rest, round up, increase in size while developing a large central vacuole, and send out a germ tube, generally two." Marshall Ward had described the process in P. gracile:- the zoospores lose their flagella and vacuole on coming to rest, and develop an "envelope and several large refractive granules;" the granules are used up in the elongating germinal tube, and a vacuole forms in the spore, becoming larger and larger. Middleton has made the following statement about the genus as a whole:- "After an active stage in which the zoospores move rapidly about in water, they encyst and assume a spherical form varying from 4 to 18 μ, mostly 8 to 12 μ in diameter. Zoospores ..... ordinarily germinate by the formation of a germ tube."

The production of a miniature sporangium by zoospores of P. dictyosporum, described by Raciborski, was regarded by Butler as "an intermediate condition, in which the germ filament from the zoospore, in cases where it failed to reach a filament of the host, formed a sporangium at the tip, which opened to emit one zoospore only." A miniature sporangium is occasionally produced in P. anandrum, P. oligandrum, P. vexans, and P. undulatum, according to Drechsler (1946); sometimes a hypha of considerable length is formed before the tip swells into a sporangium, and a number of septa may be formed behind the protoplasm as it advances; the sporangium discharges in the usual way, with a vesicle.

Repeated emergence. Cornu (1872) was the first to observe that the zoospores do not always germinate to form a mycelium. He wrote: "Les zoospores dans tous ces genres germent en donnant lieu à un filament ...., ou bien elles émettent des zoospores semblables à elles-mêmes (Ex. Pythium proliferum et ses var.) Les deux modes de germination peuvent se présenter dans la même espèce."

The behaviour of zoospores of P. diacarpum under certain conditions has been described by Butler, as follows:— A zoospore "after coming to rest, loses its cilia and becomes surrounded by a wall. After a short period a vacuole appears, and at the same time a short tube is put out from the wall of the spore. This may be rather more than the diameter of the spore in length. The changes in the vacuole and tube appear to correspond to those which occur in the ripe sporangium....." "The apex of the tube opens, and the contents flow out and lie at the mouth of the tube. Whether they are retained here by a vesicle similar to that of the sporangium was not determined," but evidently the movements of the spore suggested that it was confined by a vesicle. The account continues:- "The extruded plasma gradually acquires the usual kidney shape, becomes free from the end of the tube, develops cilia, and swims away."

Similar behaviour of the zoospores of P.Butleri has been described by Drechsler (1930): "The somewhat large primary zoospore ..... produces an evacuation tube ....., the protoplasmic contents in the meantime displaying a vacuole of increasing size. Finally the refringent tip of the evacuation tube yields and the contents flow out to collect at the orifice, the streaming requiring usually from thirty to forty-five seconds. The discharged protoplast soon begins to exhibit writhing movements, which, as the cilia make their appearance and a grooved reniform shape is assumed, become increasingly energetic though restricted within a confined space. Usually about twenty minutes after the cessation of streaming the now violently active zoospore dashes away as a freeswimming body. The restriction in amplitude of movement of the secondary spore previous to its ultimate liberation is interpreted as evidence of a confining vesicle."

Further, Höhnk (1933) has reported it in P. epigynum: "In two cases observed, a zoospore formed an exit tube by means of thinning of the extension of the consistent membrane. The one was 4 μ, the other one 12 μ in length. The plasma followed the growing tip at a short distance. In correspondence to the growth a vacuole appeared within the spore plasma which subdivided into two when the plasma, breaking the thinned membrane at the tip of the tube, slowly escaped, forming a sphere at the outside. Parallel with this process the refractive granules decreased in number and probably also in size. The flowing out took nineteen and sixteen minutes, respectively, the pause at the orifice seventeen and thirty-two minutes, respectively. During this time the vacuole was reduced, the spore became elongated, and the movement of the cilia caused the departure as a biciliate zoospore."

