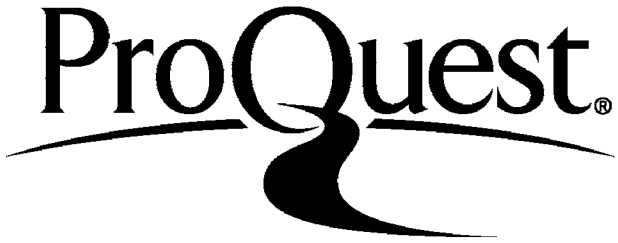
STUDIES IN PHYCOMYCETES.

I. Investigation of some of the Water Moulds of the Hogsmill River.

II. Production of Conidia in the Genus Phytophthora.

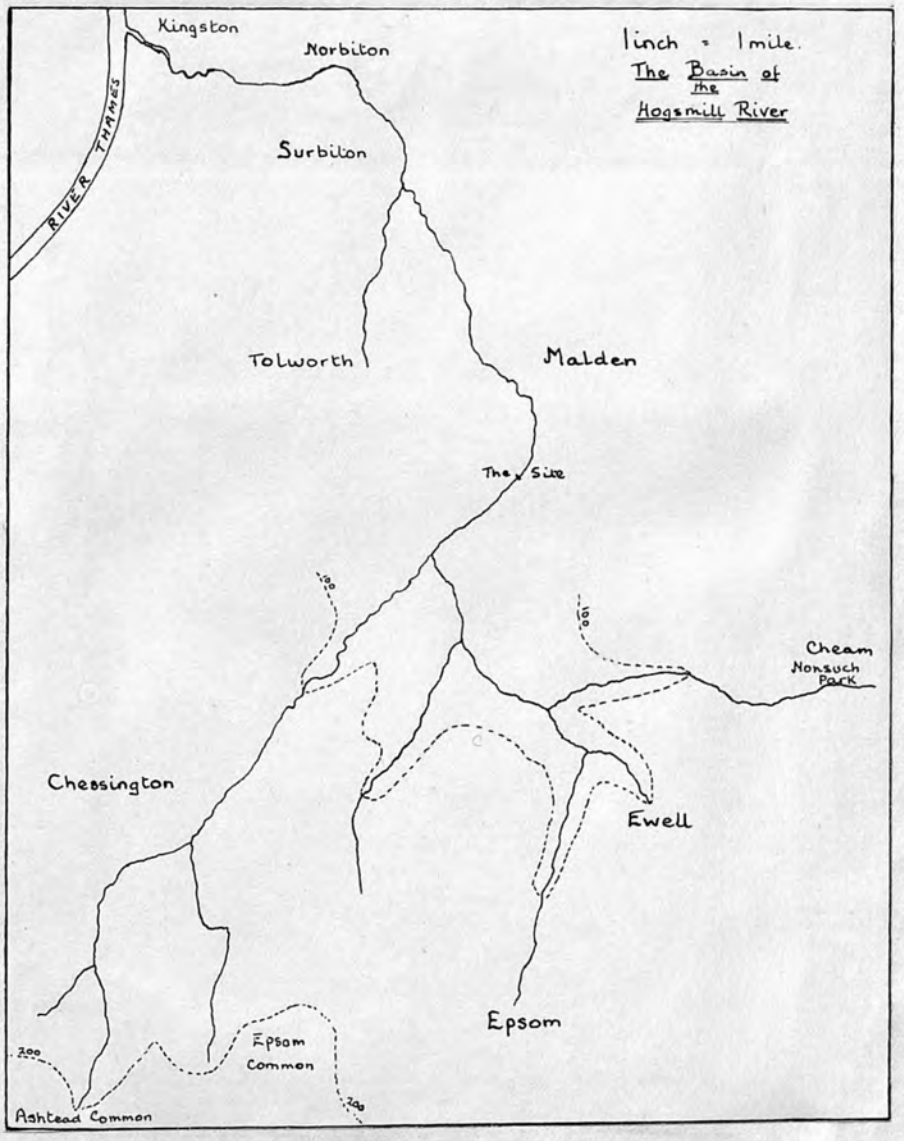


INVESTIGATION OF SOME OF THE WATER MOULDS OF THE HOGSMILL RIVER.

I. THE RIVER.

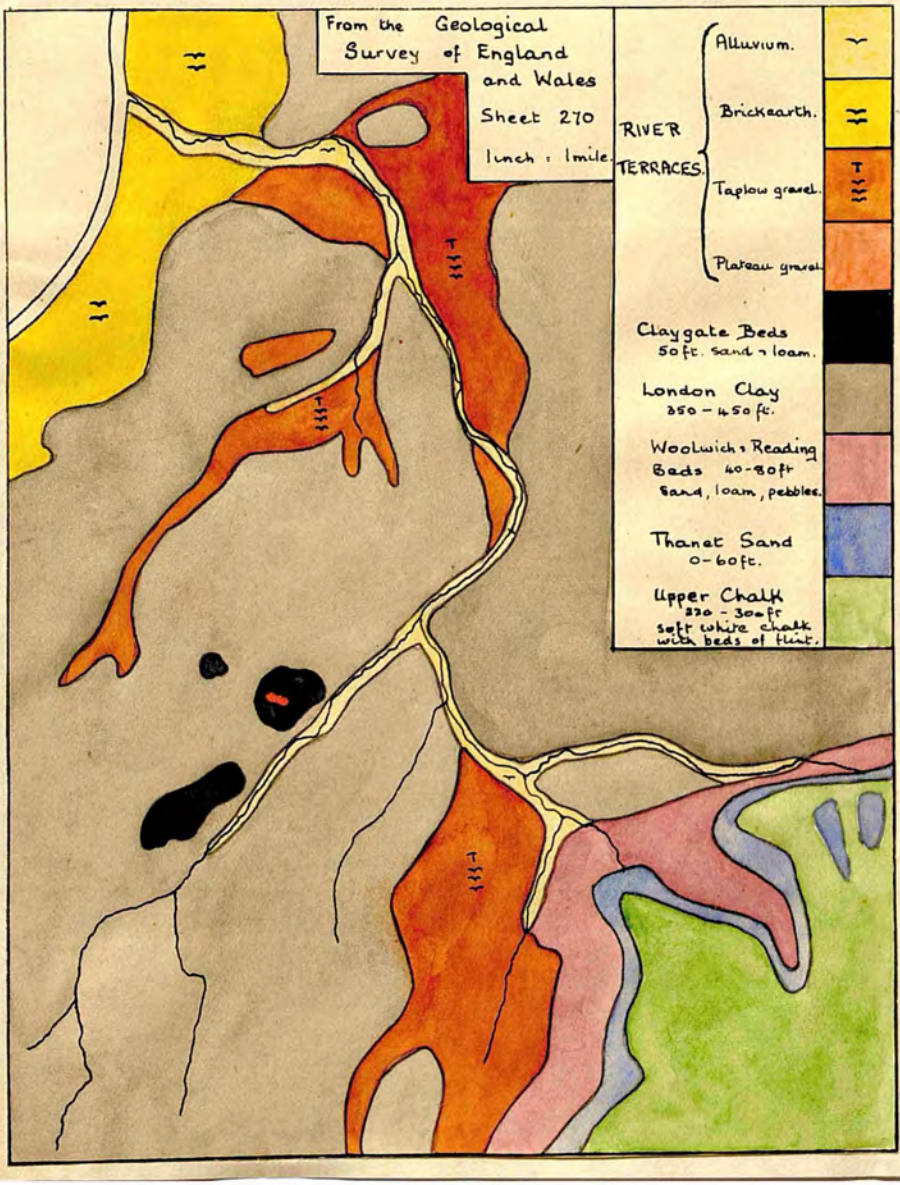
(a) Introduction

The Hogsmill river is a minor tributary of the Thames, some seven or eight miles long, rising in Ewell (Surrey) and entering the main river at Kingston. It has its source in the High Street at Ewell, where there are four lakes alongside the village street and in them springs can be seen as turbulent



areas in the mud or sand with bubbles arising therefrom. The springs are very constant and maintain a considerable flow

throughout the year, the lakes varying only about a foot in level. The geological formation there (Dewey and Bromhead 1921) is Thanet sand above the Upper Chalk and the sand is everywhere waterlogged at its base and throws out powerful springs. The Hogsmill river flows northwards through Ewell and its bed lies in a formation of Woolwich and Reading sand and pebbles. It soon enters London Clay receiving on its left bank a stream from Epsom. This tributary derives its water from two intermittent springs in Epsom and then flows through a terrace of Taplow gravel before entering the main stream. The latter



after receiving this tributary, flows along the north-east margin of the terrace, with London clay on the right bank. In the middle of this region there enters from the east a tributary which has its origin in a spring in Nonsuch Park, Ewell where again the Thanet Sand meets the Upper Chalk and gives rise to springs as in Ewell High Street. After leaving the Taplow Gravel region the Hogsmill river enters completely into the London clay region and in the next mile or so receives two tributaries on its left bank. The first is small but the second is quite a considerable stream with branches arising in the neighbourhood of Ashstead Common and Epsom Common. After the junction of this last tributary, the main river takes an abrupt turn to the north-east and flows through what is considered to be the abandoned valley of the River Mole (a neighbouring Thames tributary to the west) which once flowed into the Wandle (neighbouring tributary to the east). After flowing for about two miles in this valley, the river turns northward and enters again the Taplow gravel which flanks its banks until it reaches Kingston. During this stretch it receives but one large tributary and that from the west at Tolworth. Finally in Kingston the Hogsmill river turns west-north-west through a river terrace of Brickearth to its junction with the Thames near Kingston Bridge.

In addition to the tributaries mentioned, the river also receives the waters of various small brooks and ditches, the effluents of the sewage works at Ewell, Epsom, Malden and

Kingston and the effluents of mills and factories along its banks.

The drainage area of the basin is 26.7 square miles and half of this is a chalk area. The annual rainfall of the region is not high being :-

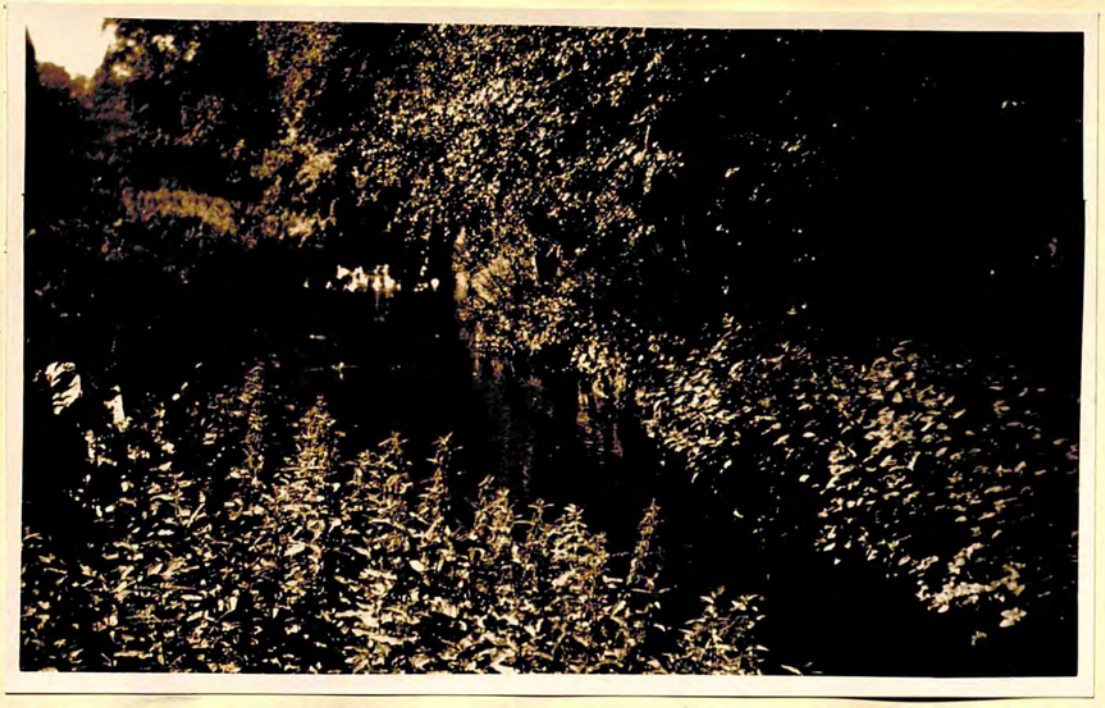
At Epsom	27.5 - 30 ins.
At Ewell	25 - 27.5 ins.
At Kingston	18 - 25 ins.

For the monthly fall during 1938 see graph (Fig. 4.)

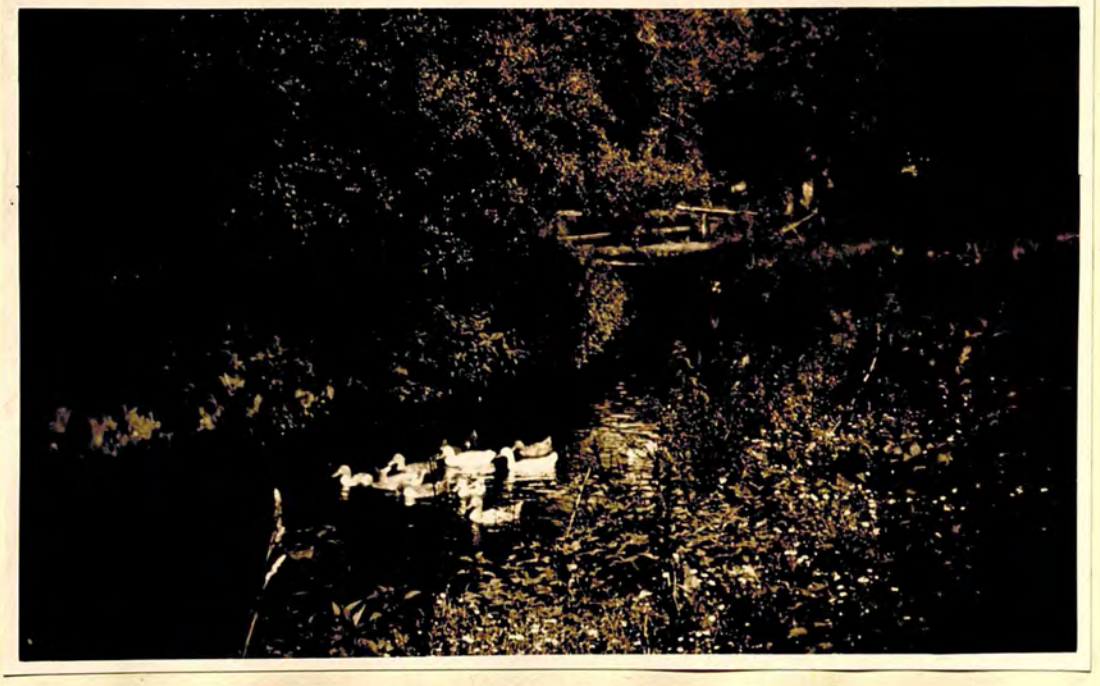
The Hogsmill river, though once famous for trout, has not been a completely rural stream for some centuries. From 1589 - 1613 there were twelve gunpowder mills on its banks between Ewell and Malden and although these have long since disappeared their place has been taken by two flour mills and a brick, tile and pottery works. These mills and other works along the banks together with the sewage effluents must have been the source of entry of considerable foreign matter in the past and have doubtless contributed to the varied fungus flora.

(b) The Site.

