

T H E S I S

Presented for the  
DEGREE of DOCTOR of PHILOSOPHY,  
UNIVERSITY of EDINBURGH

on

RESEARCHES on TWO DISEASES of ECONOMIC PLANTS  
caused by PHYTOPHTHORA SPECIES,

by

CHARLES E. FOISTER, B.A., Dip. Agric.,(Cantab.)

---

June, 1931.



TABLE of CONTENTS.A Disease of Atropa belladonna L.  
causing a Wilt and Root Rot.

	<u>Page.</u>
Introduction	1
Distribution	2
Pathology	3
Symptoms of the disease.	3
Economic Effect.	5
Microscopical Examination of Diseased Plants.	6
Isolation of Causal Fungus.	8
Pathogenicity.	10
The "Pinking" Phenomenon.	15
Infection of Potatoes.	20
Spread of the Disease.	22
Climate in Relation to the Disease.	27
Control of Disease.	30
Life History and Morphology	34
Cultural Studies.	34
Life in the Soil.	38
Longevity.	41
Review of other workers' results.	41

	<u>Page.</u>
Morphology	43
General Description.	43
(a) Mycelium	43
(b) Sexual Organs	44
The Amphigynous Antheridium	44
The Paragynous Antheridium	48
(c) Asexual Organs	49
Measurement of the fungus	54
(a) Mycelium	54
(b) Sexual Organs	54
(c) Asexual Organs	60
Physiology	63
Relation to Leonian's Physiological Key	63
Temperature Relations	64
Discussion of Results	69
Diagnosis	72
Addendum on Cytology	73

The WHITE TIP DISEASE of LEEKS

caused by

Phytophthora Porri nov.spec.

	<u>Page.</u>
Introduction	74
Historical Review	75
Distribution	78
Economics	79
Pathology	80
Symptoms	80
Microscopical Examination	81
Isolation and Culture	82
Pathogenicity (and Control)	88
Progress and Spread of the Disease	93
Morphology and Life History	98
Cultural Studies	98
Influence of Depth of Medium	101
Life in the Soil	103
Morphology	104
General Description	104
(a) Mycelium	104
(b) Sexual Organs	105
(c) Asexual Organs	108
Repetitional Diplanetism	108



	<u>Page.</u>
Measurement of the Fungus	111
(a) Mycelium	111
(b) Sexual Organs	111
(c) Asexual Organs	115
Physiology	117
Relation of Temperature	117
Relation of Light	119
Relation to Leonian's Physiological Key	120
Taxonomy	123
(a) Parasitology	123
(b) Type of Sexual Organs	125
(c) Measurements of Allied Species	129
Discussion	133
Diagnosis	135
SUMMARY	137
REFERENCES	141-146
PLATES	I - XXVIII.

A DISEASE OF ATROPA BELLADONNA L.

CAUSING A WILT AND ROOT ROT.

## I N T R O D U C T I O N .

---

In the last few years a disease has appeared in beds of cultivated Atropa belladonna which has increasingly become of economic importance. The symptoms differed from those associated with fungi and insect pests previously found to attack this plant.

The disease herein described was first recorded by Westerdijk and Van Luijk in 1920, as occurring in Holland (58), where they had studied it since 1918. No other record occurred till 1926, when Alcock ( 1 ) reported the disease as occurring in certain drug gardens in Edinburgh.

The writer was shown this disease in 1928, and as it proved of interest from pathological, taxonomic, economic and mycological points of view, was selected as part of the researches upon certain species of Phytophthora, a genus of fungi becoming increasingly distributed and dangerous to all classes of cultivated plants.

The writer is grateful to Messrs Duncan Flockhart and Company for supplying material and seeds.

DISTRIBUTION.

It has been noted that this disease of Belladonna has been recorded in Holland and Scotland. Often diseases of the lesser economic plants are not brought to the notice of Mycologists, especially as the observers are quite ready to ascribe the causes to factors of a non-organic nature, such as weather. Even should the growers record these diseases themselves, it is not always in periodicals readily accessible or likely to be noticed by Mycologists. To cover the ground prior to the time when abstracting journals were commenced, a certain amount of correspondence was made with various drug firms and trade organisations in this country and abroad. The names of these firms and Associations, particularly abroad, were kindly supplied by J. Rutherford Hill, Ph.C., Secretary to the Pharmaceutical Society of Edinburgh. In England, several firms had noted a wilt of Belladonna in their gardens before, but did not associate it with fungous attack. Its rareness and slightness did not cause these firms to investigate the disease. This state is quite unlike the disease area investigated in Edinburgh, where it is serious. The writer was fortunate in obtaining some slightly diseased material from one of those firms in England, and found in the decayed tissues the oospores of the fungus which herein is ascribed as the cause of the disease. Cultures/

Cultures were obtained from this material which in every respect resembled those made from the typical Edinburgh material. By enquiries to firms, etc., in countries where Atropa belladonna is grown as an economic plant, namely in France, Austria, Hungary, Czecho-Slovakia and America, it was found that the disease was unknown in those countries. Up to the present time, therefore, the disease is known only in Holland, Scotland and England.