Höhnk has observed that such behaviour results when fresh water is added after a zoospore has settled; Drechsler wrote in 1930, "the conditions favouring the production by encysted zoospores of a second swimming stage without the interposition of a vegetative phase apparently are the same as those that favour zoospore production generally." It is stated in Middleton's monograph that six swimming stages may occur successively . A resting period, however, is necessary between two such periods of activity; in P. epigynum, according to Höhnk, this lasts five hours.

The Use of the Terms "Repeated Emergence" and "Diplanetism."

A discussion.

Much controversy has taken place over the terminology to be used when zoospores indulge in more than one motile period. In an account entitled:- "Polyplanetism and zoospore germination in Saprolegniaceae and Pythium," Höhnk (1933) has attempted to clear up the confusion. The salient points in this account are listed and discussed below:-

. The distinction of "spore germination" from "planetism".

Statements: "Spore germination is well distinguished from planetism." The production of a miniature sporangium at the end of a germ hypha is a phenomenon of the former, not of the latter.

Comments: This seems a natural view, especially as some growth and multiplication may take place during the occurrence of this phenomenon, though these are but scanty, owing to environmental factors; the resulting sporangium produced precociously on what may be regarded as an undeveloped mycelium, is necessarily puny.

. The meaning of the term "diplanetism."

Statements: Diplanetism (dis = twice, planos = swarming) "means that only two movements are present regardless of the form and shape of the zoospores."

Comments: This return by Höhnk to the derivation of the word is open to severe criticism. Obviously, reference must be made to the author of the word, and consideration given to the special circumstances for which it was coined. As Leitgeb first used "diplanetism" to describe two successive swimming stages of morphologically different zoospores, this meaning must be retained; and as the zoospores of both Pythium and Phytophthora are always of the "secondary" type, it is clear that they do not exhibit diplanetism at all.

The comparisons drawn in this paper, by Höhnk, and in 1907 by Butler, between the processes of sporulation in Pythium and Saprolegnia, show as quite untenable Atkinson's view that the "real diplanetism in Pythium is manifest in the escape of the "spore origins" from the prosporangium (first stage) and their subsequent final differentiation and swarming outside (secondary stage.)" Such an interpretation is of historical interest only.

3. "Dimorphism" and "Polyplanetism."

Statements: In certain members of the Saprolegniaceae, diplanetism is accompanied by "dimorphism." As the zoospores are able to swarm more than once, diplanetism is "only a limited case" of "polyplanetism."

Comments: According to Leitgeb's use of the term "diplanetism" it implies "dimorphism," and the latter term is therefore superfluous. It may usefully be retained, however, to assist in elucidating the terminology.

The need for the term "polyplanetism" does not arise, as no instance has yet been described in which more than two morphologically different stages have been distinguished. Amoeboid movements can hardly be regarded as planetic.

4. The influence of environment.

Statements: "Spores react to environmental conditions by means of germination, swarming, encystment, or amoeboid movement. The main factors for the appearance of any one of them are the amounts of available food and water. Germination depends more upon food than water. It always takes place when sufficient food is present and at least a minimum amount of water. Resting and swarming appear within a relatively large amount of water which dilutes the food too much to attract the zoospores and stimulate growth. Amoeboid movement, however, seems to be related to unsatisfactory water conditions."

Comment: These observations are in accordance with those of other investigators in the genus Pythium, and are supported by the reports of Couch and Salvin, on the behaviour of zoospores of the Saprolegniaceae.

In conclusion:- the liberation by an encysted zoospore of a secondary spore like the first is of common occurrence in both Pythiaceae and Saprolegniaceae. It is distinct from the diplanetism that also occurs in the Saprolegniaceae, and is best regarded as "repeated emergence."

An historical account of the use of the term "diplanetism."

When Cornu reported in 1872 the liberation by zoospores of secondary spores like themselves, he did not supply a name for the phenomenon, and Butler believed himself to be the first to report "diplanetism" in Pythium, in describing it in P. diacarpum in 1907.