The site of the present investigation is situated in one of the few remaining rural areas along the course (for position see map 1.). At this point the river is in the abandoned valley of the Mole and is about 17½ feet wide, flowing between banks about 2½ feet deep, bordered by meadows and overhung on the left bank by trees, hawthorn, elder and ash. The bed consists



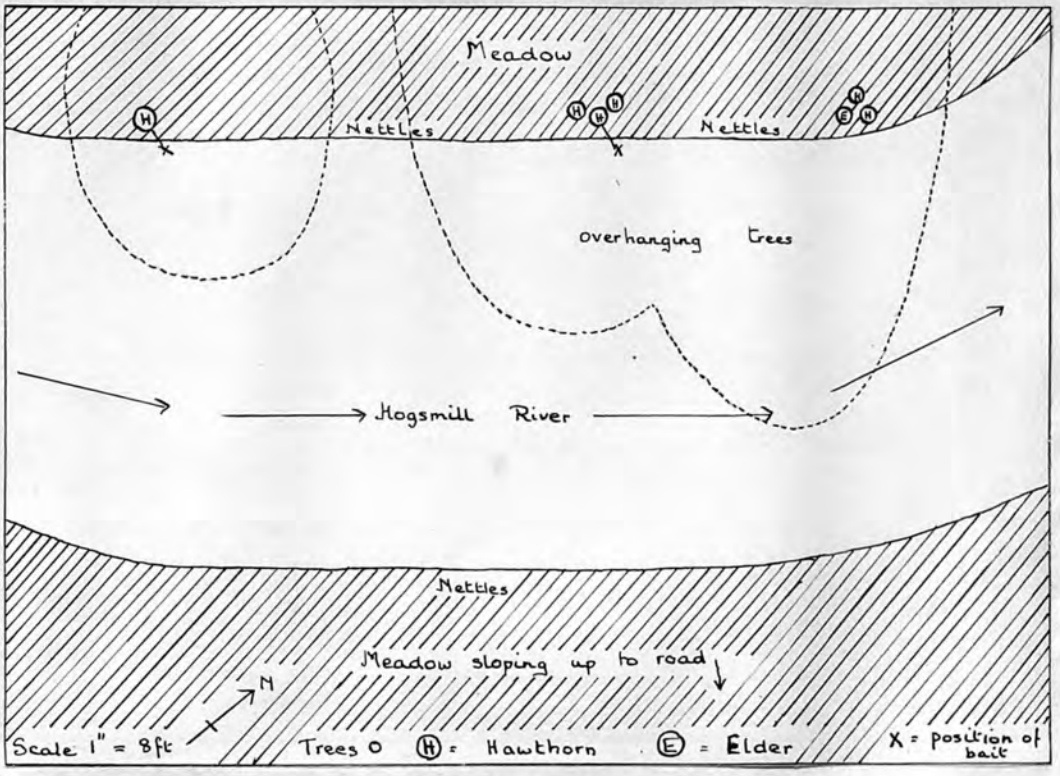
The Hogsmill River.
Site of the investigation, looking upstream.



The Hogsmill River.
Site of the investigation, looking downstream.

of soft black mud 5 - 6 inches deep at the margins.

About once a year (in mid June 1938) the river is cleared



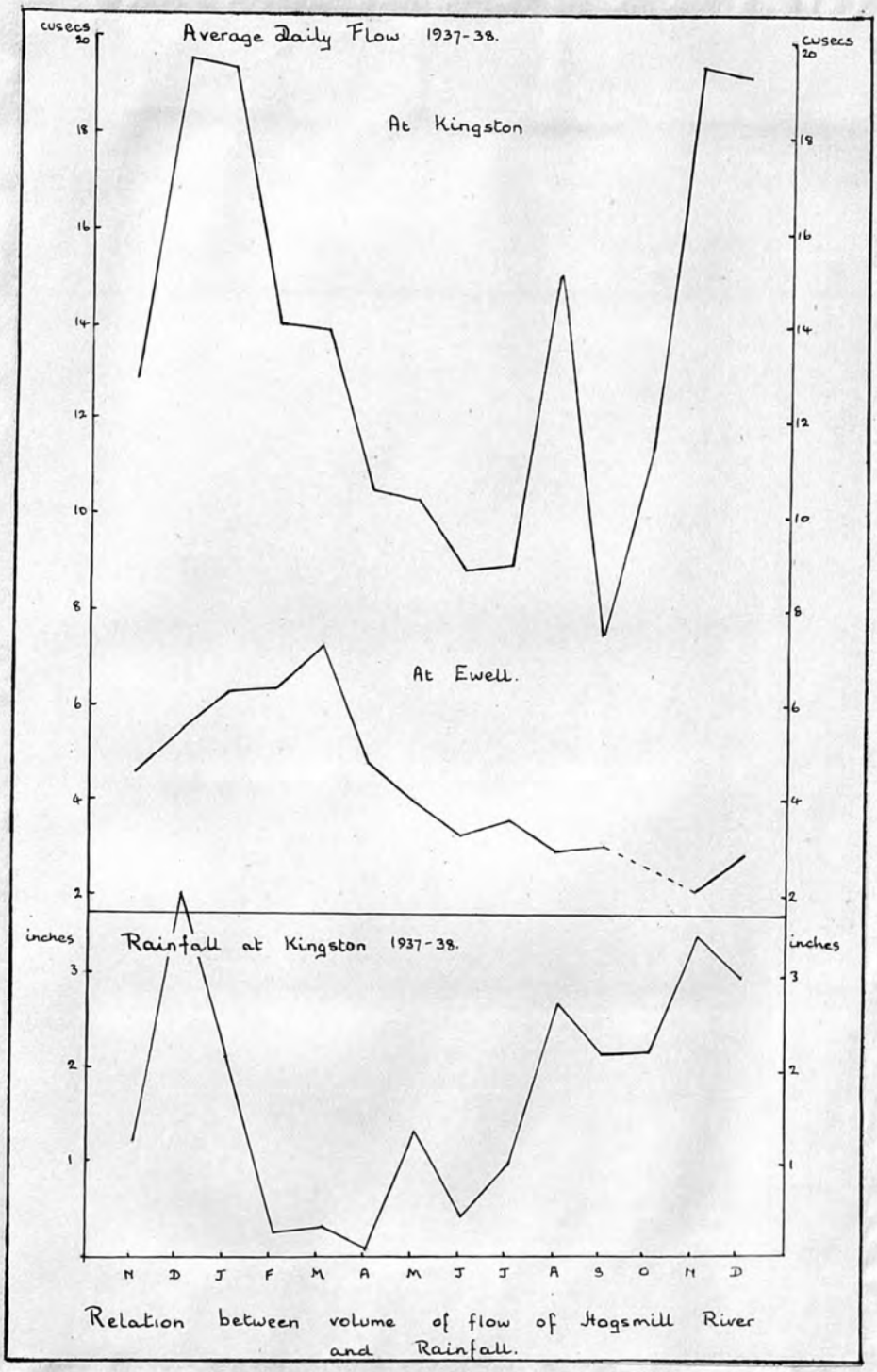
of water plants and the banks trimmed and cleared of weeds by the Surrey County Council. Consequently there is no aquatic vegetation apart from a little Cladophora and microscopic plant life thus affording the exposed bed no shade or protection. The overhanging trees are on the north side and the river at this point has the full benefit of the sun's rays.

(c) Depth and Flow.

The river is usually fairly shallow, showing an average depth of 4 - 5 inches at the bank in summer and 6 - 7 inches in winter, It is about 6 inches deeper in midstream. Normal rain showers do not alter the depth considerably (up to 3 inches increase) but continuous heavy rain gives a rise of a foot or more. Twice during January 1939 and once during the previous

winter the river overflowed its banks. About twenty four hours afterwards the level was down again to average showing that the flood water is soon carried away.

A good flow is maintained throughout the year, due partly to the strength of the springs at the source and partly to the sewage effluents. There are no official records of it ever having dried up. The Councils at Ewell and Kingston have installed continuous recorders of the volume of flow, and measurements have been taken for a number of years. The accompanying graphs show the average daily flow for each month from November 1937. I am indebted to the staff of the Borough Sewage Works at Kingston-on-Thames for the figures from which they were compiled. Examination of these graphs and similar ones for the preceding years shows that at Ewell the flow of the Hogsmill River rises steadily to a maximum in the spring (usually in March though it has occurred in February and as late as May) and then falls steadily to a minimum in November. This rise and fall shows no relation to the rainfall of the district (given on the same figure) which rises and falls irregularly though usually with a maximum in winter. The river maximum is always a month or two behind the rain maximum and the lack of correlation is due to the fact that the river at Ewell derives the bulk of its water from the springs which depend on the underground water table. This will be at its highest after the winter rains have percolated through (i.e. in early spring) and it is at this time that the springs will be strongest and the river at its highest



Relation between volume of flow of Hogsmill River and Rainfall.

level. At Kingston on the other hand the flow shows a fairly close correlation with the rainfall of the district.

At the site of the investigation therefore the flow will depend on three factors :- 1) the springs which will give a basic flow having a maximum in spring and a minimum in autumn. 2) the sewage and mill effluents which will be fairly constant all the year round and will add to the basic flow.

3) the rainfall drainage of the area above the site which will superimpose on the basic flow an irregular and intermittent variation in level with a maximum usually in January and a minimum in October.

The rate of flow at normal times is about o.8 miles per hour in midstream and about o.5 miles per hour near the bank.

(d) Temperature.

It was not possible to take continuous temperature records and none at all were taken during the first year. The following have been observed this year.

	maximum	mimimum
January	14.5°C	4.5°C
February	12.3°C	5.5°C
March	12.3°C	6.0°C
April	18.0°C	8.0°C

There are no official records of the river ever having been frozen over and it is fairly certain from personal acquaintance that such has not occurred during the past five years. The temperature taken soon after the cold spell of Christmas 1938, was 6.°C. It is probable that the higher temperature of the sewage effluents passing in maintains the temperature level somewhat above that which would occur naturally and prevents extensive freezing.

(e) Chemical Analyses.

I am indebted to the Thames Conservancy for the following

analyses which they have permitted me to quote.

	At the source 19 March 1938	Below Ewell Mill 19 March 1938	At Kingston ¼ mile from mouth 5 May 1938
Sodium chloride	3.4	3.6	7.5
Oxygen from potassium permanganate	0.02	0.04	0.41
Saline ammonia	0.0005	0.0005	1.6
Albuminoid ammonia	0.0005	0.0005	0.05

Measurements all in parts per 100,000 parts of water.

The considerable increase between the source and outflow is due to the sewage effluents which enter en route.

The hardness of the water at Ewell is 19.

(f) pH values.

1938.	June 1.	8.1
	June 16.	8.0
	June 27.	7.9 - 8.0
	July 11.	7.8 - 7.9
	Sept. 12.	7.8 - 7.9
	Sept. 27.	7.8
	Dec. 15.	7.6
1939.	Jan. 2.	7.3
	Jan. 9.	7.4
	Mar. 8.	7.4
	Mar. 31.	7.2
	April 30.	7.4
	May 31	7.6

The above recordings are for the surface water. The bottom water is always slightly more acid especially in the drier weather (June) when differences of 0.2 - 0.4 were observed. In January the difference was only 0.1. The difference is probably due to acid set free by decay in the bed of the river and is less when the surface waters are made more acid by rain water.

The recordings show that there is a definite annual variation in the pH of the river with a maximum in winter and spring and a minimum in summer.

An interesting comparison between the observations in sections (c), (d), (e) and (f) and the corresponding ones for the Thames can be made by referring to Rice (1938) who collected similar data while working on the Phytoplankton of the Thames. The pH values there showed a similar variation (7.25 - 8.5), the maximum being in the spring and the minimum in the summer or autumn. Rice attributes this variation to the removal of carbon dioxide by green plants in summer and to the large ammount of decaying matter in autumn.

(g) Summary of weather conditions during the period of the investigation.

The chief features of the weather were as follows. There was a cold spell with servere frosts and local snow at the beginning of December 1937 and lasting about a week. January 1938 was very warm with only one frost and that a slight one. The spring of 1938 was very dry the rainfall in February,

March and April totalling only 0.68 in. . This was reflected in the volume of flow of the river at Kingston (top graph fig. 4.). Although there was over an inch of rain in May the river continued to fall at Kingston until July. This was probably due to the fact that the rainfall was absorbed by the dry ground and never reached the river. At the site the river was very low right up to the beginning of September. The autumn of 1938 was fairly mild until a cold spell with heavy snow began on December 16 and lasted for twelve days. At the end of January 1939 there were floods and the river overflowed its banks.

Literature Cited. Part I.

Dewey, H. and Bromhead, C.E.N. 1921. Memoirs of the Geological Survey of South London. Explanation of sheet 270.

Rice, C.H. 1938. Annals of Botany new series vol. II.

Whittaker. 1912. The water supply of Surrey.

II. The Water Moulds.

(a) Methods. The usual methods of setting bait were employed, the bait being contained in discarded preserved fruit tins with holes punctured in the bottom and sides to permit the passage of water through the tin. Tomatoes were used principally as these gave the most satisfactory results; rapidity and variety of infection,& the facility of examination provided by the thin transparent easily removable skin. Twigs of horse chestnut, ash, oak and rose as well as grapes, hips, barberries, apples, red currants and black nightshade berries were employed either as bait in the stream or for fresh inoculations in the laboratory but nothing was found on these that was not also found on tomatoes under similar conditions.

After removal from the stream the bait was washed thoroughly in tap water and kept in glass jars in tap water, the latter being refreshed every few days. Cultures containing a single species only could be obtained fairly easily by placing a pustule or the zoospores of the type required with fresh bait. As the moulds seem to grow quite well with attendant bacteria and protozoa it was not thought necessary to attempt pure cultures except in the case of the Pythium spp. and Pythiomorpha (Phytophthora) spp. which were transferred to agar cultures for identification.

(b) List of species identified.

LEPTOMITACEAE.

Rhipidium continuum Cornu = Rh. europaeum von Minden.

The life history of this species was followed during the winter months when it appeared most abundantly and it was found to correspond closely with the description and figures of Berners (1931). It was not encountered all the year round as some investigators find (Lund 1934, Sparrow 1936) but attacked the bait only from November to February. It infected tomatoes rapidly, visible pustules appearing in 9 - 12 days and in two cases as rapidly as 4 and 7 days. Oospores were frequent and appeared after 4½ to 7 weeks the usual time being 5 weeks. This is longer than the time noted by Lund (1934) who obtained oospores after 23 days. The thallus was always of the compact plateau type bearing sporangia and oospores round the edge and never giving elongated hyphal branches with reproductive organs, suggestive of the variety compactum found by Forbes (1935).

Rhipidium americanum Thaxter.

This was not so abundant as the preceding species, in fact during the winter 1937-38 it was encountered only in January on twigs. During the succeeding winter it appeared occasionally on bait in November, December, January and February. It infected tomatoes rapidly visible pustules appearing in four days. No sexual organs were seen although occasionally spherical thick walled spores were found without however a definite antheridium but this form was so different from the preceding species even when growing side by side with it on the same tomato that it was concluded that it was R.

americanum.

GONAPODYACEAE.

Gonapodya prolifera (Cornu) Fischer.

This species was extraordinarily sporadic in its appearances. It would appear abundantly covering a tomato and then suddenly die out even though fresh bait were put in to obtain a succession of growth. It suggests that the fungus requires a substratum at a certain stage of waterlogging and decay and that fresh material is not suitable. Apple slices were tried but other hyphal Phycomycetes gained a foothold more readily and soon overran the later appearing Gonapodya. The best growth appeared during a hot spell in July 1938 and this does not coincide with findings of Sparrow (1933) who states that Gonapodya grows best at very low temperatures (8°C.). No reproductive organs other than zoosporangia were seen and these gave zoospores freely and the hyphae proliferated new sporangia several times within the original sporangium; the new ones never protruded beyond the previous one.

BLASTOCLADIACEAE.

Blastocladia Pringsheimii, Reinsch.

As is usually the case this species was the most abundant and constantly appearing of all the water moulds, apparently inactive in the stream only in August 1938. This may have been due to the fact that the river had been very low during the summer and perhaps during a wet summer it may continue its activity unabated. It appeared also in its usual variety

of form. On the whole however two types of thallus were encountered more frequently than the others, 1) which has been called the globose form and 2) which has been called the branched form, corresponding to types A and C of Lloyd (1938). Resistant sporangia appeared intermittantly, sometimes in profusion, and varying in shape from almost spherical, through shortly clavate ones, to elongated clavate forms which looked as if they were ordinary thin walled sporangia converted into resistant sporangia; some of the resistant sporangia were successfully germinated.

Blastocladia gracilis, Kanouse.

In the early part of the year this species was sometimes more abundant than the preceding one but appeared in lesser quantities in the other months and disappeared altogether from July to October 1938. Its most striking feature is the rapid and prolific production of resistant sporangia which appear within fourteen days from the time of inoculation. They are more constant in shape than those of B.Pringsheimii being oval with a truncate base and having a more transparent wall with less conspicuous pits. In Denmark Lund (1934) found that B. gracilis occurred all the year round but only in waters which were neutrally or constantly alkaline. While he examined only lakes, ponds and pools it is interesting to find that it occurs in the constantly alkaline Hogsmill river thus extending Lund's generalisation to the case of a different type of water.

Blastocladia ramosa Thaxter.

Occasional pustules of this species were encountered in the winter months. Release of zoospores was observed but no resistant sporangia were found.

PYTHIACEAE.