#### PATHOLOGY.

##### Symptoms of the Disease.

The fungus causing this disease may attack the host from the earliest period of plant growth. When the seedlings are just appearing they may be killed off in patches, the young leaves turning yellow, wilting and then dying. In the field, a few may pull through, but the majority die, and it becomes necessary to resow, though not in the same ground. Westerdijk and Van Luijk (58) do not refer to this stage of the disease but Alcock (1) does, adding that the mycelium of the fungus may be seen as a mould in the dead patches.

The second stage of the disease is found in the plants approaching the maximum size attained in the first year of growth, the age varying from plant to plant. In these plants, from the end of July onwards till the end of October, the first sign of disease is a wilt of the leaves, followed by a wilt of the upper stems (Plate 1, figs. 1 & 2). The leaves and stems then turn yellow and ultimately die. If these plants are pulled up, they break in the region of the first inch of soil. This is because the stems there are rotten. By probing deeper, the stock and the roots will also be found rotten in advanced cases, while in others - particularly if still young - the rot will not have extended so far.

In the third stage, the second year plants which are quite large, and when healthy may be taken for shrubs, do not develop so many stems nor appear so robust as uninfected plants. The stems are not rotten and the cause is only found by digging up the stock. Then it is found that all the first year roots are thoroughly decayed, yet a few new and so far uninfected roots have been formed near the surface of the soil. These new roots are sufficient to keep the already formed stems and leaves alive but are not sufficient to allow additional growth. Here and there too, are found gaps in the rows. By digging in these gaps one discovers thoroughly decayed first year plants/



plants which have not been able to produce those few roots sufficient to keep them alive.

By a study of many affected plants it seems that the fungus enters the tissues from the soil and grows chiefly in the conducting system of the host, spreading later to the cortex and pith. On cutting open a wilted stem the tissues are found to be browned for a distance above the actual decay, being most prominent in the conducting strands. The brown discoloration also extends downwards into the roots, ultimately becoming general when decay sets in.

#### ECONOMIC EFFECT.

Both Alcock ( 1 ) and Westerdijk and Van Luijk (58) described symptoms similar to those above enumerated, but did not discuss the economic extent of the injury. To fully appreciate this, account must be taken of the commercial practice. To extract the drug Atropine, the leaves are collected at regular intervals for treatment. Later, the stems are collected for the same purpose and finally the stocks and roots. A certain proportion of the plants are/



are left for second year growth and the leaves, stems, stocks and roots again collected in the second year. Naturally, wilted leaves and stems do not yield the same drug requirements as the healthy tissue and where the stocks and roots are decayed, the loss is obvious. Second year roots of healthy plants are expected to average 18" in length and very often reach three feet, being thicker in proportion. But the diseased plants only yield roots of three to twelve inches in length, with a rare one as large as 18" and not as thick as the healthy roots (Plates 2 & 3). In very bad cases no yield is obtained. In an average season, when rainfall is moderately plentiful, diseased beds may have only a quarter the yield of a normal, healthy bed. The destruction of seedlings, too, causes more loss than is at first indicated. The re-sowing, if resorted to, necessarily causes delay in the production of the right sized plants and may even delay appreciable collection until the second season.

#### MICROSCOPICAL EXAMINATION OF DISEASED PLANTS.

In sections of diseased stems in slightly browned areas and in advance of this area, a great quantity of a large, non-septate mycelium is found both/

both intra and inter-cellularly (Plate 3, fig.6). In decaying tissue there is less non-septate mycelium this having died as the result of the entrance of secondary fungi with septate mycelia. The latter mycelia vary greatly in width and character, and prove, on growing in artificial culture, to be those of the common saprophytic fungi living in the soil and which really cause the decay of the tissues, already weakened by the first fungus, with the help of many putrefactive bacteria which also gain entrance. It is the presence of the mycelium in the conducting tissues particularly that causes wilt, by diverting the plant foods from the leaves. Later the rot, which is caused by the fungi and bacteria which follow the causal fungus, cuts off all food and water from the upper portions and death follows quickly.

In the decayed tissues are also found numerous dark spherical bodies or oospores with thick walls, enclosed in thin walled oogonia with an attachment which is recognised as the antheridium, typical of fungi belonging to the group Phycomycetes. These belong to the causal fungus and will be described later.

ISOLATION/

ISOLATION OF CAUSAL FUNGUS.

The causal fungus is not difficult to obtain in artificial culture. It was obtained first on Quaker Oat Agar medium by taking pieces of diseased tissues, preferably just in advance of the brown discoloration, after sterilising the exterior of the affected stems with some quick disinfectant, methylated spirits being used mostly. The advantage of methylated spirits is that it evaporates very rapidly, and so, beyond killing external fungi, has less chance of killing the internal fungus it is desired to culture. Other disinfectants such as a 1:1000 solution of Mercuric Chloride or Chlorine Water, although washed off with sterile water, nearly always seem to penetrate sufficiently to kill the very delicate fungus which causes this disease. All instruments are sterilised in the flame a second time after cutting open the stem externally disinfected, and the selected tissue cut out and transferred to the tube of medium as quickly as possible. In twenty-four hours the hyphae of the fungus are seen growing out from the tissue over the surface of the medium, and in three days the amount of growth is considerable. In one week the slope of medium is fully covered and there is plenty of aerial mycelium. On examining the fungus in this culture spherical/

spherical bodies apparently similar to those found in the decayed tissues of diseased Belladonna were found. At first the only contaminations were bacteria, but by sub-culturing the edges of advancing colonies these were soon eliminated.