Atkinson, however, in 1909, stated his belief that this phenomenon observed by Butler "cannot properly be brought into line with diplanetism as it exists in Saprolegnia," and that it is simply the production of "secondary spores."

The occurrence in a Saprolegnia-like fungus of two swimming stages was observed in 1869 by Leitgeb, who described the zoospores as follows:- "Schwärmsporen vor der Häutung mit 3 Vacuolen und oval; nach der Häutung mit einer Vacuole und bohnenförmig." He accordingly name the fungus Diplanes saprolegnioides. It emerged, however, that such behaviour is usual, not only among species of Saprolegnia, but also in other genera of the Saprolegniaceae. As Saprolegnia and Diplanes are synonymous, therefore, the latter name has been abandoned, while the terms "diplanetic" and "diplanetism" have been retained to describe the zoospores and the peculiar behaviour which they exhibit.

Lechmere, in 1910, defined his use of the term "diplanetism" in Saprolegniaceae as follows:- "This term is applied to describe the occurrence of two motile stages in certain species and genera. The two forms of zoospore are different." "The second form of zoospore resembles that which is characteristic of the Peronosporeae."

Weston, in 1919, showed that although Dictyuchus is not truly diplanetic, the "first" swimming stage being suppressed, several motile periods may be undergone in the "second" stage. He called this behaviour "repeated emergence," regarding it as comparable with the phenomenon shown by Pythium, and stated his belief that it is possibly of general occurrence throughout the Saprolegniaceae, and is "brought about by certain favourable conditions." He came to the conclusion that the application of the terms "monoplanetic" and "diplanetic" then customary might be misleading, and necessitate "a modification of our conception of the condition of monoplanetism and diplanetism in the Saprolegniaceae." Repeated emergence in the Saprolegniaceae has also been demonstrated by Höhnk (1933), and by Salvin (1940).

Drechsler, however, returned in 1930 to the term "diplanetism" as Butler had applied it. Pythium is included in his discussion on "the repetitional diplanetism in the genus Phytophthora," in which a variety of terms is used freely and apparently without discrimination, for the same phenomenon. These terms are listed below:-

diplanetism,
diplanetic development,
repetitional development,
repetitional diplanetism,
repetitional diplanetic development.

The liberation of a zoospore from a "miniature sporangium" produced at the apex of a "germ sporangiophore" is referred to both as "indirect diplanetism" and as "indirect repetitional development."
The following conclusion is stated:- "Diplanetism was observed in various species of Pythium with filaments and with lobulate sporangia, as, for example, P. butleri, the encysted structure discharging its undifferentiated contents through a slender evacuation tube into a small vesicle where they are fashioned into a single motile spore. In Pythium, as in Phytophthora, therefore, repetitional development follows the course generally characteristic of zoospore formation in the genus."

Since 1930, the application of the term "diplanetism" in Pythiaceae has been subjected to much criticism, and Drechsler did not use it in 1941, when he gave a new species the name of P. palingens. In this species, "an iterated swarming stage" was so evident that from the "repeated emergence and renewed animation so freely displayed by its zoospores, the fungus was given a specific name defined in the familiar phrase "born again"." Again, in 1946, Drechsler used only the term "repetitional development."

In 1931, Matthews expressed her view in the statement, "since the spores in both swimming stages in Pythium are alike, we prefer to follow Atkinson in considering this type only as a repeated emergence rather than true diplanetism, which is found in some of the Saprolegniaceae.