Pythiomorpha (Phytophthora)

During the investigation forms were frequently found which answered to the description of Pythiomorpha. On the whole there seemed to be two types 1) a type with smaller sporangia which resembled P. gonapodyides Petersen, and 2) a type with larger sporangia and wider hyphae which is referred to as the larger form in the ensuing account and in the monthly summary. Type 1 was found thirteen times on old bait which had been in the stream either in November, December 1937 or January 1938 and three times similarly from October to December 1938. It was obtained only in culture in other months. This is the form which was found to be parasitised by a Chytrid (for a full description of this parasite see final section). The larger form was encountered seven times on bait which had been in the stream in the months from November 1937 to January 1938. Some of these forms were isolated in agar cultures (bean, quaker oat, cornmeal and malt). On agar alone they produced no reproductive organs except spherical swellings resembling chlamydospores (and in one case a few misshapen sporangia) but when portions of the infected agar were transferred to water or Petri solution abundant obpyriform, non-papillate sporangia

were produced. Secondary sporangia were produced either by proliferation through an empty sporangium or by sympodial growth from below. The tendency to sympodial growth was more pronounced in portions floating at or near the surface of the water whereas proliferation took place more on portions 2-3 centimetres below the surface. This phenomenon has been noticed by other investigators and has been attributed to the fact that at the surface the more abundant air supply contributes to more rapid growth, and branching occurs before the sporangia are ready to dehisce or because they are inhibited from doing so by insufficiant water supply.

It seemed that these forms were undistinguishable from species of Phytophthora belonging to the group which is characterised by its obpyriform, non-papillate sporangia and showing proliferation through empty sporangia and producing reproductive organs sparsely or rarely (except chlamydospores) on solid media. The species of Phytophthora included in this group are :- P.cambivora, P.cinnamomi, P.cryptogea, P.Drechsleri, P.erythroseptica, P.megasperma and P.richardiae. The present forms most nearly resembled P.cambivora and P. cryptogea. In view of the frequent occurrence of Phytophthora spp. in the soil and their affinity for moisture it seems curious that aquatic forms have not been recorded. It suggests that some of the Pythiomorpha spp. described in the past may have been Phytophthora spp. which have migrated from the

soil into the water.*

Oospores associated with the Pythiomorpha type of sporangium were found only once and these were under the skin of a grape taken from the stream in December 1937.

One case of diplanetism in the zoospores was noticed, these having been discharged from sporangia from resuscitated mycelium on grape skins which had been stored in agar from January to April.

Pythium

A Pythium sp. with large spherical sporangia frequently made its appearance in most months on bait which had become old and decayed. It was the only fungus recorded in the stream in August 1938. The sporangia were abundant and always spherical with a short lateral discharge tube. If they did not give zoospores the sporangia persisted for months on the bait and finally germinated by hyphae if given fresh media. No sexual organs were obtained either on the bait or in cultures of agar, carrot slices and hemp seed., and it has therefore not been possible to identify this species yet.

Pythiogeton von Minden.

A pythium-like form with oval eccentric sporangia on

---

* The status of the genus Pythiomorpha in relation to these species of Phytophthora is now being investigated in the Royal Holloway College laboratory and it therefore seems best to defer the identification of the forms mentioned above until this investigation is completed.

very thin hyphae was encountered occasionally (once in abundance) but the discharge of zoospores was not witnessed. It is referred temporarily to this genus. The sporangia were usually terminal but occasional subterminal ones were observed.

WORONINACEAE.

A parasite corresponding very closely to Pleolpidium inflatum Butler was found and isolated. It is described in detail in the last section.

SAPROLEGNIACEAE.

Species of Saprolegnia and Achlya occurred frequently on suitable bait but it was decided not to include identification of these in the present investigation.

(c) Monthly Occurrence of Identified Species.

In this list are first recorded (under the heading "stream") those species actually found in a certain month on bait taken from the stream in that month. It is well known that species are not always apparent on the bait when it is removed from the water but appear subsequently as the material is watched in the laboratory. Any forms thus appearing in subsequent months are recorded under the month in which they were first noticed but the reader is referred also to the month in which the bait was taken from the stream. In general a species once obtained can be kept growing in culture through the subsequent months by continually refreshing the water and providing fresh

substratum. Those thus kept on in culture are not included in this list but a few sporadic appearances of forms not purposely cultured are noted.

With regard to inoculation of the bait in the stream it is worth noting that in the winter (November and December) pustules were visible on tomatoes after 10 days In the summer the period is much longer and in May no pustules were visible after 23 days and in July and September none were visible until fourteen to seventeen days.

a - abundant, f - frequent, o - occasional

R.S. - resistant sporangia O.S. - oospores

br - branched form gl - globose form

T - tomato fruit C - horse chestnut twig

G - grape A - ash

H. - hip R - rose

V - variety of bait

NOVEMBER.

	1937				1938	
	Stream				Stream	
a	B.Pringsheimii (br)	T		a	B.Pringsheimii (br)	T
o	" " (gl)	T		f	B.gracilis	T
o	B.gracilis	T		o	R.continuum	T
o	B.ramosa	T		o	R.americanum	T
a	R.continuum	T			From October	
				o	Pythiogeton sp.	T
				o	G.prolifera	T

DECEMBER.

1937				1938		
	Stream				Stream before December 15th.	
a	B.Pringsheimii (br)	T		a	B.Pringsheimii (br)	T
o	" " (gl)	T			Stream after cold spell.	
o	B.ramosa			f	B.Pringsheimii (br)	T
a	R.continuum + O.S.	V		o	B.gracilis	T
f	P.gonapodyides + O.S	G		f	R.continuum	T
o	Pythiomorpha (large)	T		o	R.americanum	T
	From November				From October	
f	R.continuum O.S.	T		f	Pythium sp.	T
o	P.gonapodyides	G				
o	Pythiomorpha (large)	T				

JANUARY.

1938				1939		
	Stream				Stream	
f	B.Pringsheimii (br)	T		f	B.Pringsheimii (br)	T.A
o	B.ramosa	R		f	B.gracilis	T
f	R.continuum	T.R		a	R.continuum	T
o	R.americanum	C		o	R.americanum	T
f	P.gonapodyides	V			From October	
	From November			o	B.Pringsheimii R.S.	T
o	Pleolpidium inflatum?	G.T		f	Pythium sp.	T
	From December				From December	
o	B.gracilis	T		f	Pythium sp.	T
f	R.continuum O.S.	H.G.		f	B.gracilis R.S.	T

FEBRUARY.

1938				1939		
	Stream				Stream	
f	B.Pringsheimii (br)	T.C		f	B.Pringsheimii (br)	T
o	" " (gl)	R		a	B.gracilis + R.S.	T
o	B.gracilis + R.S.	A.C		o	R.continuum	T
f	R.continuum + O.S.	T.C		o	R.americanum	T
o	P.gonapodyides	R			From November	
	From November			o	P.gonapodyides	T
o	Pythium sp.	T		a	Pythium sp.	T
				f	B.Pringsheimii R.S.	T

MARCH.

1938.				1939.		
	Stream.				Stream	
f	B.Pringsheimii	G		f	B.Pringsheimii	T
a	B.gracilis + R.S.	G		a	B.gracilis + R.S.	T
	From January			o	R.continuum	T
f	B.Pringsheimii R.S.	T.A			From January	
f	B.gracilis + R.S.	A		a	B.Pringsheimii R.S.	A
o	R.americanum	T				
o	Pythiomorpha (large)	G				

APRIL.

1938

	Stream	
f	B.Pringsheimii	T.G
f	B.gracilis	T.G
	From January	
f	R.americanum	C.A
o	P.gonapodyides	C

1939

	Stream	
f	B.Pringsheimii	T
a	B.gracilis + R.S.	T
	From October	
o	P.gonapodyides	T

MAY 1938

	Stream	
o	B.Pringsheimii	T
o	B.gracilis	T
	From November	
o	G.prolifera	H

JUNE 1938

	Stream	
o	B.Pringsheimii	T
	From November	
f	G.prolifera	C.R
	From dried twigs	
	B.Pringsheimii (gl)	
	From May	
f	Pythium sp	T

JULY 1938

	Stream	
f	B.Pringsheimii (br) + R.S.	T
	" " (gl) + R.S.	T
	From May	
f.	B.Pringsheimii R.S.	T
	From dried twigs	
a	G.prolifera	T
f	Pythiogeton sp.	T

AUGUST 1938

	Stream	
a	Pythium sp.	T

SEPTEMBER 1938

	Stream	
o	B.Pringsheimii (small immature plants)	T
o	B.gracilis	T
f	Pythium sp.	T

OCTOBER 1938

	Stream	
f	B.Pringsheimii (br)	T
f	B.gracilis R.S.	T
	From September	
f.	B.gracilis R.S.	T
	From dried twigs.	
o	B.ramosa	T

(d) Attempts to germinate resistant spores.

Rhipidium continuum.

No investigator has yet reported the germination of the oospores of this genus. Berners (1931) kept some for a year without success. Various methods were tried in this investigation, also without success. Tomato and grape skins bearing oospores were stored by the methods which had proved successful with Blastocladia Pringsheimii resting spores (Blackwell 1938). Some were kept in the laboratory and some on the roof and some in a refrigerator (temp. 34 F.) for 3, 4 or 5 months. On transporting from store to water it was noticed that some spores had much thinner walls than others (see figure). This may have been a prelude to germination but no germination was obtained even after keeping the spores for a year and putting them with fresh bait. Alternate drying and wetting, treatment with potassium permanganate (McKay 1937) and transference to Petri solution and agar met with no success.

It was noticed in December 1938 that whereas previously there had been little R.continuum on tomatoes from the stream, after a severe cold spell lasting about 10 days it appeared in abundance. This suggests that perhaps the sudden cold had brought about the germination of the previous winter's oospores and thus given the increased production. It is possible that similar methods in the laboratory - considerable chilling of spores of the right age - might effect germination

in this species as with Phytophthora spp. (Rosenbaum 1917, Blackwell 1937)

Blastocladia Pringsheimii.

As Miss Blackwell was germinating the resistant sporangia of this species successfully in the Royal Holloway College laboratory it was decided to try the resistant sporangia from the Hogsmill river. Tomato skins which had borne plants with resistant sporangia in March 1938 were kept in water until July. They were then allowed to dry slowly in the empty jar from July 25 to September 21. The skins were then flooded with fresh tap water. One day later a large percentage of the resistant sporangia showed cracks and papillae, and eventually discharged zoospores. Some of these were allowed to settle down on the slide and were seen to germinate by a tube. Motile zoospores were put with fresh tomatoes and young plants (not visible to the naked eye) were produced in 24 hours.

Twigs of rose, ash and horse chestnut which had borne plants with resistant sporangia in January and February were kept in water until June and were then allowed to dry for a week or two. On putting them into water with a tomato, plants of the globose type visible to the naked eye were produced in 4 days. Other twigs bearing resistant sporangia and dried from August to October, then treated in the same way gave visible pustules on tomatoes in 6 days. This of course is only indirect evidence that re-inoculation came

from the germinated resistant sporangia but in all the many cases of germination with Miss Blackwell's resistant sporangia the old thallus has never been seen to resuscitate.

Blastocladia gracilis.

Indirect experiments similar to those tried with B. Pringsheimii were also performed with B.gracilis. Twigs bearing resistant sporangia were dried from August to November and were then put in water with a tomato. Plants of Blastocladia gracilis were visible on the tomato after 7 days

In connection with these drying experiments it is interesting to note that Lund (1934) found no paucity of water moulds in shallow pools subject to drying.

LITERATURE CITED PART II.

Berners, A. 1931. Planta 15.

Blackwell, E.M. 1937. Nature 140, p.933.

Forbes, E.J. 1935. Trans. Brit. Myc. Soc. XIX.

Lloyd, D. 1938. Trans. Brit. Myc. Soc. XXI.

Lund, A. 1934. Mem. de l'Acad. Roy. des Sc. et des Lett. de Dan. Copenhagen Sect. de Sc. 9 series, t VI, I.

McKay, R. 1937. Nature 139, pp.758 and 802.

Rosenbaum, J. 1917. Journ. Agric. Res. VIII.

Sparrow, F.K. 1933. Ann. Bot. 47, p.517.

Sparrow, F.K. 1936. Journ. Linn. Soc. Lond. vol. L, no.334.

PLATE I.

Rhipidium spp.

Figs. 1 - 4 R.continuum

Fig. 1. a - e Stages in development of thallus from germinating zoospore.

Fig. 2. Young thallus with sporangial bearing hyphae just growing out.

Fig. 3. a - c Stages in the growth of the sporangial bearing hyphae.

Fig. 4. a, b Normal sporangia of R.continuum
(a) with protoplasm divided into zoospores round central vacuole
(b) sporangium showing lateral dehiscence pore.

Fig. 5. R.americanum young plant from horse chestnut twig.

PLATE I

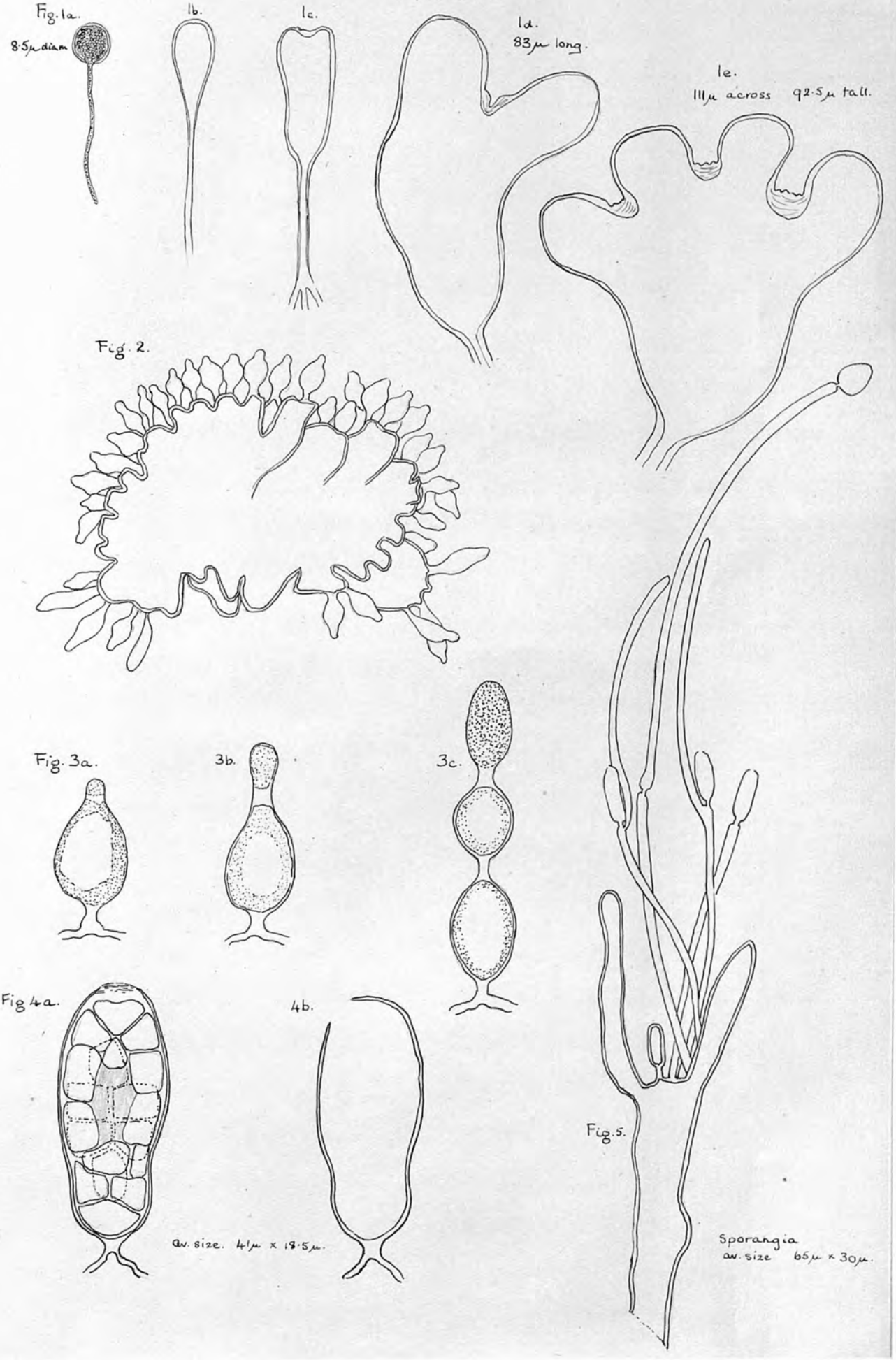


Fig. 1a.
8·5μ diam
1b.
1c.
1d.
83μ long.
1e.
111μ across 92·5μ tall.
Fig. 2.
Fig. 3a.
3b.
3c.
Fig 4a.
4b.
av. size. 41μ x 18·5μ.
Fig. 5.
Sporangia
av. size 65μ x 30μ.