The fungus can also be induced to produce certain fructifications called conidia or summer-spores, when slips of diseased tissue are placed as aseptically as possible into tubes containing various liquids, being held in place by a plug of cotton wool. These pieces can also be placed in petri dishes with some liquid and this method is more convenient for microscope examination. (Plate 4, fig.7). Ordinary sterilised water, tap water, (Edinburgh tap water is reasonably pure and even bacteria are rarely found in it), sterilised bog-water and a solution of one part of Potassium Nitrate in one thousand parts of sterile water can be used. As bog-water is exceedingly difficult to obtain, it may be substituted by water from a tub containing water-weeds, algae and the usual aquatic micro-fauna, this being boiled before use. Other chemicals than Potassium Nitrate can be used, as will be shown in the section on the Physiology of the fungus, but this chemical causes the outgrowth of the conidia to be more rapid and more abundant. The fact that these conidia are produced by the tissues in various liquids, and that the oospores are produced naturally in the decayed tissues, supports the contention that the fungus in culture/

culture is the same, since the latter produces oospores in culture and also conidia when pieces of the culture are placed in the same liquids as sufficed for the purpose with the tissues. Referring back to the conidia produced by the diseased tissue in liquid culture, it is possible to obtain the fungus in culture on the solid media by removing the piece of tissue from the tube or petri dish on to a flamed slide with a drop of sterile water, and examining this under low magnification tease off with flamed needles the fungal hyphae with the conidia. Once a mass is teased away, it can be picked up by a needle and placed in a tube of Quaker Oat Agar, on which the conidia germinate quite readily and as good a culture obtained in this way as by the direct tissue method. A single conidium can be separated in a similar manner and likewise placed in culture; this germinates and produces a colony but very much slower than when more than one is placed in the tube.

#### PATHOGENICITY.

On obtaining the oospore-producing fungus in culture, experiments to test not only that the fungus in culture was the same as that in the diseased/



diseased tissues, but also to test its ability to cause the disease became necessary, for it is a common experience that many fungi isolated from diseased plants are not the cause but the sequelae of some other causal agent.

The first infection experiment did not prove pathogenicity, but nevertheless suggested that a species of Phytophthora might be held responsible. Seedlings of Atropa belladonna only eleven weeks old (78 days) were planted in three pans containing soil, these having been sterilised in the autoclave at 20 lbs. for half an hour. There were fourteen seedlings to each pan. One pan (a) was left uninfected as a control, the second (b) was infected with the fungus isolated from diseased Belladonna plants, and the third (c) infected with a species of Phytophthora which had been stated to cause this Belladonna disease by Westerdijk and Van Luijk (58), namely, Phytophthora erythroseptica. This species was first recorded and named by Pethybridge (39), as a parasite of the Potato tuber in Ireland, its specific name being given because when diseased tubers are cut, a pink colour develops in a short time. For reasons which will be discussed later, Westerdijk and Van Luijk (58) identified the Belladonna parasite as this species, while Alcock (1) tentatively suggested that the parasite was a variety of P.erythroseptica. The object in this and later experiments was to see if the/  
the/

the authentic P.erythroseptica, as isolated from diseased potatoes by Pethybridge himself, a culture of which was kindly forwarded by him, would cause the same disease as the unknown Phytophthora actually isolated from naturally diseased Belladonna.

After 31 days pots (b) and (c) were dead and dying respectively, and in another month pot (c) was dead also. Pot (a), the control, remained healthy till it was discarded four months later. (Plates 4, fig. 8). But as the dying seedlings in pots (b) and (c) were so small and the stem accordingly very thin, it was difficult to dissect out a piece of the inner tissue only. Most of the seedlings were well rinsed in sterile water, but apparently this was not sufficient to prevent the carrying in of external fungi on the stems when these were cut open. Those which were rinsed in proper sterilants, such as methylated spirits, failed to give anything in culture, as apparently the sterilant killed both external and internal fungi. Cultures taken from the water-rinsed seedlings gave saprophytic fungi, and although some mycelium was seen in these cultures which suggested the presumed causal Phycomycete, apparently the saprophytic forms were too abundant and swamped the Phycomycete. To have tested the pathogenicity of both the Belladonna Phytophthora, and of Phytophthora erythroseptica, they should have been isolated from the/



the artificially infected plants, and grown in culture. It was significant at the time, however, that the infected pots died, while the control pot remained healthy though treated exactly like the other two pots in all respects except inoculation. To avoid contaminated water, freshly distilled water was used for watering all the pots.