The following statement was made by Middleton in 1943:- "Zoospores are monoplanetic and ordinarily germinate by the formation of a germ tube. Repeated emergence has been observed rarely in species possessing large zoospores, the spores sending out on evacuation tubes from 2 to 3 μ wide and from 5 to 30 μ long, capped by a vesicle in which usually a single, though rarely from two to six zoospores are formed. A single zoospore of some species may undergo as many as six repeated emergence .... This process has been erroneously referred to as diplanetism. Since only one type of swarmspore is produced a true diplanetic condition does not exist; rather, typical spore germination merely recurs; this phenomenon is termed repetitional emergence or secondary spore formation." This statement clearly expresses Middleton's decision against the use of the term "diplanetism" in the genus Pythium, but also shows some inconsistency: thus, while it is stated that zoospores "ordinarily germinate by the formation of a germ tube," repeated emergence is also referred to as a recurrence of "typical spore germination."

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APPENDIX I

A HISTORY OF THE TAXONOMY OF THE GENUS PYTHIUM

The genus *Pythium* was created in 1858 by Pringsheim, who described it as follows:

"Schwarmsporen aussen vor der Oeffnung der Sporangien aus deren Inhalt gebildet, sich nicht häutend. Schläuche die entlierten Sporangien weder durch wachsend noch seitliche Sporangien treibend. Oosporen einzeln in jedem Oogonium."

P. monospermum and *P. entophytum* were the first described species. *P. entophytum* was transferred to *Lagenidium entophytum* by Zopf in 1890, and *P. monospermum* then became the type species.

In a discussion of Pringsheim's assignment of the generic name *Pythium* to "the group of organisms under consideration," although Sideris wrote in 1932 that "it was doubtless on account of the morphology of either their emptied prosporangium or the type of injury caused to the roots of plant seedlings," he stressed the former alternative. Thus, he explained that the "emptied prosporangia of *Pythium* resemble in shape a jug, the equivalent Greek word of which is *pithos* and the diminutive *pithion*. The type of root injury caused to plant seedlings by various members of the genus *Pythium* is a rot, the equivalent Greek word of which is *pythos*, from the verb to cause rot." Sideris then proceeded to the illogical conclusion, "only those members of pythiaceous organisms possessing a pithiod prosporangium should be assigned the generic name *Pythium*," in spite of the fact that the species on which the genus was founded, does not produce a "pithiod prosporangium," (sic) but a filamentous one. According to Middleton (1943), "*P. monospermum* is usually reported as a saprophyte from either insect cadavers or plant debris," and Pringsheim himself had described it as growing on decayed mealworms. It seems most probable, therefore, that Pringsheim had in mind the word *pytho*, and assigned the name *Pythium* to the organisms that he described, on account of their association with decay.

There have been many attempts at the classification of the genus, as the following list of events shows:-

1858 PRINGSHEIM founded the genus on a species with filamentous sporangia, P. monospermum, and placed it in the family Saprolegniaceae.

1881 DE BARY pointed out the relationships of Pythium to Phytophthora, and placed it in the family Peronosporaceae.

1888 Berlese and de Toni returned to Pringsheim's classification placing Pythium in Saprolegniaceae.

1892 FISCHER referred Pythium to the Peronosporaceae, and created three subgenera:-

Pythium (Peronosporaceae):
- Aphragmium - filamentous sporangium not delimited by a septum.
- Nematosporangium - filamentous sporangium delimited by a septum.
- Sphaerosporangium - spherical or lemon-shaped sporangium.

Fischer further divided the subgenus Sphaerosporangium:-

Orthosporangium - sporangium attached to the sporangiophore during zoospore formation.
Metasporangium - sporangium attached or deciduous, releasing zoospores or germinating directly.

1897 SCHRÖTER adapted Fischer's classification, creating a new family, Pythiaceae, which with Leptomitaceae, Saprolegniaceae and Peronosporaceae, constituted the group Saprolegniales. Fischer's subgenus Nematosporangium was raised to generic rank, and made to include all species with filamentous sporangia. Fischer's subgenus Aphragmium was retained, as a subgenus of Nematosporangium, distinguished from the subgenus Eu-nematosporangium, by its not delimiting the sporangium by a septum. Thus only species with spherical or limoniform sporangia remained in the genus Pythium. Since the genus was founded on a species with filamentous sporangia, Schröter's classification is unsound. It may be represented as follows:-

Pythiaceae:
- Nematosporangium - sporangium filamentous (Eunematosporangium; Aphragmium)
- Pythium - sporangium spherical or limoniform

1899 Gobi proposed a classification of *Pythium* based on the presence or absence of septa cutting off the reproductive parts.