PLATE II.

Gonapodya prolifera.

Fig. 1. Portion of plant showing candelabra-like branching.

Fig. 2. Release of zoospores from double necked sporangium. Spores inside sporangium are amoeboid.

Fig. 3. a - g Stages in dehiscence of sporangium.

Fig. 4. Zoospore after release.

Fig. 5. Release of zoospores from secondary sporangium within primary sporangium.

PLATE II

Fig. 1.

Sporangia
av. size 146μ x 27μ.

Fig. 2.

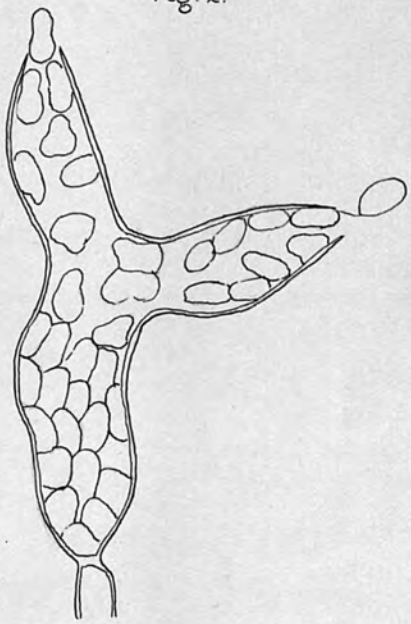
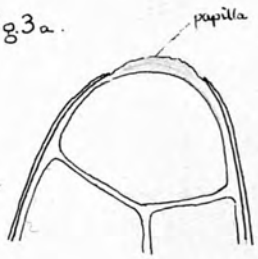
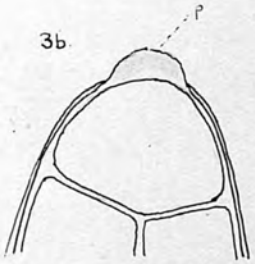


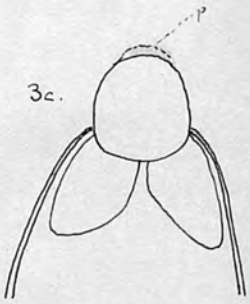
Fig 3a.



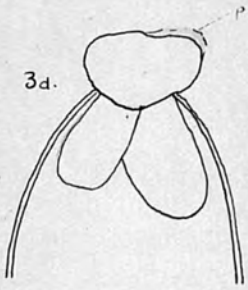
3b.



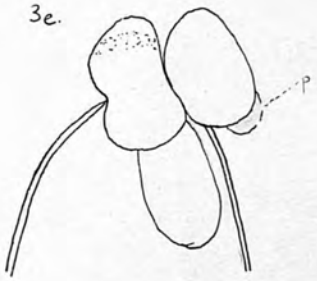
3c.



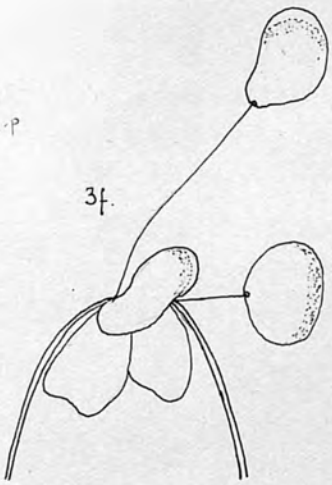
3d.



3e.



3f.



3g.

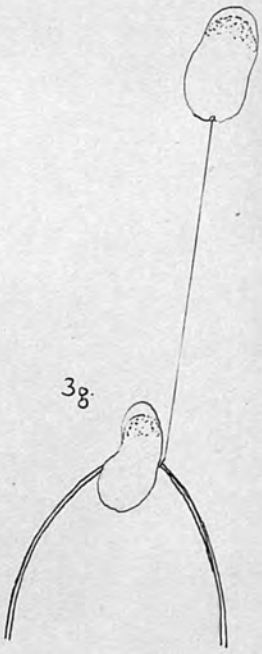


Fig. 5.

Fig. 4.

7μ x 3·5μ.

PLATE III

Blastocladia spp.

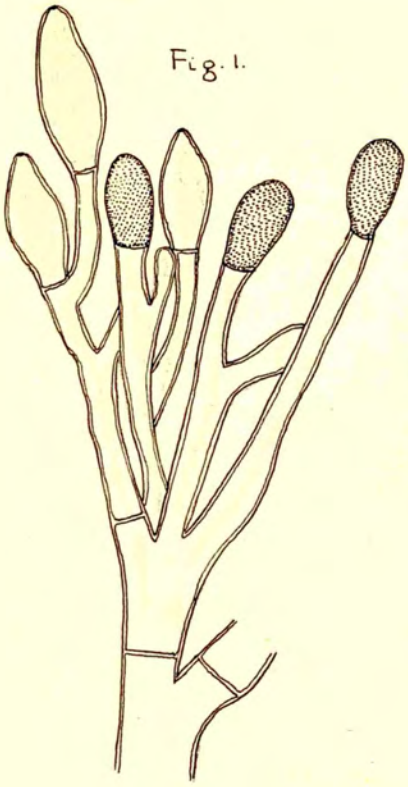
Fig. 1. B.gracilis showing branching, sporangia and resistant sporangia.

Fig. 2. B.ramosa with three sporangia.

Fig. 3. a - c Formation of zoospores in B.ramosa
(a) conspicuous areas with vacuoles
(b) sudden swelling of areas to form zoospores and disappearance of vacuoles.

PLATE III

Fig. 1.



Sporangia 78 μ × 27·5 μ av. size.
resting spores 44·5 μ × 28 μ av. size

Fig. 2.

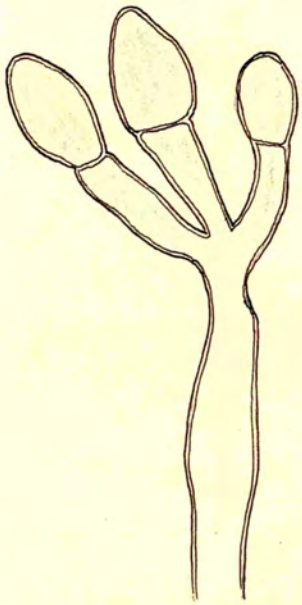
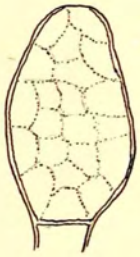


Fig. 3a.

3b.



3c.

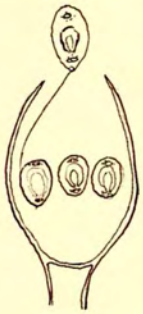


PLATE IV

Pythiomorpha (Phytophthora) spp.

Figs. 1 and 2 Large form.

Fig. 1. a, b Showing crowded sympodial growth at surface of Petri solution (bean agar culture)

Fig. 2. a - e Variety of sporangial shapes on tomatoes

Figs. 3 - 6 Pythiomorpha gonapodyides.

Fig. 3. Sympodial and proliferating growth of sporangia in shallow water.

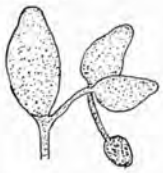
Fig. 4. a - d Variety of sporangial shapes on tomatoes.

Fig. 5. a - e Variety of sporangial shapes in Petri solution (agar cultures).

Fig. 6. Repetitional diplanetism of zoospores.

PLATE IV.

Fig. 1a.



1b.

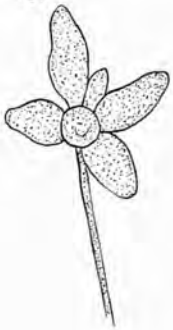
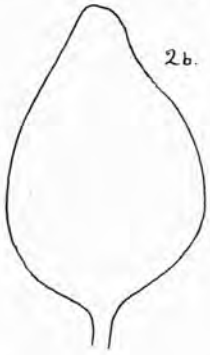
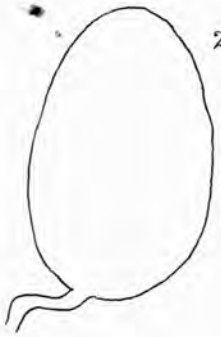
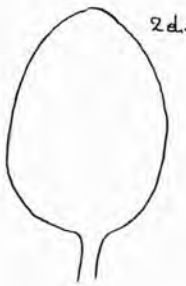


Fig. 2. sporangia sizes 44–63μ x 33–40μ.







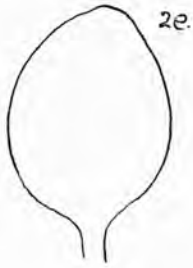


Fig. 3.

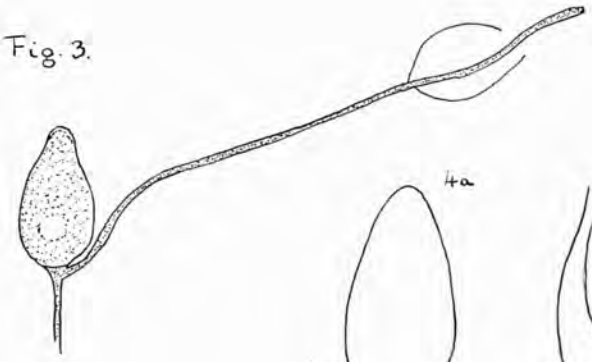


Fig. 4. sporangia av. size 39μ x 20μ

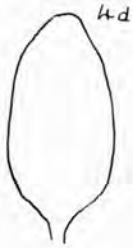
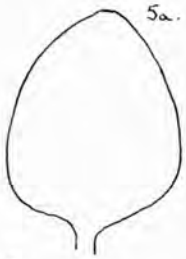
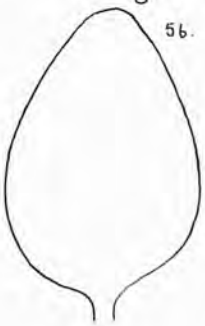
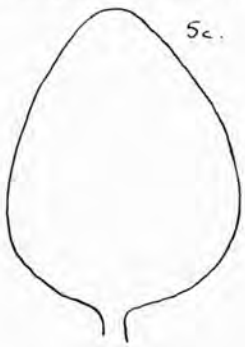


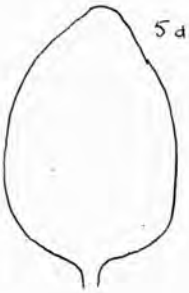
Fig. 6.

Fig. 5. Sporangia av. size 55μ x 37μ









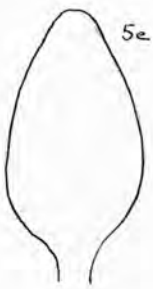


PLATE V.

Figs. 1 - 3 Pythiogeton uniforme Lund?

Fig. 1. a and b Subterminal sporangia with remains of hyphal tips.

Fig. 2. a, b, and c Showing variety of sporangial shapes.

Fig. 3. Sporangia with discharge tubes forming.

Figs. 4 - 6. Pythium spp.

Fig. 4. Form with small sporangia in pustules on tomato.

Fig. 5. Form with large sporangia on tomatoes.

Fig. 6. Formation of discharge tubes in larger form.

PLATE V

Fig. 1a. 1b. Fig. 2a. 2b. 2c.

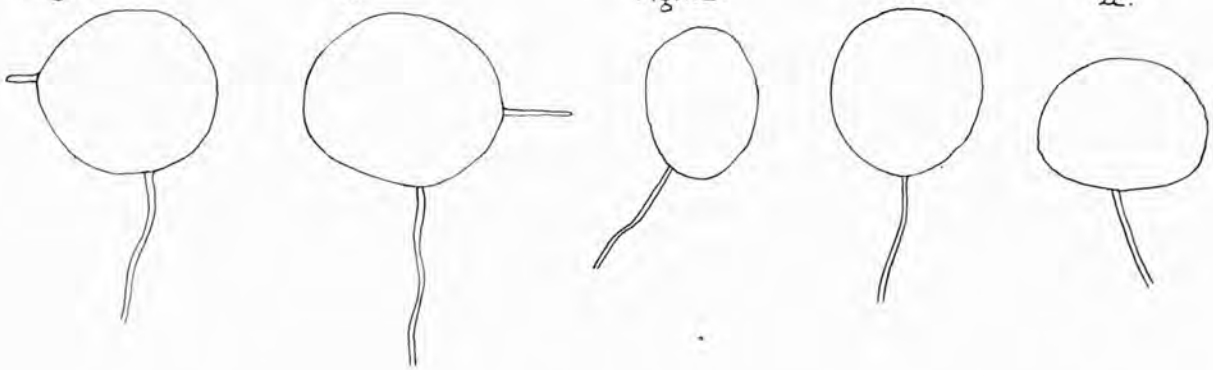
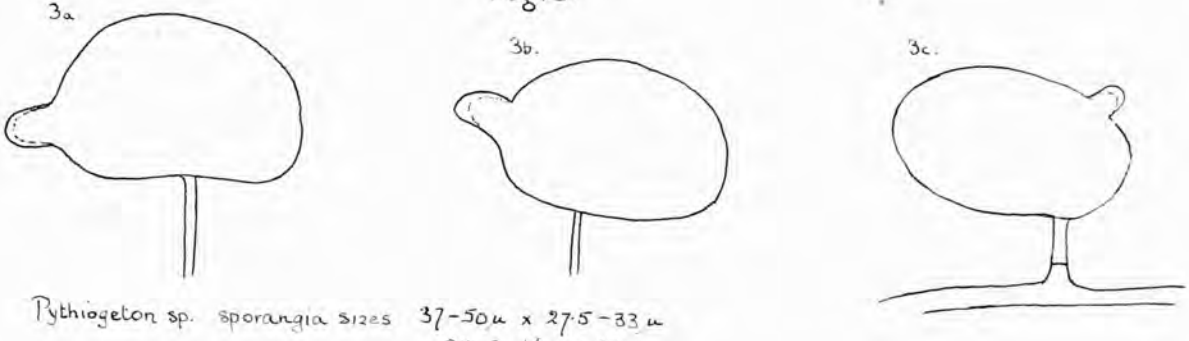


Fig. 3.

3a 3b. 3c.



Pythiogeton sp. sporangia sizes 37-50μ x 27·5-33 μ
av. = 46μ x 28μ.

Fig. 4.
sporangia sizes 16·5μ - 28μ diam.

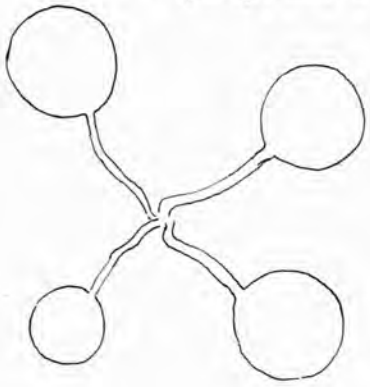


Fig. 5.
5a. 5b. sporangia sizes 22μ - 29μ diam.

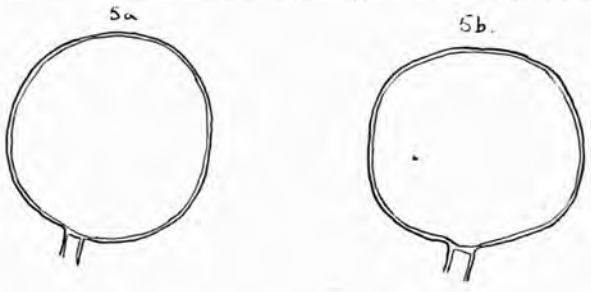


Fig. 6.

6a. 6b. 6c.

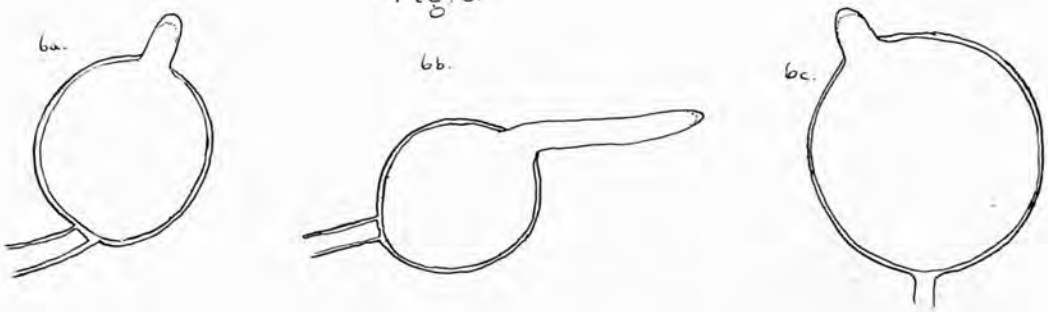


PLATE VI.