In a second series of infection experiments started in September, 1929, older seedlings were used. These were about four inches high and three were transplanted into each of twelve pots containing soil, these having been previously sterilised in the autoclave. The plants were well washed free of soil before replanting in the sterilised soil. They were then left for a week to recover from any shock (Plate 5 fig. 9). They were watered with distilled water. In Table 1 is shown the treatment and the results. After twelve days numbers 1, 2, 4 and 7 were beginning to wilt and two days later were severely wilted and dying (Plate 5 fig.10). Three days later these particular plants were taken up, washed free of soil, rapidly rinsed in methylated spirits to disinfect them externally, washed again to free from methylated spirits, then cut open aseptically and inside tissue cultured on Quaker Oat Agar. When microscopically examined, plenty of non-septate mycelium was found.

Table 1.  
Infection Experiment No.2.

Pot No. : No. of : Plants :	Treatment 9/9/29.	Results 8/10/29.
1. : 3. :	Into Stem Below Soil Level : <u>Phytophthora "atropae"</u>	:Oospores found :in culture.
2. : 3. :	Into Stem Below Soil Level : <u>Phytophthora "atropae"</u>	:Oospores found :in culture.
3. : 3. :	On Stem Below Soil Level : <u>Phytophthora "atropae"</u> .	:Still healthy.
4. : 3. :	Into Stem above Soil Level : <u>Phytophthora "atropae"</u>	:Oospores found :in culture.
5. : 3. :	Control. No Fungus.	:Still healthy.
6. : 3. :	Control. No Fungus.	:Still healthy.
7. : 3. :	Into Stem Below Soil Level : <u>Phytophthora erythroseptica</u>	:Oospores found :in culture.
8- : :		
12. : 15. :	Discarded owing to slight : blemishes.	

The Phytophthora from diseased Belladonna will be named for the present P."atropae" to distinguish it from the authentic P.erythroseptica and for easy reference. It is not implied at present that it is a new species. It will be seen from this experiment that both Phytophthora "atropae" and P.erythroseptica can cause a wilt of Atropa belladonna, when inoculated "into" the stem below soil level. P."atropae" also causes death when inoculated into the stem "above" soil/

soil level, while it did not prove pathogenic in this instant when merely placed against the stem under the soil level. This would suggest that a wound is necessary before P."atropae" can cause a disease. Yet, as previously stated, beds of untransplanted seedlings grown from the seed often go down to this disease. Wounding is out of the question there. The argument for necessary wounding is thus much weakened, but in the field the transplanting of seedlings cannot be done without some kind of wounding and this may help to increase the severity of attack.

#### THE "PINKING" PHENOMENON.

When Westerdijk and Van Luijk (58) described this disease in Holland, they recorded the infecting of Potato tubers with the Phytophthora from Atropa belladonna and the subsequent pinking of the flesh when these tubers were cut. This was an artificial infection in the Laboratory and not a natural infection in the field. They considered this as part evidence that the Phytophthora from Belladonna/

Belladonna was the same as P.erythroseptica. Alcock ( 1 ) on the other hand stated that, in the field where the Belladonna disease was found and where potatoes were subsequently grown, she never found a single tuber diseased with "Pink Rot"; the common name for the disease produced by Phytophthora erythroseptica. In this case the point is that no natural infection resulted. But Alcock ( 1 ) also tried an artificial inoculation and a rot resulted, but no reddening. The fact that P."atropae" fails to infect potato tubers naturally in the field will be considered later when the identity is discussed. Reinking (42) stated that Phytophthora Faberi Maublanc  $\phi$  causing diseases of Rubber, Cocoa, Coconut, and other tropical plants, caused a pinking of the flesh of artificially inoculated potato tubers. That this pinking of diseased tissue is not a test for the presence of Phytophthora erythroseptica only is contended as a result of experiments conducted upon potato tubers using various species of Phytophthora. In Tables 2 and 4 are given lists of species and their effects on tubers, while in Plates 6 and 7 are shown water paintings of the most prominently pinked tubers.

$\phi$  This is at present considered P.palmivora, Butler (16) by Ashby ( 5 ). Butler originally named it Pythium palmivorum (15) in 1907. Maublanc called it Phytophthora Faberi (34) in 1909, and Coleman erected the name P.Theobromae (18) but withdrew it later. Tucker (53), Gadd ( 25, 26 ), Lester-Smith ( 33 ), and Leonian ( 32 ), stress the similarity of P.palmivora and P.Faberi.

Table 2.

Phytophthora species inoculated 6/5/30 into heel end of potato tubers to test ability to cause "pinkings" when cut.

No.	P o t a t o	Spe- cies:	R e s u l t s.
1.	Sharpe's Express	:1.	:Very Pink, especially heel-end
2.	Epicure	:1.	: " " " " "
3.	Sharpe's Express	:2.	: " " " " "
4.	Epicure	:2.	:Fairly " " " "
5.	Sharpe's Express	:3.	:Moderately Pink near " "
6.	Epicure	:3.	: " " " " "
7.	Sharpe's Express	:4.	:No Pinking.
8.	Epicure	:4.	:Very Pink.
9.	Sharpe's Express	:5.	:Slight "
10.	Epicure	:5.	:Very slight Pink.
11.	Sharpe's Express	:6.	:Slight Pink.
12.	Epicure	:6.	:No Pinking.
13.	Sharpe's Express	:7	:Very slight Pink.
14.	Epicure	:7	: " " "
15.	Sharpe's Express	:-	:No Pinking.
16.	Epicure	:-	: " "

Table 3/



Table 3.