1907 BUTLER criticised Gobi's proposed classification, on the grounds that he did not believe that any species with filamentous sporangium formed a septum delimiting the sporangium, although he recognised the "vegetative formation of septa." He believed it to be "at least probable that Pringsheim was misled by a purely vegetative occurrence." While returning to Fischer's classification, Butler preferred to drop the subgenus Nematosporangium altogether, and to retain the subgenus Aphragmium for species with filamentous sporangia:-

Pythium
- Aphragmium - sporangium filamentous, not cut off by a septum.
- Sphaerosporangium - sporangium spherical, oval, etc., cut off by a septum.

1915 Edson created the new genus *Rheosporangium* for a fungus that he called *R. aphanidermatus*, as he could find no suitable genus in von Minden's "Saprolegniineae." von Minden accepted De Bary's classification of *Pythium* in Peronosporaceae, and therefore did not include it in Saprolegniaceae.

1923 Fitzpatrick favoured the separation of *Pythium*, with spherical sporangium, from Nematosporangium, with filamentous sporangium. He suggested the merging of the genus *Pythium*, as he conceived it, and the genus *Phytophthora* into one genus which with *Pythiogeton* and *Pythiacystis* should make up the family Pythiaceae.

1930 Drechsler, recognising types of sporangia for which Schröter's classification made no provision, considered it best "to retain the genus *Pythium* in its more inclusive sense, as employed in the writings of De Bary and Butler."

1930-2 Sideris attempted a revival of Schröter's classification, while including *Phytophthora* in the family Pythiaceae, with *Nematosporangium* and *Pythium*. He saw no need to subdivide the genus *Nematosporangium*.

1931-2 Sparrow stated his conviction that some species, as for example P. monospermum, possess truly filamentous sporangia not associated with any lobulate swellings, and pointed out that there is "no taxonomic justification for _Nematosporangium_." As "some name must be given to the lobulate and sphaerosporangial forms other than _Nematosporangium_ and _Pythium_," he tentatively suggested the retention of Edson's genus _Rheosporangium_ for the former, and the merging of the latter with _Phytophthora_ to form a group which might be called either _Phytophthora_ or _Sphaerosporangium_.

1931 MATTHEWS preferred "to follow Butler in retaining the genus _Pythium_ in the older sense." She saw no sharp distinction between Sideris' groups _Nematosporangium_ and _Pythium_. Her comment on Sparrow's proposition of union of _Pythium_ (as he conveived it) with Phytophthora was, "we do not think that this last assemblage would help matters."

1943 MIDDLETON, in his taxonomic study, followed "the generic tenets of Butler and Matthews" and retained the genus "in the older and broader sense." With regard to the separation of _Pythium_ and _Phytophthora_, he made the following statement:- "The genus _Phytophthora_, though related to _Pythium_ in the broader sense, comprises a group of species easily distinguishable from _Pythium_."

APPENDIX II

DISCUSSION ON THE RELATION BETWEEN PYTHIUM AND PHYTOPHTHORA

Discussing the Peronosporaceae, Butler has written:- "The genus *Pythium* is separated from all the rest by liberating its zoospores in an imperfectly differentiated state into a bladder at the mouth of the sporange, in which differentiation is completed." Butler recognised also other minor distinguishing features, but expressed a belief that none of these differences is absolute.

Indeed, it is often very difficult at first to distinguish between aquatic species of *Pythium* and *Phytophthora* which are growing saprophytically, intermingled on the same material. It has been pointed out by Blackwell, Waterhouse, and Thompson (1941) that species of both genera have been described under the invalid name "Pythiomorpha."