Rhipidium continuum.

Probable stages in preparation for germination of oospore

Fig. 1. Ripe oospore (with persistant antheridium) as stored in December.

Figs. 2 - 4 After soaking in water on removal from store at end of April.

Fig. 2. Oospore with slightly thinner wall and swelling protoplasm.

Fig. 3. Wall thinner still, protoplasm more extensive, oil drops disappearing.

Fig. 4. Wall very thin, coarsely granular protoplasm fills spore.

PLATE VI

Fig. 1.

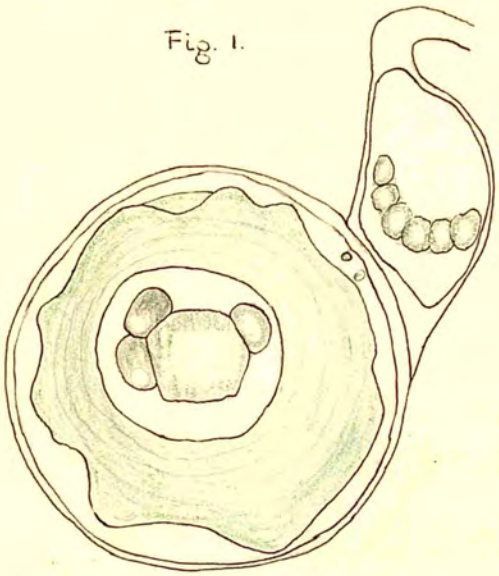


Fig. 2.

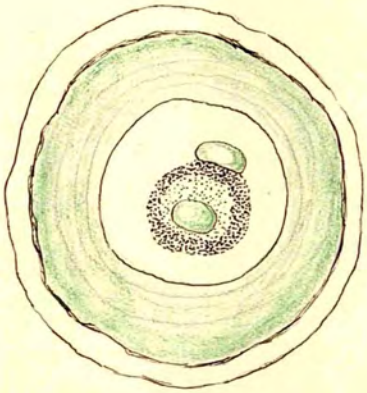


Fig. 3.

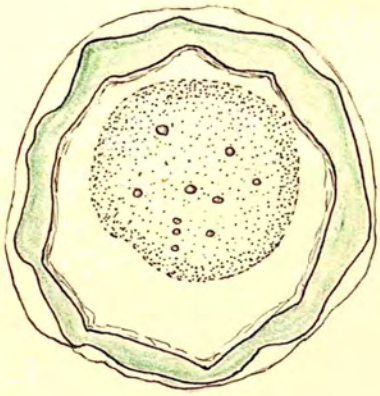
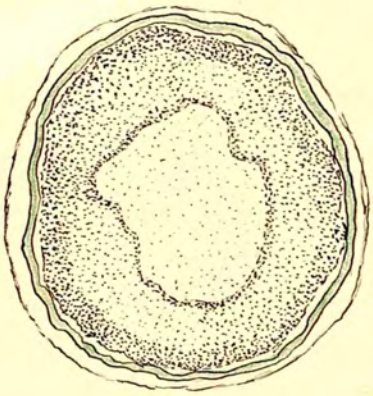


Fig. 4.



Sizes of oospores 26μ – 30μ diam.

PLATE VII

Blastocladia Pringsheimii.

Germination of resistant sporangia.

Fig. 1. a - e Development of papillae and appearance of zoospore initials.

Fig. 2. Discharge of zoospores.

Fig. 3. a and b Showing variety of shapes of resistant sporangia (papillae formed).

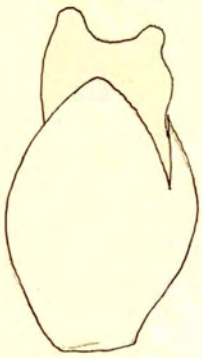
Fig. 4. a and b Germinating zoospores in water 12 hours later.

Fig. 5 Nine young germlings in crack of tomato skin 24 hours later.

Fig. 6. Two young plants on tomato 36 hours later.

PLATE VII

Fig. 1a. 44·5μ × 27μ.



1b.

1c.

Fig. 2.

89μ × 24μ

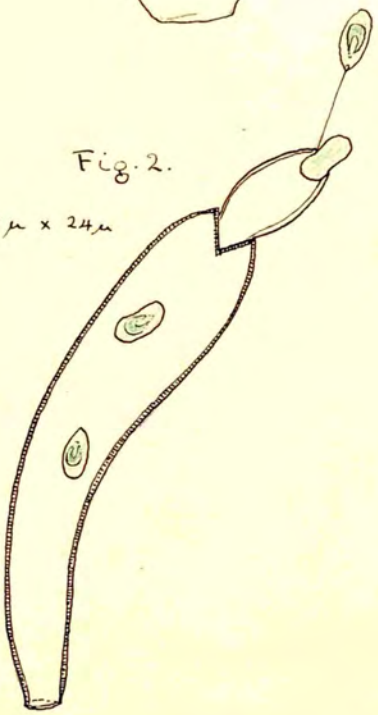
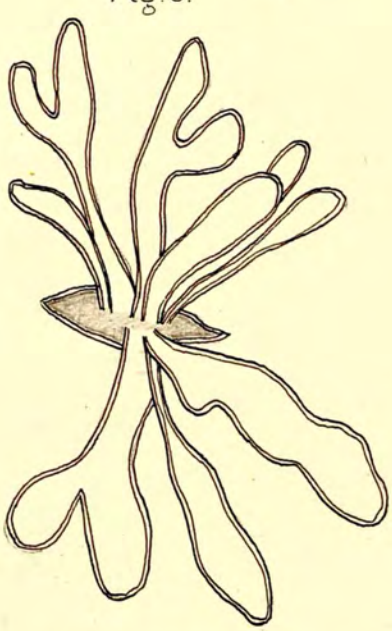


Fig. 3a.

3b.

Fig. 6a.

Fig. 5.



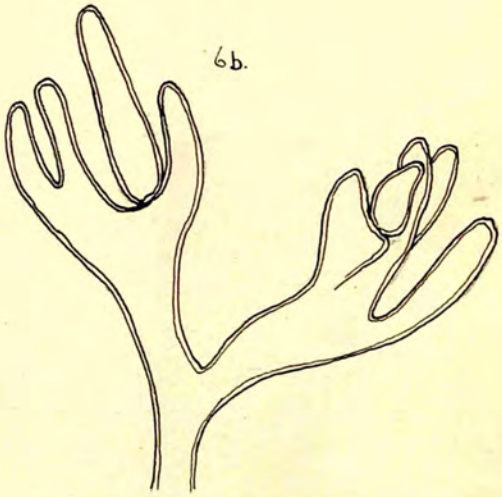
av. size 60μ tall.

Fig. 4a.

5μ diam

4b.

6b.



(e) A detailed description of a chytrid allied to Pleolpidium inflatum Butler.

Among the species of the chytridiaceous genus Pleolpidium discovered by Butler (1907) infecting Pythium intermedium, one was described which differed from the others in having bi-flagellate zoospores. The species was kept in the same genus and named Pleolpidium inflatum pending the discovery of the resting spores although, as Butler added, "the two cilia separate it widely from the other species". Since this first description there has not been any other record of the occurrence of this species, so far as I can ascertain, until the present appearance of a very similar one.

The parasite effects its entry into the hyphae of the host by means of zoospores which pierce the wall. The protoplasm then passes in and mingles with that of the host and is indistinguishable from it. The sporangia of the parasite are formed within the host wall, inside what presumably would have been a sporangium of the host. The parasitic sporangium completely fills the host sporangium and its wall is intimately fused with that of the host. The parasite causes little change in the form of growth of the host apart from occasional hyphal swellings and a difference in the shape of the sporangium.

The parasite appeared during an investigation of the water

moulds of the Hogsmill River in January 1938 on a mould on tomato and grape skins in three quite separate cultures kept in different places. The tomatoes and grapes had been used either as bait in the river or for re-inoculation experiments in the laboratory. After the tomatoes and grapes had broken up, the skins were kept from November to the end of January in glass jars with frequent changes of tap water and examined at intervals to ascertain whether any other moulds had appeared. During the preceding fortnight there had been much Pythiomorpha gonapodyides on the grape. When the parasitic sporangia were noticed portions of infected skin were transferred to quaker oat agar in Petri dishes, and a good growth of the parasitised fungus was obtained. The host when isolated was found to be a Phytophthora species.

DESCRIPTION OF PARASITISED HOST.

On the fruit skins the hyphae were fine (2.5μ diam.) and the sporangia smaller than in culture and pear shaped (30μ long). There was little hypertrophy of the host hyphae. On quaker oat agar the hyphae were much coarser (up to 8μ diam.) and often more swollen below the sporangium. (Fig. 1.) In both cases the hyphae were non-septate except in the older parts. Parasitised sporangia were produced sparingly on quaker oat agar medium but when the culture was flooded with water innumerable sporangia developed (600-800 per sq. mm.). They varied very much in shape and size being pyriform or oval in fresh cultures (Fig. 2.) and oval or spherical in older cultures . (Fig. 3.) (variation

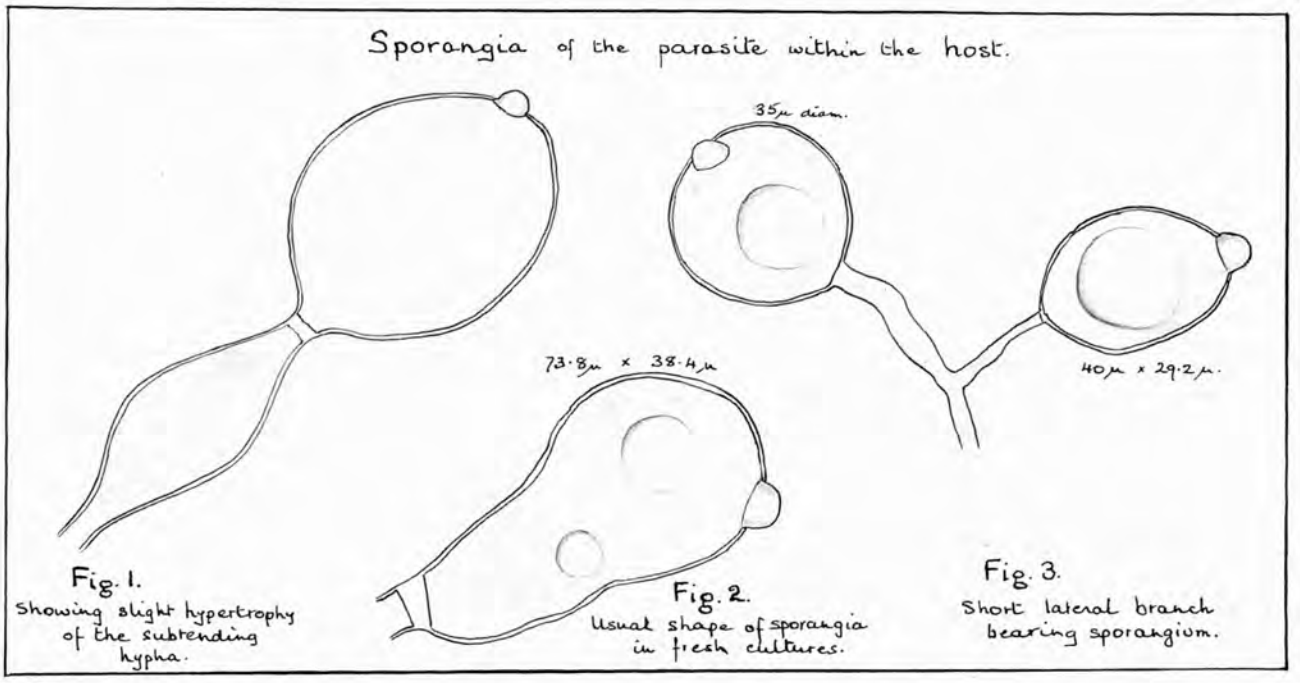


Fig. 1.
Showing slight hypertrophy of the subtending hypha.

Fig. 2.
Usual shape of sporangia in fresh cultures.

Fig. 3.
Short lateral branch bearing sporangium.

25μ- 74μ in length 18μ - 48μ in breadth, the usual size being about 38μ x 32μ.) (Fig. 4.) The sporangia were always terminal on the main hyphae or on short lateral branches. No intercalary ones were ever observed and there was no sympodial growth or proliferation through empty sporangia. The wall of the sporangium was thick, about double the thickness of a normal Phytophthora sporangium and at the base delimiting it from the supporting hypha it formed sometimes a thick plug (5μ deep) (Fig. 11.) or protruded as a hemispherical swelling into the sporangium, (Fig. 5.) or in a few cases appeared double. (Fig. 6.) The

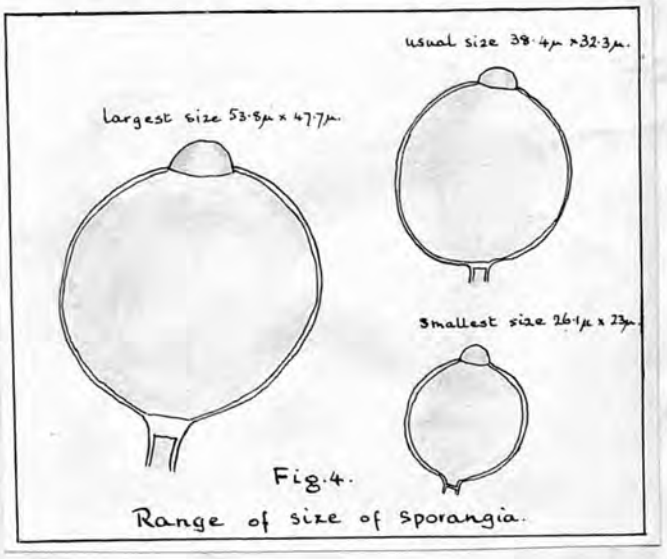


Fig.4.
Range of size of sporangia.

common wall gave a pale purplish red colour with chlor-zinc-iodide. The inner wall of the sporangium of the parasite could not as a rule be distinguished from that of the host in any part, but in one isolated sporangium in a culture that had become partially dried the entire parasitic sporangium with papilla had contracted within the host sporangium and it was outlined by a thin membrane. (Fig. 7.) The papilla was single in most cases though there were several examples with two (Fig. 8.) and a few with three, papillae. The papilla was usually

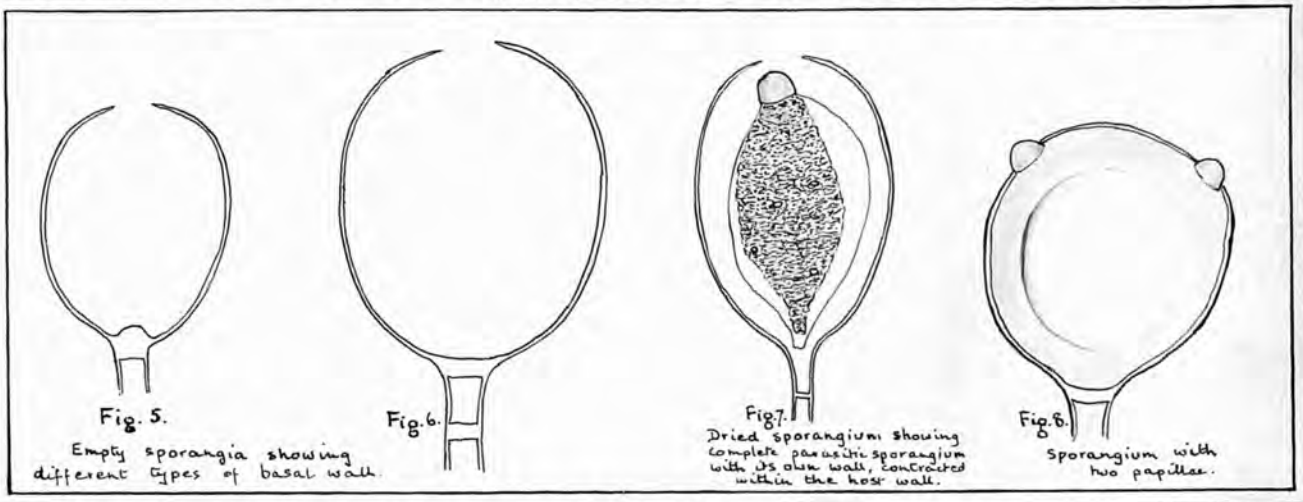


Fig. 5. Empty sporangia showing different types of basal wall.