List of Phytophthora species used in experiment of  
Table 2.

No.	Name.	Original Source.	Isolated by	Sent by
1.	Atropae	Belladonna	C.E.Foister	----
2.	Cryptogea	x Tulip(shanking)	C.E.Foister	----
3.	Erythroseptica	Potato tuber	G.H.Pethybridge	same.
4.	parasitica	Tomato fruit	C.E.Foister	----
5.	Pini	Pinus resinosa	R.G.Pierce	S.F.Ashby.
6.	Richardiae	Richardia	unknown	S.F.Ashby.
7.	Syringae	Apple fruit	C.E.Foister	----

x P.cryptogea was originally described attacking Tomatoes by Pethybridge (40), but first described by the writer (24) on Tulips.

Table 4./

Table 4.

*Phytophthora* species inoculated 5th March, 1931, into heel end of Potato tubers to test ability to cause "pinking" when cut. Sharpe's Express and Epicure used with each species.

No.:	Species:	Sent by	Results	12th March, 1931.
1	: Arecae	: Lister : Institute	: No rot.	: No pinking.
2	: Boehmeria	: do.	: " "	: " "
3	: Cactorum	: do.	: " "	: " "
4	: Cambivora	: Ashby	: Very slight rot.	: No pinking.
5	: " (Walnut)	: do.	: " "	: " " "
6	: Capsici	: do.	: " "	: " " "
7	: Cinnamomi	: do.	: " "	: " " "
8	: citricola	: Lister : Institute	: No rot.	: " "
9	: Colocasiae	: Ashby	: Slight rot.	: No pinking (Epicure).
10	: hibernalis	: C.B.S.	: Very slight rot.	: No " "
11	: hydrophila	: Ashby	: Good rot.	: Good " "
12	: infestans	: (Local: Brown)	: Slight rot.	: No " "
13	: Meadii	: Ashby	: Very slight rot.	: No " "
14	: Melongenae	: C.B.S.	: " "	: " " "
15	: Mexicana	: C.B.S.	: " "	: " " "
16	: Nicotianae	: C.B.S.	: " "	: " " "
17	: Paeoniae	: C.B.S.	: " "	: " " "
18	: palmivora	: C.B.S.	: " "	: " " "
19	:	: (Local: Foister)	: " "	: " " "
20	: tabaci	: Lister : Institute	: " "	: " " "
21	: terrestria A.	: C.B.S.	: " "	: " " "
22	: do. B.	: C.B.S.	: " "	: " " "
23	:	: Control	: No rot.	: " "
24	: Erythroseptica	: Pethybridge : (Dual control)	: Good rot.	: Good pinking.

C.B.S. = Centraal Bureau voor Schimmel-cultures, Baarn.  
A = Pethybridge strain; B = Sherbakoff's strain.



It is seen that Phytophthora "atropae", P. Cryptogea, P. erythroseptica, P. hydrophila and P. parasitica cause a good flush when infected tubers are cut, while P. Pini, P. Richardiae and P. Syringae cause a faint flush. If one includes Reinking's record of P. Faberi ( = P. palmivora ), although the writer did not obtain a pink flush with it, this makes nine species which cause a good or weak pink flush.

#### INFECTION OF POTATOES.

As Alcock had not found any diseased potato tubers in a field heavily infected with the Belladonna pathogen, an experiment was conducted to see if potato plants would die when infected. Brooks ( 9 ) states that potato tubers are infected via the stolons and that a wilt may result if the stems are attacked by Phytophthora erythroseptica. Four varieties of potato tubers were sprouted in damp moss after being externally disinfected and were kept as free from spore infected air as possible. After twenty-five days they were planted in sterilised pots of earth and infected after another sixteen days in the stems just beneath soil level, cotton wool being placed round the place of infection. The results are given in Table 5.

Table 5 /

Table 5.

Pot:	Variety.	Treatment	R e s u l t.
1.	Arran Chief.	<u>P."atropae"</u> inside base of shoots.	Collapse of stems. Tissues below and one new tuber browned.
2.	do.	Control	Perfectly healthy.
3.	Eclipse	<u>P."atropae"</u> amongst roots.	No wilting but the only new tuber developed a pink flush when cut. (Obviously diseased).
4.	do.	Control	Turned yellow as watering had been omitted. Fifteen healthy new tubers.
5.	Epicure	<u>P."atropae"</u> inside base of shoots.	No wilt. Some roots diseased and two new tubers diseased.
6.	do.	<u>P."atropae"</u> amongst roots.	No wilt. Old tuber and two new tubers diseased and turned pink.
7.	do.	Control	Perfectly healthy.
8.	Sharpe's Express	<u>P. erythroseptica</u> inside base of shoots.	Collapse of stems. No tubers diseased.
9.	do.	<u>P. erythroseptica</u> amongst roots.	Perfectly healthy.
10.	do.	Control	do. do.