Fitzpatrick (1923) and Sparrow (1931) have proposed the merging of *Phytophthora* and those species of *Pythium* which possess spheroid sporangia into one genus; however, it seems that these *Pythium* species show enough distinguishing features, in common with those which poseess filamentous sporangia, to justify a position separate from *Phytophthora*.

These features are discussed below in turn:-

1. The differentiation of the zoospores within the sporangium (see p. 176)

The reports of many investigators, on a number of different species, indicate that various stages in the differentiation of the protoplasm are reached before its emergence from the sporangium into the vesicle. Thus, while Sparrow (1931b) has described the ejected protoplasm of *P. adhaerens* as a "smoothly granular, homogeneous mass," Büsgen has written of the sporangium of *P. gracile*, also filamentous, "der Inhalt der Sporangien schon vor dem Austreten in volkommen getrennte Portionem zerfallen war." This is a further stage in differentiation than Büsgen has described for *P. proliferum*, but both he and Hesse have suggested that at least a foreshadowing of cleavage occurs within the sporangium.

De Bary (1887) recognised that "transitory beginnings of division" are sometimes shown before the protoplasm streams into the vesicle; "there it breaks up at once into a number of swarm-spores,....." He has observed also that in Phytophthora "the swarm-cells are not formed in the same way as in Pythium but inside the original membrane of the sporangium,...."

Although Butler has criticised the accounts of Hesse and Büsgen for their strong indications of cleavage, though apparently transitory, within the sporangium, his description of the "lumping" of the protoplasm (see p.) and its mode of emergence (see p.), and his use of the term "final fashioning" for the development undergone by the zoospores within the vesicle, make it clear that he believed a considerable degree of organisation to be reached by the protoplasm before its discharge. Moreover, in 1910, he described in a fungus which he was then calling P. palmivorum a type of sporulation in which "segmentation into zoospores occurs within the sporangium, and is complete or nearly complete before the papilla opens." According to Butler, in this fungus the protoplasm breaks up into freeswimming zoospores immediately after streaming out of the sporangium, but, "owing to the pressure of the mass within the sporangium it is not possible to distinguish the individual zoospores before escape, but it is evident that they must be fully formed before the rupture of the papilla, since they separate at once after escape, and also because as soon as pressure is reduced by extrusion of part of the sporangial protoplasm, the remainder may segment while still inside and emerge as fully formed mature zoospores." Such behaviour resembles that which usually occurs in Phytophthora, and in 1919 Butler re-named this fungus Phytophthora palmivora.

The same fungus, however, shows other ways of sporulating, the method at the opposite extreme from the one described above being regarded by Butler as the "most characteristic, though by no means the most common." It is the method which caused him to place the fungus in the genus Pythium at first, believing it at that time to be "that which is practically universal in the genus Pythium and which is the only absolute mark of distinction of this genus from Phytophthora." This "characteristic" method involves the formation of a "thin gelatinous vesicle into which the protoplasm passes in a uniform granular mass." It is interesting to note that it is more frequent in the warmer months of the year.

Variation in the behaviour of different sporangia of the same species is a common phenomenon. It has been observed several times in P. proliferum. Cornu recorded it in 1872 as follows:- "Chez le P. proliferum de Bary, la sortie des zoospores a souvent lieu comme chez le P. monospermum; dans d'autres cas, les zoospores, toutes formées dans le sporange, s'échappant directement au dehors: la durée, depuis la rupture du sporange jusqu'à la dissemination des zoospores, n'est que d'une à deux minutes, tandis que, dans l'autre cas, elle était de vingt à trente minutes; la vésicule se montre encore, mais pendant quelques instant seulement." Marshall Ward's conception of the usual behaviour of P. proliferum appears to be illustrated by the statement: "Even as the last granules passed slowly up the axis of the beak, the slowly writhing mass of protoplasm began to divide up into separate blocks." He has described, however, one sporangium in which "a peculiar stage was passed through, during which the contents partially divided up and again became granular." When the contents flowed out they immediately became divided up into "five actively amoeboid masses." The whole process from the first rush of protoplasm to the swimming away of the spores took only thirteen minutes. In sporangia of the material observed by Höhnk (1933), "the plasma became differentiated into parts somewhat larger than the final spores. Their outlines were angular, but the separation was not complete, for connecting threads were visible. The separating membranes were of varying visibility in different sporangia." "The fact that in several cases the discharged plasma within the vesicle had an irregular outline suggested that the former differentiation into spores was still present." "However, more frequently the discharged plasma did not seem to be subdivided."