Fig. 6.

Fig. 7. Dried sporangium showing complete parasitic sporangium with its own wall, contracted within the host wall.

Fig. 8. Sporangium with two papillae.

terminally placed or was occasionally slightly lateral and was hemispherical in shape arising so abruptly from the general contour of the sporangium as to give the appearance of being stuck on. It stained deeply with cotton blue in lactic acid. The protoplasm of the hyphae and young sporangia was granular and there was nothing to distinguish that of the parasite and host. Soon after the sporangium was delimited the protoplasm became hyaline with a large central vacuole (or occasionally several vacuoles). (Figs. 1 and 2.)

DEVELOPMENT OF SPORANGIUM OF THE PARASITE AND EMISSION OF ZOOSPORES.

The development of the parasitic sporangium at the end of a host hypha and the emission of zoospores from several sporangia was observed in hanging drop cultures. (Fig. 9.) The end of a

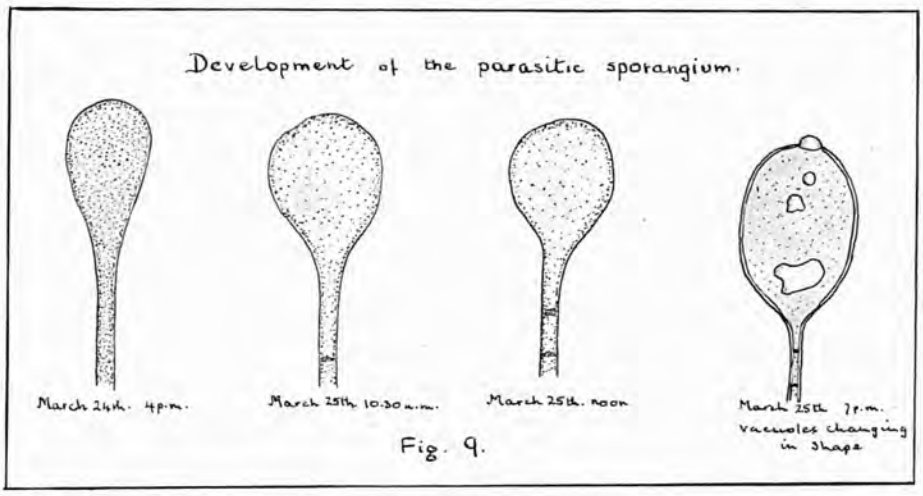


Fig. 9.

hypha radiating from a portion of an agar culture began to swell about four o'clock one afternoon. By ten thirty the next morning the swelling was about four times the diameter of the hypha and spherical in shape and was cut off by a transverse wall. The protoplasm which had been granular now became hyaline and vacuoles appeared which changed in shape and moved about. The sporangium reached its full size by the evening but the papilla did not appear at once and the growth of it was not observed. In most sporangia when fully developed there was a large central vacuole. Upon the addition of fresh water the central vacuole dispersed leaving the protoplasm quite clear except occasionally

for a few small dark granules clustered in the centre. (Fig. 10.)

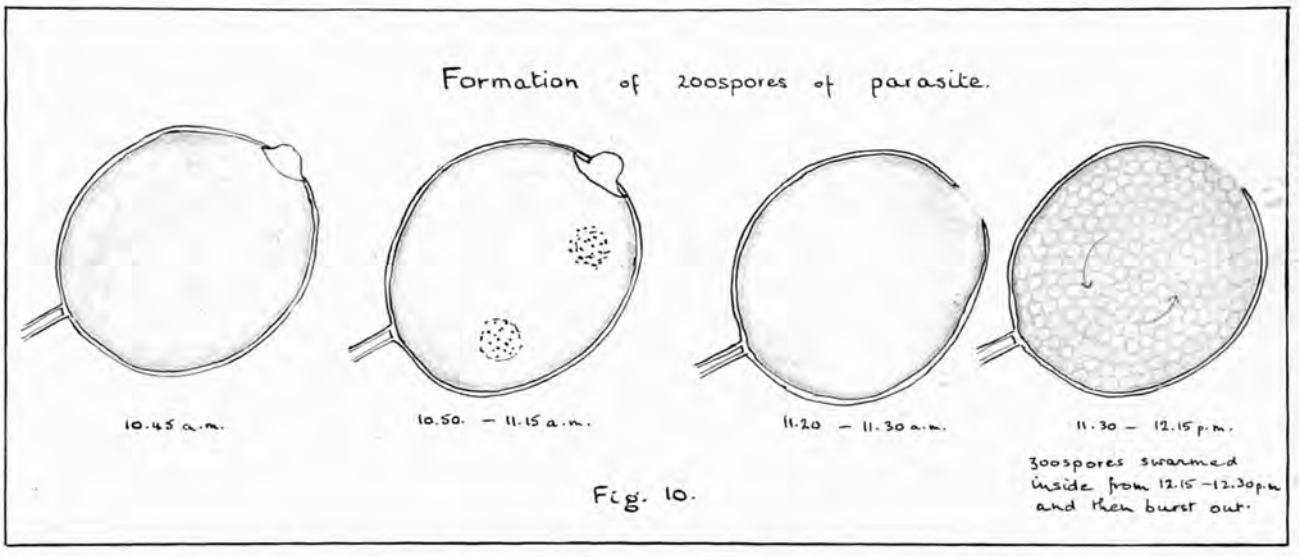


Fig. 10.

Then the protoplasm was seen to be dividing up into what looked like zoospore initials but in ten minutes this appearance had vanished, the protoplasm becoming hyaline once more. It remained so for about half an hour and then the papilla suddenly vanished. It went so quickly that it was not observed whether it dissolved into the surrounding water or whether it was drawn into the sporangium but the space in the wall which it had occupied could be seen quite clearly. Soon after this the protoplasm presented a fine reticulate appearance and it was soon seen that the zoospores were being formed but in this case the initials were much smaller (about a quarter of the size) than those seen earlier on. (Figs. 10 and 11.) When the zoospores were quite clearly defined (in about $\frac{1}{2}$ - $\frac{3}{4}$ hour) a heaving movement commenced and gradually the contents began to move round in an anti-clockwise direction. This movement, very slow at first, gradually became more rapid until the zoospores were swirling round at

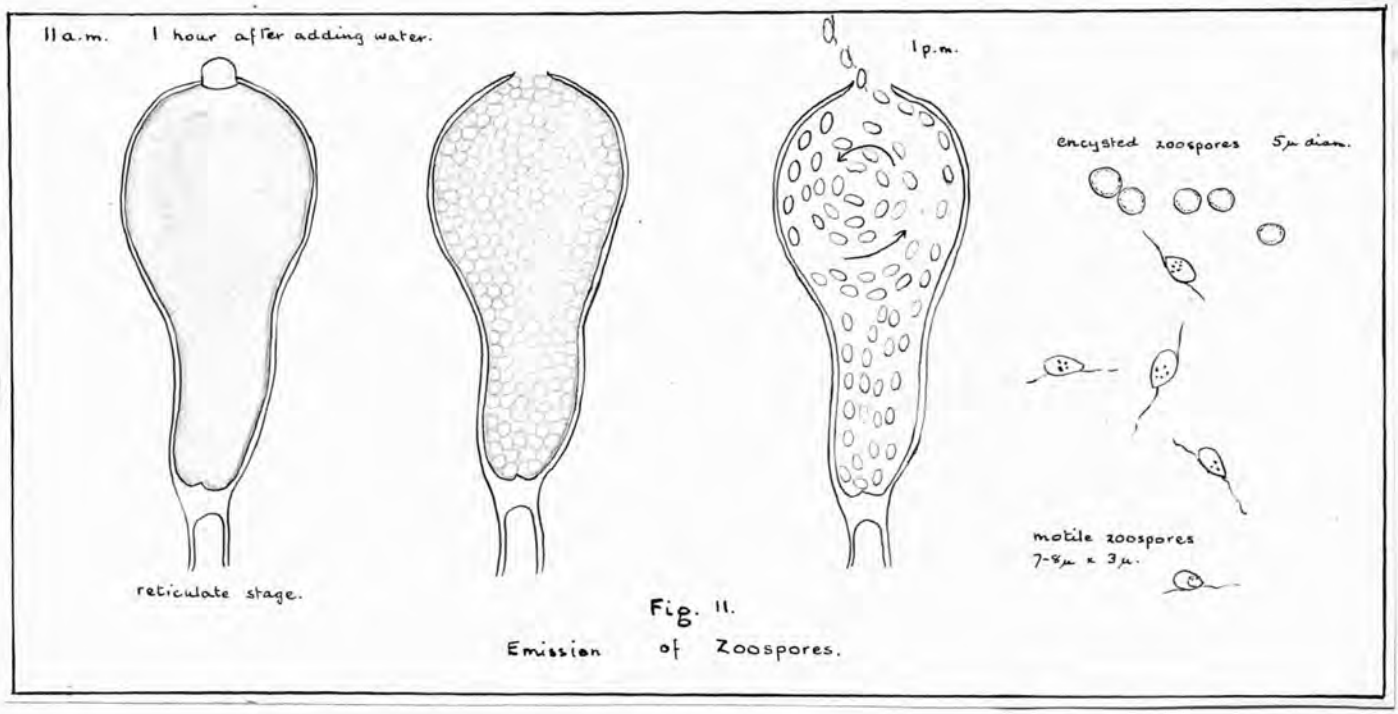


Fig. 11.
Emission of Zoospores.

a great speed. The swarming continued for about ten minutes until suddenly the membrane across the pore left by the papilla burst, and out shot the zoospores one by one at a tremendous speed. The sporangium took some minutes to empty and in spite of the large number (estimated at 1,500 - 2,000 in a medium sized sporangium) it was very rarely that any zoospores were unable to escape. (It is probable that, owing to the adverse conditions in a hanging drop culture, the times given are somewhat longer than those that would occur in a Petri dish or in nature.) The <u>zoospores</u> (Fig. 11.) were small (5μ - 8μ long) pear shaped with two flagella the slightly longer one directed anteriorly. The protoplasm was clear except for a few bright granules placed centrally or nearer the pointed end. The zoospores swam for twenty four hours or more if they did not reach suitable hyphae to infect. If however the culture was young they were soon seen hanging in rows on the young hyphae.

Those which settled down in the water rounded off and encysted. In becoming attached to the hyphae they seemed to hang on by means of the flagella, the pointed end of the zoospore being directed to the hypha. In one case penetration was observed. (Fig. 12.) The zoospore after attaching itself gradually

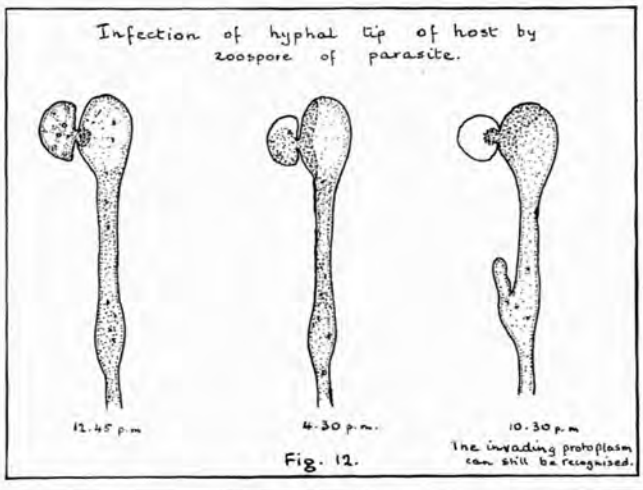


Fig. 12.

became flattened against the hyphal wall until it was hemispherical in shape. A slender tube was put out which pierced the wall and then the contents of the zoospore, now granular all over, passed gradually in taking about twelve hours to do so in a hanging drop culture. At first the penetrating protoplasm could be seen distinctly but soon it became indistinguishably intermingled with that of the host. No resting spores were found either on tomato skins or in agar cultures even after two months.

INOCULATION EXPERIMENTS.

Altogether five inoculation experiments were successful. The hosts used were (1) the original host isolated from its

parasite (2) Pythiomorpha gonapodyides, a culture from Mrs. Topping. Portions of agar were cut from the host culture and put into water and left for a day or two until a radiating hyphal growth had formed. The portion was then halved and to one half was added either a portion of agar culture already parasited or the zoospores of the parasite only, the other half acted as a control. The water was changed at intervals.
The first evidence of activity on the part of the parasite was the production, or increased production of normal sporangia of the host giving off normal zoospores, the controls showing no such production or increase. The second evidence was the production of parasitised sporangia in gradually increasing numbers.

Inoculations of original host.

Experiment 1.

March 3. Portions of host mycelium on agar in water.

March 10. Radiating hyphae, no reproductive organs,
Zoospores of parasite put in.

March 12. Normal sporangia on host, none on control.

March 15. Normal and parasitised sporangia on host:
control with a few normal sporangia.

March 30. Host covered with masses of sporangia of parasite.

Experiment 2.

March 20. 11.a.m. Portions of host mycelium on agar put in water
with parasitised mycelium on agar.

March 20. 5.p.m. Numerous normal sporangia on host:

March 22. none as yet on control

March 24. Parasitised sporangia on host:
control with a few normal sporangia.

Experiment 3.

March 24. Portion of control from Experiment 2 put in water with zoospores of parasite.

April 1. Parasitised sporangia produced:
control with a few normal sporangia.

Inoculations of Pythiomorpha gonapodyides.

Experiment 4.

March 18. Portion of agar with mycelium in water and parasitised mycelium.

March 24. Parasitised sporangia very numerous:
control, only sex organs.

Experiment 5.

March 15. Portion of agar with mycelium in water.

April 20. Radiating hyphae, no sporangia.
Fresh water added and portion of parasitised mycelium.

April 23. Parasitised sporangia on host. No control.

There were several inoculation experiments made on the host and one on Pythiomorpha gonapodyides which induced no parasitism. This may be due to reduced vigour of parasite or of its zoospores or reduced vigour of hosts.

Inoculations were also tried on Rhipidium europæum on tomatoes and on Rhipidium americanum on ash twigs and no parasitism appeared.

As the parasite disappeared during the following vacation it was not possible to test its pathogenicity for Pythium intermedium the host on which Butler found the similar parasite. Therefore it was not possible to determine whether the present species was identical with Pleolpidium inflatum.

CONCLUSIONS.

It is clear that the present parasite is not very specialised as it is known to attack two different genera of pythiaceous fungi. It is therefore quite possible that it might also parasitise Pythium species and therefore be identical with the original species. The points of difference noticed in comparing it with Butler's description are :-

(1) Host. Apart from the fact that it was found only on Pythium intermedium, Butler also noted that only soil species of Pythium were attacked and that an aquatic Pythium species bearing a parasite was never seen. The present parasite has so far been found only on Phytophthora and Pythiomorpha species and only on aquatic ones.

(2) Hypertrophy. The excessive hypertrophy of supporting hyphae described as due to Pleolpidium inflatum was not found in the present case: only a few supporting hyphae were slightly swollen

(3) Size of sporangium. The diameter of the largest Butler noticed was 85μ. The largest here was 74μ. This difference is not so important because the size of the sporangia is very

variable in both cases and slightly different conditions such as temperature (Butler worked at Antibes) might make a considerable difference in the activity of both host and parasite.

(4) Zoospores. The zoospores in the Pythium parasite were of a rather more kidney shape than the pear shape prevailing in the present cultures.

There is little doubt that the parasite described here is either identical with or very closely to Pleolpidium inflatum (Butler) and under ordinary circumstances one would have little hesitation in giving it that generic name. The nomenclature of Pleolpidium inflatum is however in an unfortunate position for two reasons. One is that Butler's original naming was only provisional owing to the fact that the zoospores were biflagellate and that no resting spores were encountered whereas other members of the genus, though very like Pleolpidium inflatum in other respects, were uniflagellate and produced resting spores. The flagellation of the zoospore is now considered to be an important diagnostic feature and therefore on these grounds the provisional name of P. inflatum cannot be retained for Butler's species. In addition the original naming of the genus Pleolpidium by Fischer has fallen into

disfavour. Sparrow (1938) considers that in the segregation of the members of Cornu's original genus into two genera the name Rozella should have been kept for the species now called Pleolpidium. On these grounds then it seems that the generic name Pleolpidium for Butler's fungus should be discarded and another generic name obtained which would include it and the present species.