This does show that in the field referred to no special point should be made of the absence of natural infection as, in the experiment, the species that should have caused a wilt of potato plants and a pink rot of tubers - P. erythroseptica, did so in a much less degree that P."atropae" which was not expected to cause any disease in growing plants.

Spread of the Disease.

It had been a matter of conjecture as to how diseases were spread in the field up to 1800. Following that time the discovery of the various sexual and asexual reproductive bodies in various orders of fungi cleared up one difficulty and the researches that have been conducted, chiefly since 1900, on the environmental factors influencing the dispersal or survival of these bodies have in most cases clearly shown how, when and why diseases spread. <sup>x</sup>

In the particular group of fungi to which P."atropae" belongs, certain facts have been known for some time. It is known that the conidia of the Peronosporales generally are detached from their conidiophores when mature and are blown to other plants where they germinate and infect the leaves. The oospores, where they are formed, have been proved to enable the fungus to over-winter and initiate the disease in the next spring or summer. It is known in the case of Phytophthora infestans that epidemics are produced as a result of the production and dispersal of the conidia. It was therefore thought that the conidia of P."atropae" might be produced in nature to help in the spread of the disease. But the writer has never seen conidia on diseased leaves or stems in the field or in pot experiments. It seems that Pethybridge's (39) observation on the absence of aerial conidia in nature in the case of P.erythroseptica is/

x.

The writer has summarised the mycological side of this subject up to 1929, with a full bibliography (23).

is similar to the writer's observations. Both of these Phytophthoras produce typically aquatic conidia which do not become detached but germinate in situ.

Conidia are produced on soil cultures kept moderately damp in petri dishes and they may also be produced naturally in the field on the surface of the soil with a humid atmosphere and damp soil. That these act as conidia, i.e., become detached and blown on to healthy leaves, infecting them, is rather unlikely for three reasons. Firstly, because of the absence of detachment when grown artificially; secondly, because if they did act in such a manner, there would be no reasonable supposition that they could not be produced on aerial tissues where they have not been found; and thirdly the fact that the leaves and stems wilt and do not show signs of direct infection but show every indication of an attack at the base of the plant, food supplies being thereby cut off. It is supposed that the conidia germinate by zoospores which are disseminated in the soil by films of water and actually carried some distance by the downward flowing water on sloping beds. This is partially supported by the observation that the spread in the diseased beds seemed chiefly in the direction of the rows which pointed down the slope. As has been observed in many cases where soil diseases are supposed to be spread by such methods as just suggested, the rate of spread of this disease is not rapid and has/

has no similarity to the epidemics of Potato Blight caused by the wind-blown conidia of Phytophthora infestans. Jones & Drechsler (27) consider the flood water partially responsible for the spread of Root Rot of Peas, caused by Aphanomyces euteiches. It is not supposed that the conidia of P. "atropae" germinate in the field by a germ tube, as they do sometimes in the Laboratory, according to the external factors, because, as will be shown later, tube germination takes place at high temperatures and zoospore germination at low temperatures, and the climate of the Edinburgh district will never have a mean temperature sufficiently high to permit natural tube germination. The conidia presumably are formed on the surface of the soil as oxygen conditions are optimum there; they may also be formed under the surface soil but must be relatively few in comparison with the surface production as they were rarely found deep in the petri dish cultures. Should the conidia have been formed on the aerial parts of diseased Belladonna and have proved of primary importance in the aerial spread of the disease and infection of healthy plants, there would have been some object in conducting control experiments to find the exact conditions and periods of their production on, and infection of, the aerial parts. But as they are not produced on the leaves and are not as important in the spread of the disease as the conidia are in the case of Phytophthora infestans there /



there was no need for such research, for the results if there were any positive ones, would not have been applicable to natural facts.

The importance of the resting spore or oospore in the spread of the disease must not be overlooked. It is likely, and indeed highly probable, that during the fairly cold winters experienced in the Edinburgh district the mycelium inside the diseased host plant and that living in the soil will be killed. This means that the oospores must be the means of survival of the organism over the winter. As they are formed in nature very readily in the rotten tissues of the host plant, they are probably distributed in small pieces of plant debris in digging, harrowing and other cultural activities. Oospores may be carried in damp soil adhering to implements or boots to land so far uninfected, and though these may not be effective in causing disease in the first year, they may grow in a few years sufficiently to cause noticeable disease. Alcock ( 1 ) suggested that the disease was introduced from abroad by the spores of the fungus lurking "in some scrap of a solanaceous plant, tobacco, henbane, thorn-apple or datura, or belladonna, etc." This suggestion is impossible to check as it is not perfectly certain when the disease really started as it may have been present before it was brought to the notice of Alcock in 1924. It is rather similar to a disease of Strawberries/

Strawberries, chiefly prevalent in the Lanarkshire Valley, which lately has been found to be associated with a Phytophthora species. Alcock et al (3) found that the earliest date of the Strawberry Root Rot or Red Core Disease approximately coincided with the cessation of the use of horse manure and the commencement of the use of town manure usually obtained from ships; and therefore probably brought from the Mediterranean where a closely allied fungus, Phytophthora Gambivora, causes the Ink Disease of Chestnuts. So it is not outside the bounds of possibility that the Belladonna disease was introduced in some way from abroad.