Variation also occurs in P. aphanidermatum, for although the protoplasm usually flows out in an apparently undifferentiated state, Edson has observed that "cleavage may occur within the sporangium" (his conception) "without its escape from the wall of the presporangium."

Similarly, in the genus Phytophthora, instances in which the zoospore mass was incompletely divided before its escape from the sporangium have been described by Dastur and Butler (1910), for Phytophthora parasitica and Phytophthora palmivora respectively. In the latter, changes in temperature seem to be to some degree associated with variations in sporulation. Drechsler has observed that in P. marsipium, "deficiency in the supply of water may often fail to inhibit sporangial discharge, even while it is causing premature disintegration of vesicles, with the result that numerous discharged protoplasmic masses, deprived of their protective coverings, undergo degeneration on a relatively enormous scale." It seems entirely feasible, therefore, that such environmental factors as aeration, light, state of vigour of the protoplasm, etc. may also affect the process, and that in a number of instances, descriptions have been given of material which was not performing under optimum conditions. That protoplasm undergoing differentiation should exhibit such sensitivity is not surprising, since the influence of environmental factors at other stages in the life history is obvious.

It is clear, therefore, that there is considerable justification for the view expressed by Fitzpatrick in 1923, and revived by Sparrow in 1931-2, that the "attempt to determine the exact points in sporangial discharge at which zoospore formation begins and ends, for use as a basis for segregation of the species of this general group into the two genera, *Pythium* and *Phytophthora*, seems at best an unsatisfactory procedure."

2. The formation of a vesicle.

The formation of a vesicle, from the papilla of the sporangium, has been regarded as characteristic of the genus *Pythium*. A vesicle has, however, been reported many times in species of *Phytophthora*. Rosenbaum has described the occasional formation of an evanescent vesicle in *Phytophthora cactorum*, *Phytophthora arecae*, and *Phytophthora parasitica*. In *P. parasitica*, according to Dastur, two methods of discharge may be observed; in the first, the papilla dissolves early, and the zoospores are discharged at once, although "sometimes a little granular fluid flows out before them;" in the second, "the papilla swells or is blown into a hemisphere by the inrush of the zoospores. It disappears before all the zoospores have left the sporangium. Those that get into it remain stationary, huddled together for a few seconds, before dashing away." Wormald has described the vesicle of *P. cactorum*:- The zoospores "cling together, surrounded by a vesicle, just outside the mouth of the sporangium, for about a second before suddenly dispersing. The vesicle is derived from the hyaline plug forming the papilla; this is pushed out as a spherical transparent body which rapidly increases in size as the zoospores rush in. When its diameter is about equal to the length of the sporangium the vesicle suddenly becomes ruptured and disappears from view, the enclosed zoospores dispersing immediately.

Having, in 1919, transferred the fungus causing bud rot of palms from the genus *Pythium* to *Phytophthora*, despite the "characteristic" formation of a vesicle (although this was sometimes extremely evanescent and sometimes absent), Butler, in 1924, stated his view of this species as "one of those forming a connecting link between the genera *Pythium* and *Phytophthora*."