The family which contains the forms most nearly related to the two under consideration is the Woroninaceae, which includes four genera (Fitzpatrick 1930 and Gaumann 1925) viz:- Pseudolpidium, Olpidiopsis, Rozella (Fischer's naming- see Sparrow 1938) and Woronina. The features exhibited by members of this family are:-

1) Thallus plasmodial and mingling freely with the protoplasm of the host and not producing a mycelium.
2) Parasitic and completely within the host at all stages.
3) Sporangia multispored.
4) Zoospores laterally biflagellate.
5) Thick walled resting spores in the host.

The two fungi under consideration show all these features except (5). They differ from the other genera however in that the sporangium completely fills the hypha of the host and its

wall is fused with that of the host and is undistinguishable. from it. In the four genera named the sporangia lie loosely in the host hyphae. If this feature constitutes a generic difference then a new genus must be formed to house these two nameless species. The question remains as to whether this can be done while the resting spores are still unknown.

If the parasite produces no resting spores it must continue its life either by reinoculation of the host or by prolongation of the zoospore stage. The former case seems to be more probable although more precarious. It was noticed in inoculation experiments that the vigorous hyphae were soon covered with swarms of attached zoospores. If however the host is as extensively parasitised in nature as it is in culture, being almost entirely transformed into parasitic sporangia, no such re-inoculation would be possible. In this connection it is interesting to note that in the inoculation experiments the introduction of the parasite seemed first to induce the production of normal host sporangia. This would ensure the provision of new young host plants for the subsequently produced parasite zoospores. Fischer (1882) observed instances in the case of Rozella "in which the presence of the parasite did not interfere with the normal life of the host even to the extent that normal sporangia and zoospores were formed and it was only later on when secondary sporangia were forming that the parasite gained the upper hand". This seems to be a similar phenomenon although Fischer did not attach any

significance to it.

If there is a prolongation of the zoospore stage it may be either as a cyst or in the active state. Many of the zoospores were seen to encyst and many continued their activity for twenty four hours without abatement. Their subsequent history was not followed but it has often been observed that among the bacterial colonies on bait from the stream there are numerous small zoospore-like creatures strongly resembling those of Chytrids. It is probable therefore that water moulds both saprophytic and parasitic may be able tp exist for considerable periods in that stage.

Note. Since the thesis has been prepared some doubt has been cast on the naming of Mrs. Topping's isolation of Pythiomorpha gonapodyides. Mr. Ashby has examined it and suggested it that it might be Phytophthora megasperma (Drechsler). The matter is still being investigated.

Literature cited.

Butler, F.J. 1907. Mem. Dept. Agric. India. vol.I. no.5.

Fischer, A. 1882. Jahr. fur Wiss. Bot. von Pringsheim 13.

Fitzpatrick, H.M. 1930. Phycomycetes.

Gaumann, E.A. 1925. Comparative Morphology of Fungi. Translated dy Dodge 1928.

Sparrow, F.K. 1938. Mycologia vol. 30.

THE PRODUCTION OF CONIDIA IN THE GENUS *PHYTOPHTHORA*

BY

G. M. WATERHOUSE, B.Sc.,
Royal Holloway College

REPRINTED FROM THE
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY
VOL. XV, PARTS III AND IV, 21 MARCH 1931

CAMBRIDGE
AT THE UNIVERSITY PRESS
PRINTED IN GREAT BRITAIN

[Reprinted from the
TRANSACTIONS OF THE BRITISH MYCOLOGICAL SOCIETY
Vol. XV, Parts III and IV, 21 March 1931.]

THE PRODUCTION OF CONIDIA IN THE GENUS PHYTOPHTHORA.

By G. M. Waterhouse, B.Sc., Royal Holloway College.

The object of the investigation was to examine the influence of various conditions on the production of conidia in the genus *Phytophthora*. The species investigated were:

P. Cactorum, *P. cryptogea*, *P. Fagi* } Temperate.

P. Colocasiae, *P. palmivora* (= *P. Faberi*), *P. parasitica* } Tropical.

Material. Cultures on potato agar, ground Quaker-oat agar, bean juice agar and Knop agar were kept in both Petri dishes and test tubes, and temporary cultures in water were made as follows: small squares of agar infected with the mycelium were removed from slopes or plates with a sterile scalpel and transferred to tap or distilled water in watch glasses or small specimen tubes.

Method. In some cases the conidia were counted to determine exactly the actual increase during the period of the experiment, and for this purpose cultures on bean agar were used exclusively as this medium is more transparent than Quaker-oat or potato agar. A surface section was taken with a razor and the conidia were counted *in situ* by means of a squared micrometer eyepiece. Very few conidia fell off during the operation, so that a fairly accurate count was obtained and the number per square millimetre could be calculated.

The cultures were kept either in an incubator at 22° C., or on the laboratory bench at 15°–20° C., and the following conditions were investigated: (*a*) medium, (*b*) water, (*c*) air, (*d*) light, (*e*) temperature, (*f*) age of culture.

(*a*) *Medium.* The nature of the medium on which the fungus is growing affects the conidial production to a considerable extent. Most of the species of *Phytophthora* hitherto examined have been found to grow quite well in culture on solid media, as parasites on living tissue, or as facultative saprophytes on bean, oat, potato and corn agar; and to produce conidia to a greater or less extent. Exceptions however have been reported by Pethybridge (1913) in *P. erythroseptica*, and by Coleman (1910) in *P. Arecae*, where conidia were usually absent in culture. A similar lack of conidia in culture was found by Wormald (1919) in *P. Cactorum*, but later they were obtained quite readily

in this species by Rose (1924), by Rosenbaum (1917), and by other workers. They were also obtained abundantly during the present investigation. The most favourable medium for conidial production is different for each species but in general these reproductive organs are never so numerous on nutrient media as on the host plant or on living tissue. *P. Syringae* appears to be an exception to this rule, as Wilson (1914) stated that the conidia of this species are unknown in nature. A similar lack of conidia on the host was noticed by Pethybridge (1913) in *P. erythroseptica*, whereas they occur in watery extracts. Jones, Giddings and Lutman (1912), studying the effect of different kinds of media on *P. infestans*, showed that the fungus made a more favourable growth on a medium which gave a neutral reaction, and that it was more sensitive to an alkaline medium than to an acid one.

In the present investigation all the species except *P. cryptogea* produced conidia freely on the media used, cultures on bean and Knop agar giving them most abundantly. *P. cryptogea* did not form conidia on bean or Quaker-oat agar at 22° C. This is a result comparable to that obtained by Robinson (1915) investigating an unknown species on asters (probably *P. cryptogea*).

(*b*) *Water.* All workers have agreed that moist conditions are essential for the production of conidia. McAlpine (1910), for example, stated that he obtained conidia of *P. infestans* on potato tubers under moist conditions, and since that time various workers have made similar observations for other species. Vowinckel (1926) determined the actual moisture content necessary, and showed that unless there was a 73 per cent. saturation on the leaves or a 64 per cent. saturation on the tubers of a potato plant, no conidia of *P. infestans* would be produced.

Even if conidia are readily formed under these moist conditions, the presence of excess water materially increases their abundance. Thus Pethybridge and Lafferty (1919) found that if tissues infected with *P. cryptogea* "are allowed to stand in water many more spores are formed." Leonian (1925) requiring abundant conidia for examination grew his various species actually in water. This increased production is also evidenced in nature by the increased prevalence of the parasite during the more rainy periods, obviously due to an abundance of conidia, coupled no doubt with an easy production of zoospores. This has been described for *P. Phaseoli* on bean pods by Clinton (1906), for *P. parasitica* on castor oil by Dastur (1913), and on rhubarb by Godfrey (1923), and by others.

Some species are so dependent upon moisture for the formation of conidia that they will not produce them until growing completely in water. Thus Pethybridge (1913) obtained abundant

conidia of *P. erythroseptica*, which were otherwise rare, in watery extracts of soils, and in rain water, to which a portion of infected agar or tuber had been added. In the same way Coleman (1910) obtained no conidia of *P. Arecae* in culture until portions were transferred to water, and Klebahn found the same treatment was required for *P. Syringae*. Robinson (1915) obtained the conidia of the species on asters by this method when other methods failed.

The results of this investigation are in accordance with those obtained by previous workers. Conidia of *P. Cactorum*, *P. palmivora*, *P. Colocasiae*, *P. parasitica* and *P. Fagi*, which are obtained readily on solid media, showed a considerable increase in numbers when the fungus was grown in water. Thus portions of an agar culture, where the mycelium was one day old and showed no conidia, produced them in abundance after twelve hours floating just on, or just under, the surface of the water. The numbers obtained by counting the conidia on portions in water, and on the controls on solid media, are shown in Table I.

Table I. *Cultures of* P. Cactorum *on bean agar at 22 ° C.*

Number of conidia per square millimetre		
At beginning of observations	After one night in water	After one night left on solid medium
1·5	4·0	1·75
0·0	9·3	4·9
0·48	26·5	13·5 (2 nights)
18·4	65·5	22·6 (2 nights)

Increased production in water was also shown by the number of conidia formed on each sympodium. Thus in *P. Fagi* there was an average of four to six conidia on conidiophores growing on solid media, and six to twelve on those growing in the water. Again with *P. Cactorum* there were never more than five conidia per conidiophore on solid media, but generally more in the water.

In accordance with the observations of other workers it was found that *P. cryptogea* did not produce conidia on solid media under normal conditions. Growth in water was therefore tried but the methods which had succeeded with other species gave no results here, periods of one, two and three days at 30° C., 22° C. and 16°–19° C., both in the light and in the dark being tried. Following an observation by Robinson (1915) that conidia of his species which closely resembled *P. cryptogea* were obtained after sixteen to twenty-one days in water, longer periods at 22° C. were tried, the water being changed periodically to keep down bacteria and to prevent accumulation of staling products. A few conidia appeared after fourteen days' immersion. One culture on Quaker-oat agar in water showed several after five days, and changing the culture to fresh water caused many

more to be produced after two more days. It is probable that conidia could be obtained after still shorter periods of immersion under more favourable conditions.

(*c*) *Air.* It has been generally observed from the time of de Bary that conidia are usually found on the surface of the host; the internal hyphae making their way out into the air before producing conidiophores. An exception to this was noted by Jones, Giddings and Lutman in 1912 who state that the conidia of *P. infestans* may be borne internally. Wormald (1919) showed experimentally that the conidia require air for their formation. He kept cultures of *P. Cactorum* in a closed vessel and conidial formation ceased. The only exceptional species appears to be *P. erythroseptica*, which was found by Pethybridge (1914) to produce conidia of an "essentially aquatic type," none being formed on aerial hyphae. It is assumed that oxygen is the necessary constituent of the air.

Definite experiments were carried out to ascertain whether the hyphae produce more conidia at the surface of the water than below it. Portions of agar cultures of *P. Fagi* were placed in tap water in small specimen tubes. Some portions were allowed to float on the surface, others were completely submerged. The cultures were kept on the bench.

Table II. *Production of conidia of* P. Fagi; *in cultures* (1) *floating on,* (2) *submerged in, water.*

Medium	Conditions	Time left	Floating	Submerged
Quaker-oat agar	Dark	1 day	Many conidia	Few
Bean juice agar	Light	1½ days	,, ,,	,,
,, ,,	Dark	2 ,,	13 per sq. mm.	11 sq. mm.
,, ,,	Light	2 ,,	42 per sq. mm.	0
Quaker-oat agar	,,	3 ,,	Abundant	Very few

Similar results were obtained with cultures of *P. Colocasiae*, where portions of agar were taken from the extreme edge of the radiating growth of the mycelium, which was consequently very young. Conidia were numerous on the floating portions after one night in water, but none appeared on the submerged even after five days. Again with *P. palmivora* at bench temperature the following results were obtained. After three days the floating portions bore twenty-seven conidia per square millimetre and there were six to eight on each conidiophore; the submerged portions bore twenty-five per square millimetre, with five to six on each conidiophore.

These experiments show that the conidial production is considerably reduced if the air supply is inadequate. If boiled water had been used instead of tap water, which contains some dissolved air, the numbers would probably have been reduced

still further, even perhaps resulting in the complete cessation of formation. All the results therefore point to the fact that air (*i.e.* oxygen) is a necessary factor for the formation of conidia.

The question, however, arises whether the non-production of conidia inside the host plant is really due to lack of air and whether the formation when the hyphae reach the exterior is due to its acquisition. The intercellular spaces in which the hyphae run are full of air and yet no conidia (except in one species) are formed there, not even the few that might be expected in a less adequate supply of air. Again the fact that the atmosphere of the leaf is moister than the air outside is another apparent contradiction of the conditions governing the process; but further consideration reveals the possibility that the surface film of moisture on the leaf is enough to cover the emerging hyphae. The important factor may therefore be the fresh water and not the air supply.

(*d*) *Light.* Previous investigations on light as a controlling factor in the production of conidia show rather varied results. Vowinckel (1926) working with *P. infestans* and Butler (1913) working with *P. Colocasiae* claim that light has no effect. On the other hand both Gadd (1924) and Reinking (1923) noticed that it had some influence on *P. Faberi,* Reinking finding fewer conidia in the dark. Coleman (1910) could obtain no conidia for five weeks in cultures of *P. Arecae* in darkness, but they were formed in two days in light. Dastur (1913) put forward the suggestion that alternating light and dark is essential for conidial production in *P. parasitica*: cultures kept always in the dark being sterile. It is of interest to note that in nature conidia are most frequently produced during the night and observed in the early morning.

The majority of the cultures used in the present investigation were kept in an incubator and were thus in more or less continuous darkness, except when the incubator was opened for the examination of the cultures. All the species formed conidia quite freely under these circumstances. Thus it appears that for the species examined full daylight is not essential for the actual formation of these reproductive organs, although it may have some effect on the extent of the production. Experiments were therefore carried out to ascertain if light would increase or decrease the numbers. Portions of agar infected with *P. Fagi* were used and when placed in water produced abundant conidia both in the light and in the dark at room temperature. Counting showed that sometimes there were more in the light (forty-two per square millimetre) than in the dark (twelve per square millimetre), sometimes there were more in the dark (eleven per square millimetre) than in the light (none per square millimetre). The experiments were not extended to other species but it was

noticed that conidia were always quite numerous in the dark at 22° C. Though further experiments are desirable, it appears doubtful whether light can be considered as one of the essential factors in conidial formation, although sometimes it may have an effect on the relative abundance.

(*e*) *Temperature.* Temperature affects conidial production in the same way that it influences other physiological processes, by acting as a limiting factor. The limits beyond which no sporangia are produced are not the same in the different species, and the optimum temperatures are also different. But there are indications that species found in temperate regions, *e.g. P. infestans* and *P. Cactorum*, have lower optima than tropical ones, *e.g. P. Faberi* and *P. parasitica*. Thus the optimum temperature for conidial production in *P. infestans* was found to be 16°–18° C. (Jones, Giddings and Lutman (1912)), and these workers also noted that no spores were produced below 10° C. or above 23° C. The optimum obtained by Vowinckel (1926) was 19°–22° C., and by Melhus (1915) 22°–25° C., but McAlpine (1910) obtained conidia of this species under special conditions at 27° C. Rose (1924) showed the optimum for *P. Cactorum* to lie between 10° C. and 20° C., and Uppal (1926) used a temperature of 22°–23° C. for maximum production in *P. Colocasiae*.

With tropical species on the other hand the temperature requirements appear to be much greater. Reinking (1923) could obtain no conidia of *P. Faberi* below 20° C., the optimum lying between 27° C. and 30° C., and Tucker (1926) claimed that 27° C. was the optimum. Similarly Ashby (1929) found the optimum for *P. parasitica* to lie between 27° C. and 30° C. The fact that tropical species require a higher temperature for fructification is to be expected from a consideration of the natural conditions under which they grow and produce conidia.

No definite experiments have been carried out to ascertain the effect of temperature on conidial production, but during the investigation it was noticed that differences did exist in cultures kept at different temperatures. This effect was particularly noticeable in the case of *P. Fagi*. Cultures kept in the incubator at 22° C. showed no conidia after two weeks and it was thought that this species, like *P. erythroseptica* and *P. cryptogea*, did not produce them on solid media. Cultures eight days old were then kept on the bench as well as in the incubator; the first showed abundant conidia after three days, but those at the higher temperature were still sterile. This was not due to the fact that those on the bench were in the light because, as shown above, light has no effect on the production of conidia in this species. It was assumed that it was due to the lower temperature, and to confirm this the experiment was repeated with cultures in water.