Another aspect is that of other host plants. It is quite a usual thing for Phytophthora species to attack several hosts and the importance lies in the danger of the fungus thus lurking in one host plant, from which it can spread, while it has been prevented from attacking the other by some control method. As soon as this method is stopped, the fungus may be distributed in some way from the first host plant to the now uninfected ground and all the work has to be done over again. But in this case no other host plant has been found, either wild or cultivated. Even nearly related drug plants grown in the same ground as infected Belladonna had been grown in never took the disease. Alcock (unpublished <sup>2</sup> paper) recorded the same.



Climate in Relation to the Disease.

There are three chief ways in which climate can effect diseases generally: one, by its relation to the survival of the fungus; two, by its relation to the production and dispersal of the spores by which the fungus spreads; and three, by its relation to the activity of fungus, host and disease.

The relation of climate to the survival of the Belladonna fungus has been touched upon previously (p. 25 ), where it was suggested that the low temperatures of the Edinburgh district would kill the mycelium of the fungus but not the oospores. For this district the normal extreme minima for the critical months, November to March, vary between five and nine degrees (F) of frost and, in exceptional years, as much as twenty-six degrees of frost have been experienced. De Bruyn (11) showed that Phytophthora erythroseptica withstood winter conditions, apparently down to  $-7^{\circ}\text{C}$ , by the oospores, and not by the mycelium, while P.Syringae mycelium does withstand similar low temperatures. Later she also proved that P.infestans does not over-winter by its mycelium or conidia (12 ). It is certain that various species resist cold more readily than others and that their mycelia are able to live through fairly low temperatures but the fungus under investigation, P."atropae", being somewhat similar to P.erythroseptica, it can be expected to react to temperatures/

temperatures in an approximately similar manner. Moisture also influences the survival of the fungus. Oospores survive better in dry soil at low temperatures than in wet soil. Apart from over-wintering, the wet soil tends to cause most of the oospores to germinate at the advent of warm weather. It was once believed that in this way, the oospores would be cleared out of the soil and the fungus die should there be no host plant to infect. But it is known now, and was proved by the writer, that the fungus grows and lives saprophytically in the soil and makes abundant oospores there. Thus, unless it is killed by too high or too low a temperature, there is no chance of the soil becoming free of the fungus.

In the case of Phytophthora "atropae" the conidia are only produced on the soil surface and only when a quantity of moisture is present. The subsequent spread of the fungus aided by the films of water was mentioned before. Temperature will probably have less influence in their production except near the minimum and maximum.

Temperature and moisture influence the vegetative growth of the fungus and the resulting disease. It will be shown later that the fungus grows best at approximately 23°C. (73°F), and fairly well at 18-20°C (64 - 68°F). The normal mean maximum temperature in the Edinburgh district does not rise above 60°F. till June and falls below 60°F. in October.

With/

With slight seasonal variations, this period, June to September, corresponds with the period of the disease when it flourishes amongst the seedlings and older plants. There has not been sufficient variation since May, 1928, up to the present to account for any scarcity of the disease on the grounds of too low or too high a mean temperature. In 1929 the disease was fairly reduced in comparison with 1928 and 1930, and the slightly dryer, though cooler season, seems to be correlated with this fact.

Control/

Control of Disease.

When this disease was first studied, various suggestions were carried out in the diseased fields. When sowing the seed, diseased areas were avoided and this had the desired effect for a time. But soon all the areas became diseased and it was necessary to find a treatment which would kill the fungus responsible but not harm the Belladonna. Bewley advocates a mixture called "Cheshunt Compound" for the treatment of diseases caused by Phytophthoras.( 6 ). This compound is made up of two parts of Copper Sulphate and eleven parts of Ammonium Carbonate, finely powdered, one ounce of the mixture being dissolved in two gallons of water. It does not cure diseased plants but is often effective in preventing attack by killing the disease organism. Rows of seedlings planted in infected soil were watered with this compound but no substantial control was effected.

To test out control methods, experimental pots were therefore set up. Six 8" pans with potting earth were sterilised in the oven for half an hour and next day were inoculated with living cultures of Phytophthora "atropae". They were then variously treated, as in Table 6, before or after planting the seedlings.

Table 6/

Table 6.Control of Belladonna disease 13th March, 1930.

Pot:	Soil	Treatment	Result	5th April, 1930.	
1	Infected	None	Dead.	Dying since 24/3/1930.	
2	do.	Cheshunt Compound :before planting :seedlings.	do.	do.	do.
3	do.	Cheshunt Compound :after planting :seedlings.	do.	do.	do.
4	do.	Cheshunt Compound :after planting :seedlings.	do.	do.	do.
5	Not :infected:	Control	Still healthy.		

This was repeated using Corrosive Sublimate as well, which was quite effective.

Table 7.

Pot:	Soil	Treatment	Result	14th May, 1930.	
1	Infected	None	Dead.	Dying since 1/5/30.	
2	do.	Cheshunt Compound	do.	do.	do.
3	do.	do. do.	do.	do.	do.
4	do.	Corrosive :Sublimate (1/1000)	Healthy.		
5	Not :infected:	Control	Dying.	Not watered during :last few days of experiment :in absence of writer.	