Conversely, the fungus which Leland Shanor has regarded as a new species of *Phytophthora*, and called

P. stellata, appears in the description and figures to fall much more readily into the genus Pythium. The author himself has remarked that "often the type of sporangial development strongly suggests Pythium," but that "the shape of the sporangia and the presence of a papilla indicated that it was a Phytophthora whose sporangial germination shows a close relationship to P. palmivora Butler." Such a point of view illustrates the need for a proper appreciation of the features of the two genera.

This need is indicated in Middleton's statement that the "mode of zoospore production may be retained as a criterion of generic segregation for Pythium and Phytophthora despite recurring criticisms if its value and limitations are thoroughly understood and appreciated." Middleton has also pointed out that, while "rare instances are on record wherein sporangia of Phytophthora have given rise to vesicles," "in these aberrant cases the protoplasm does not flow into the vesicle in an undifferentiated state, but rather already differentiated into zoospores." His opinion is, therefore, that the formation of a vesicle should not be over-emphasised in distinguishing the two genera, but should be used for convenience, in conjunction with other features.

3. Other features.

The value in classification of the exit tube has been emphasised by some investigators. Sideris has classed it with the "prosporangium" and the "zoosporangium" as one of "three morphologically different organs," with the function of separating the other two and serving for the passage of the protoplasm from the one to the other. The sporangium of members of his genus Nematosporangium were supposed to have a long exit tube or "emission collar," while in the "pithoid" sporangium of his genus Pythium it is short, like the neck of a jug, and in Phytophthora it is altogether absent. Matthews, however, has pointed out that the length of the exit tube in Pythium is variable and determined by environmental conditions. Indeed, there are various reports on its presence or absence, (see p.) and it is clear that it "cannot be used as a generic character." (Matthews.)

Middleton has stated:- "Sporangiophore branching is unusual in Pythium and not uncommon in Phytophthora." As he does not define the limits of a sporangiophore, it is not clear whether Middleton has taken into account the proliferation and subsporangial branching which occur in species of Pythium with spheroid sporangia.

The statement of Middleton that the "habits of growth of Pythium and Phytophthora are different enough for general macroscopic distinction" is supported by Drechsler' (1946) description of the vegetative mycelium of

P. undulatum, which "offers the generally flexuous appearance familiar among species of Pythium rather than the stiffly branching aspect common in species of Phytophthora."

Drechsler has also added, as another distinguishing feature, the width of the sporangial openings, which are usually narrow in Pythium but often "generous" in Phytophthora.

After investigating the structure of the wall in six species of Pythium and nine of Phytophthora, Thomas (1942) came to the conclusion that "there is close conformity in mycelial structure among species of a genus." He found that in both genera the basic skeleton is of chitin; superimposed on this is the cellulose, strongly impregnated with fatty acids which prevent the fixation of dyes. The older regions of the hyphae of both genera are doubly refractive to polarised light, owing to the presence of this cellulose layer, which in Pythium evidently consists of only one kind of cellulose, while in Phytophthora it is a mixture of two kinds, only one of which causes the double refraction. Moreover, although "at no time can the cellulose be made brightly anisotropic," "Phytophthora hyphae present a dimly anisotropic appearance at all stages of growth," while the younger regions of the hyphae of Pythium are isotropic. This difference is due to the presence of a layer of pectic compounds on the outside of the young Pythium hyphae, but not of those of Phytophthora. In Pythium, "the pectic material is used up and disappears after five to seven days as the hyphae reach maturity, leaving the cellulose the outside layer."

If further evidence can be provided in support of Thomas's observations, the structure of the wall may prove to be one definite criterion, among a number of ill-defined and somewhat flexible ones, in the distinction of Pythium from Phytophthora. Meanwhile, the position is summed up in the following statement by Middleton: "Though there is no one infallible feature for generic segregation of these two genera, there are a number of characteristics which make separation feasible, and though difficult to outline in abbreviated form, in practice separation is easily done."