Again it was found that very few conidia were formed at 22° C., whereas they were abundant at the lower temperature. It was interesting to note also that many more sexual organs were present at the higher temperature than at the lower.

In the other species there was not such a marked difference between the extent of conidial formation at the two temperatures, but there was a certain retardation in growth at the lower; the fungus took longer to produce conidia, although after this longer period they were as abundant as at the higher temperature. Transference of cultures to the bench probably caused a slowing down of the metabolic processes and a consequent increase in the time that must elapse before conidial production. This retardation is shown distinctly by measurements of radial growth at the two temperatures. Thus the extent of the mycelium of cultures of *P. Cactorum* on potato agar plates at 22° C. is 3·25–3·6 cm. after seven days; whereas at bench temperature the time taken for the same radial growth is eleven to twelve days.

(*f*) *Age of culture.* The length of time which elapses between the inoculation of the medium and the appearance of the first conidium varies considerably with the conditions. Under optimum conditions this period is reduced to a minimum, but no one so far has been able to state the precise nature of such conditions. The shortest periods recorded, however, may be taken as most nearly reaching that standard. These are set out in Table III.

Table III.

Author	Species	Time elapsing before conidial production
McAlpine (1910)	*P. infestans*	7 hours
Hotson & Hartge (1923)	*P. mexicana*	48 hours
Ashby (1929)	*P. parasitica* var. *nicotiana*	1–2 days
Hopkins (1925)	*P. parasitica* var. on cotton	24 hours
Gadd (1924)	*P. Faberi*	2 days
Robinson (1915)	(*P. cryptogea*?)	16–21 days

It is possible that some of these periods could have been reduced by different conditions.

The periods noted in the present investigations with cultures growing under as nearly optimum conditions as possible were:

Table IV.

Species	Time elapsing before conidial production
P. parasitica	1 day
P. Colocasiae	1 ,,
P. palmivora	1 ,,
P. Cactorum	3½ days
P. Fagi	1–2 ,,
P. cryptogea	5 ,,

It has been noticed that the period is not the same for different parts of the same culture, even when these are approximately the same age. For instance in an agar culture in a Petri dish the outer parts of the radiating growth of the mycelium seem to lag behind the central parts in the production of conidia. This was confirmed by counting the conidia in different parts. The dishes were ringed after each day's growth, so that the age of any portion of the culture could be ascertained. The rings were numbered from the centre outwards and the conidia were counted on the portions where the rings were the same age. The counts are recorded in Table V.

Table V.

Age of mycelium (days)	Position in dish	Conidia per sq. mm.
4½	Ring 1	1·5
4½	,, 3	1·6
7	,, 2	22·6
7	,, 4	14·4
7	,, 2	53
7	,, 4	24
8	,, 1	60
8	,, 2	22
8	,, 3	18
12	,, 1	107
12	,, 2	82
12	,, 3	53
12	,, 4	18
24	,, 3	196
24	,, 4	53

Presumably all the parts of the plate were under the same conditions of medium, temperature, humidity, light and air supply, and yet there is a considerable difference in the abundance of the conidia. This suggests that there is another factor or factors, influencing the production of conidia besides those already considered. The effect may be due to the more congested state of the mycelium at the centre, the part at the edge being more spread out and in this region still expending material in establishing itself on the medium. The fact that there are more conidia at the centre than at the outside does not admit the possibility that the accumulation of staling products, if occurring, is having much inhibitory effect, as is sometimes found in plate cultures.

Conclusion.

Many factors apparently affect conidial production to a greater or lesser extent. Temperature and moisture content are the most important and act as limiting factors, temperature preventing the formation both at the upper and lower limits, and water at the lower limit only, for apparently no amount of water

is great enough to check the growth. The medium also is important, the fungus showing a more favourable growth in certain nutrient cultures. It is not clear which constituents of the medium cause the greatest abundance of conidia, although it has been shown that the relative acidity or alkalinity has some effect. It is probable that the moisture content of an agar culture is important here, since a moister medium like bean juice agar gives more conidia than a slightly drier one like Quaker-oat agar. The fact that the fungus produces conidia more readily on living tissue, which always has a high water content, is in accordance with this suggestion. Air is necessary to a certain extent and its absence may sometimes limit the process but conidia can be produced even when the air supply is very limited. The action of light and darkness on the formation shows varied results, but usually it appears that light is not a limiting factor; but whether it can modify the production at all, and to what extent, requires further investigation.

The combined effect of these conditions does not explain completely the story of conidial production. Certain results are not apparently due to the action of any of them, and point to the possibility of other factors also at work. Among these might be included the state of maturation of the fungus and the density of mycelial branching and growth.

In conclusion I should like to thank Miss E. M. Blackwell, M.Sc., Botany Department, Royal Holloway College, at whose suggestion the investigation was started, for her helpful advice and criticism throughout.

Summary.

1. The effect of certain conditions on the conidial production in species of *Phytophthora* has been examined.

2. Temperature, humidity, and air supply act as limiting factors, the two latter stopping the process at the lower limit only. The presence of excess water more than doubles the number of conidia.

3. The nature of the medium regulates the production, its action probably depending on its moisture content.

4. The precise effect of light is still doubtful; its presence or absence does not appear to cause any great variation in some species.

5. The time elapsing after inoculation and before conidia are formed, *i.e.* the age of the culture, is influenced considerably by the above conditions.

6. The number of conidia per unit area depends on the density of mycelial branching.

LITERATURE CITED IN THIS AND THE PRECEDING PAPER.

ASHBY, S. F. Bud rot caused by *Phytophthora palmivora* Butler. West Ind. Bull. XVIII (1920), p. 62.

—— Strains and taxonomy of *Phytophthora palmivora* Butler (*P. Faberi* Maubl.). Brit. Myc. Soc. Trans. XIV (1929), p. 18.

DE BARY, A. Recherches sur le développement de quelques champignons parasites. Ann. des Sci. Nat. Bot. Sér. 4, XX (1863), p. 14.

BUTLER, E. J. and KULKARNI, G. S. Colocasia blight, caused by *Phytophthora Colocasiae* Rac. Mem. Dep. Agric. India, Bot. Ser. V (1913), p. 233.

CLINTON, G. P. Downy mildew of lima beans. *Phytophthora Phaseoli* Thaxt. Conn. Agr. Exp. Sta. Bull (1906), p. 304.

COLEMAN, L. C. Diseases of the areca palm (*Areca Catechu* L.). I. Koleroga. Ann. Mycol. VIII (1910), p. 591.

CURZI, M. L' eziologia della "cancrena pedale" del *Capsicum annuum* L. Riv. Pat. Veg. XVII (1927).

DASTUR, J. F. On *Phytophthora parasitica* Nov. Spec. Mem. Dept. Agric. India, Bot. Ser. V (1913), p. 177.

GADD, C. H. *Phytophthora Faberi* Maubl. Ann. Roy. Bot. Gard. Peradeniya, IX (1924), p. 47.

GODFREY, G. H. A *Phytophthora* foot rot of Rhubarb. Journ. Agr. Res. XXIII (1923), p. 1.

HOPKINS, J. C. Notes on the soft rot of Cotton Bolls in the West Indies caused by *Phytophthora*. Ann. Bot. XXXIX (1925), p. 267.

HOTSON, J. W. and HARTGE, L. A. A disease of tomato caused by *Phytophthora mexicana* sp. nov. Phytopathology, XIII (1923), p. 520.

JONES, L. R., GIDDINGS, N. J. and LUTMAN, B. F. Investigations of the potato fungus *Phytophthora infestans*. Vermont Agric. Exp. Sta. Bull. No. 168 (1912).

KLEBAHN, H. Krankheiten des Flieders. Berlin, 1909.

LAFFERTY, H. A. and PETHYBRIDGE, G. H. On a *Phytophthora* parasitic on apples which has both amphigynous and paragynous antheridia; and on allied species which show the same phenomenon. Sci. Pro. Roy. Dublin Soc. XVII (1922), p. 29.

LEONIAN, L. H. Physiological studies on the genus *Phytophthora*. Amer. Journ. Bot. XII (1925), p. 444.

MCALPINE, D. Some points of practical importance in connection with the life-history stages of *Phytophthora infestans* Mont. (de Bary). Ann. Mycol. VIII (1910), p. 156.

MELHUS, I. E. Experiments on spore germination and infection in certain species of Oomycetes. Univ. Wisconsin Agric. Exp. Sta. Res. Bull. No. 15 (1911), p. 25.

—— Germination and infection with the fungus of the late blight of potato (*P. infestans*). Univ. Wisconsin Agric. Exp. Sta. Res. Bull. No. 37 (1915), p. 1.

MURPHY, P. A. The morphology and cytology of the sexual organs of *Phytophthora erythroseptica* Pethyb. Ann. Bot. XXXII (1918), p. 115.

—— The bionomics of the conidia of *Phytophthora infestans* (Mont.) de Bary. Sci. Pro. Roy. Dublin Soc. XVI (1922), p. 442.

PETHYBRIDGE, G. H. On the rotting of potato tubers by a new species of *Phytophthora* having a method of sexual reproduction hitherto undescribed. Sci. Proc. Roy. Dublin Soc. XIII (1913), p. 529.

—— Further observations on *Phytophthora erythroseptica* Pethyb., and on the disease produced by it in the potato plant. Sci. Proc. Roy. Dublin Soc. XIV (1914), p. 179.

PETHYBRIDGE, G. H. and LAFFERTY, H. A. A disease of tomatoes and other plants caused by a new species of *Phytophthora*. Sci. Proc. Roy. Dublin Soc. XV (1919), p. 487.

PETHYBRIDGE, G. H. and MURPHY, P. A. On pure cultures of *Phytophthora infestans* de Bary, and the development of oospores. Sci. Proc. Roy. Dublin Soc. XIII (1913), p. 566.

REINKING, O. A. Comparative study of *Phytophthora Faberi* on coconut and cacao in the Philippine Islands. Journ. Agric. Res. XXV (1923), p. 267.
ROBINSON, W. "Black Neck" or wilt disease of asters. Ann. Appl. Biol. II (1915), p. 125.
ROSE, DEAN H. Leather rot of strawberries. Journ. Agric. Res. XXVIII (1924), p. 357.
ROSENBAUM, J. *Phytophthora* disease of Ginseng. N.Y. Cornell Univ. Agric. Exp. Sta. Bull. 363 (1915).
—— Studies of the genus *Phytophthora*. Journ. Agric. Res. VIII (1917), p. 233.
SZYMANEK, J. Contribution à l'étude du *Phytophthora infestans*, parasite de la pomme de terre. Ann. des Épiphyties, XIII (1927), p. 213.
TUCKER, C. M. *Phytophthora* bud-rot of coconut palms in Porto Rico. Journ. Agr. Res. XXXII (1926), p. 471.
UPPAL, B. N. Relation of oxygen to spore germination in some species of the Peronosporales. Phytopathology, XVI (1926), p. 285.
—— Toxicity of organic compounds to the spores of *Phytophthora Colocasiae* Rac. Journ. Agric. Res. XXXII (1926), p. 1069.
VOWINCKEL, O. Die Anfälligkeit deutscher Kartoffelsorten gegenüber *Phytophthora infestans* (Mont.) de Bary, unter besonderer Berücksichtigung der Untersuchungsmethoden. Arb. Biol. Reichsanst. für Land- u. Forstwirtsch. XIV (1926).
WORMALD, H. A *Phytophthora* rot of pears and apples. Ann. App. Biol. VI (1919), p. 89.
WILSON, GUY W. Studies in N. Am. Peronosporales. V. Review of the genus *Phytophthora*. Mycologia, VI (1914), pp. 54–83.

Abstract of Thesis to be presented for
M.Sc degree by G.M.Waterhouse.

STUDIES IN PHYCOMYCETES.

Part I The Water Moulds of the Hogsmill River.

A. The river itself has been examined and the following features reported on:-
1) the geological formation of the basin,
2) the volume and rate of flow and depth of water, these being correlated with the district rainfall,
3) temperature- general statement, no details,
4) chemical analyses given by the Thames Conservancy,
5) pH values which show a decrease from 7·2 in winter and spring, to 8·1 in summer.

B. A list of identified species of Phycomycetes (excluding Saprolegniaceae) is given together with their occurrence month by month.

C. The results of attempts to germinate resting spores is given. Only those for a species of Blastocladia were successful.

D. An account of a parasite (probably Pleolpidium inflatum, Butler.) including a description of the parasite, sporangia formation, zoospore liberation, penetration of the host by a zoospore, and the results of successful inoculation experiments on the host and on Pythiomorpha gonapodyides.

Part II The Production of Conidia in the Genus Phytophthora.

This is a paper (published in 1931) by the candidate. The conditions affecting conidia production i.e. medium, water, air, temperature, and age of culture were reviewed and the results of experiments investigating these factors were incorporated. These showed that:-
1) excess water more than doubled the number of conidia on solid media,
2) that conidia were produced more abundantly on floating than on submerged media,
3) light was found not to have any definite effect,
4) that temperate species e.g. P.Fagi had lower optimum temperatures as compared with tropical species.

Minimum readings for the time elapsing between inoculation and conidial production (i.e. under presumably optimum conditions) were given.
The state of maturation of the fungus and the density of mycelium were shown to have an effect on conidial production.

In addition there is another printed contribution quoted in support of the qualification for the degree.

This is a joint paper by E.M.Blackwell and G.M.Waterhouse (published in 1931) on the types of spores found in the genus Phytophthora, and their modes of germination. The candidate contributed the material extracted from papers by other workers, and also the tables in Section II. Investigation showed that water, oxygen, light, temperature, and maturation were the factors affecting germination of conidia. The results of relevant experiments by the candidate were added.

M.Sc. Thesis in Botany presented by G.M.Waterhouse B.Sc.
entitled
STUDIES in PHYCOMYCETES.

Statement showing how far the thesis embodies the results of the candidate's own research and observation, and in what respects the investigations appear to advance scientific knowledge.

Part I. The Water Moulds of the Hogsmill River.

The records here are all the result of the candidate's own research except 1) the geological summary which was deduced from the Geological Survey Sheet 270 and not from geological observations in the field, 2) the rainfall and volume of flow readings given by the Kingston Borough Sewage works, and 3) the chemical analyses from the Thames Conservancy.

No research work has ever been carried out on this river before so far as I have ascertained from the local councils within whose bounds the river is situated, and from searches among relevant scientific literature(except in the case of H.B.Guppy who studied variation in river temperature in 1894). Moreover very little work of the nature of that in the thesis has been carried out on rivers,most past recorded observations being confined to ditches, ponds, pools, and lakes. In one only of these (Lund in Denmark) has the investigation included records of the hydrogen-ion concentration, which is of importance in determining some of the species present.

Regular systematic surveys of the freshwater fungous flora are of value in advancing our knowledge of the freshwater biology of the British Isles and may prove of value not only scientifically but economically in connection with river pollution and the restocking of freshwater situations with fish. The reports of the Freshwater Biological Association of the British Isles show that our knowledge of inland waters is very scanty, and that a very small percentage has been investigated.

The species named were identified by the candidate from existing descriptions and the fact that some are not named specifically is indicative of the inadequacy of the Phytophthora-Pythiomorpha generic distinctions.

Attempts to germinate resistant spores are of value in view of the difficulty usually experienced in bringnig about germination and aids in the understanding of the conditions in nature which contribute to this phenomenon, thus bringing about the reappearance and spread of the species. In the case of Blastocladia Pringsheimii the results were useful in confirming the first complete life cycle known(Miss Blackwell's unpublished findings) with material from a source far removed from College grounds.

The parasitic species related to Pleolpidium inflatum Butler has not been previously recorded in the British Isles and no description has appeared since Butler's first account. The present record is of interest in that the host was a species of Phytophthora whereas Butler's isolation was parasitic on Pythium intermedium. The parasite was identified by Mr.Ashby from the description and figures.

Part II. The Production of Conidia in the Genus Phytophthora.

The conditions governing the production of conidia are important in view of the parasitic nature of the species of this genus and since the conidia are the reproductive organs responsible for the rapid spread of these parasites. The results of the effect of any conditions previously investigated were summarised from available literature as these results had not previously been gathered together for reference. In the confirmatory experiments carried out by the candidate systematic counts of conidia produced under the different conditions were made to give definite comparative values as this had not been done before. These values supplemented and confirmed the general statements made by other workers