The Cheshunt Compound was not only used before planting but for the first week of the experiment after planting. The Corrosive Sublimate was used once only before planting the seedlings.

The experiment was repeated in April 1931, the pots being kept at a warm laboratory temperature as heated glasshouses (for disease material) were not available, and the temperature was too low in the unheated disease glasshouse. The average temperature for the first week was 17°C, but 14°C, during the second week, owing to the fact that the pots had to be removed to another part of the building.

Table 8.

Control Experiment 10th April, 1931.

<u>Pot.</u>	<u>Soil</u>	<u>Treatment</u>	<u>Percentage dead or dying.</u>
1	Not infected	Control	0
2	Infected	None	75
3	do.	Cheshunt Compound, after planting.	50
4	do.	Corrosive Sublimate. (1/1000).	12.5.

The experiments confirmed the failure of Cheshunt Compound in the field. The success of Corrosive Sublimate is fortunate as it is an easy disinfectant to use in the field. It has been on the market for some time, made up in tablets, and when one tablet is dissolved in a stated amount of water it gives a 1:1000 solution, coloured slightly. Its poisonous nature does not matter so much in treating Belladonna as in treating vegetable crops.

In 1930, Cheshunt Compound was repeated in the diseased fields and again was reported as of next to/



to no use in checking the disease. The results of the trial with Corrosive Sublimate has been communicated to the growers and this Spring will be tried out in the field. Unfortunately the results of the field test will not be known for some time and cannot be included in this account. But considering that when Corrosive Sublimate has been found to succeed in plot tests it has also been successful in the field, it may reasonably be expected to secure a practical control of the Belladonna disease in the field.

Life History and Morphology.Cultural Studies.

These were undertaken not only to find the growth of P."atropae" on various media, but as a comparison with the authentic P.erythroseptica. Pethybridge describes the growth of P.erythroseptica on many media but mostly on plant tissues or extracts, such as Potato, Carrot, Bread, and Lima Beans. The writer cultivated both strains on various standard media, the growth on which can more readily be compared with other species. Only two abnormal media were used, -Dung Extract Agar, and Earth Extract Agar, and these were used to see if any growth resulted similar to their growth in actual soil.

On "Quaker Oat Agar" P."atropae" grows very readily and covers the slope with a white dense felt in a week. The sexual organs are produced in the aerial felt and in the medium, where they are exceedingly abundant. There is no particular plane of growth, the amphigynous arrangement of the antheridium being easily seen no matter how the slope is cut. Towards the deeper portions of the slope, fewer sexual organs are formed. Conidia have been found in culture but are not common and are smaller than those produced in water cultures. The production of conidia is uncertain and once a great abundance of these were found on one culture only. The tube and mycelium were/

were not humid and the explanation set forth by Pethybridge (39) for the occasional appearance of conidia in the case of P.erythroseptica on Quaker Oat Agar is not correct for P."atropae". Pethybridge found that when conidia of P.erythroseptica were formed it was usually in drops of water out of the medium, though a few times "rarely one or more appeared to be in or on this medium". Pethybridge (p.546) leads one to believe that conidia of P.erythroseptica on Quaker Oat Agar slants or cover glass films (Oat Extract?) were not normal and developed irregularly. The abundant conidia of P."atropae" on Quaker Oat Agar slants were normal in size and shape and measured  $32.28 \times 20.1$   $\mu$ , and were definitely borne on aerial hyphae.

On "Corn Meal Agar", P."atropae" grows as well as on Quaker Oat Agar and sexual organs are freely formed.

On "Malt Extract Agar", P."atropae" grows well and forms a white, aerial mycelium which is not so dense as on Quaker Oat unless very old. P.erythroseptica is recorded as forming oospores but no conidia on "Wort-Agar", which is very close to "Malt Extract Agar". P."atropae" forms abundant oospores and occasionally conidia. It is interesting that on this medium oospores were formed at 18-20°C. but cultures placed at 23-25°C. did not produce oospores.

Of the other media comparison between the growths of the two fungi P."atropae" and P.erythroseptica as grown by the writer will be made briefly.

P."atropae"/

P. "atropae"P. erythroseptica.Oat Extract Agar.

Very poor growth: oospores.

Poor growth: oospores.

Oat Extract - Saccharose Agar.Good surface growth.  
No oospores.

Some growth: oospores.

Oat Extract - Glucose Agar.Some surface growth.  
No oospores.Good surface growth:  
oospores.Potato Extract Agar.Good growth: abundant  
oospores and conidia.Fairly good growth:  
few oospores and very  
few conidia.Potato Dextrose Agar.Very good growth.  
No oospores. A few conidia.Very good growth.  
Abundant oospores.  
No conidia.Earth Extract Agar.Faint growth. Mycelial  
weft at tip of slope.  
Abundant oospores and  
some conidia. Rather  
similar to growth in  
actual soil cultures.Faint growth. Mycelial  
weft at tip of slope.  
Few oospores and no  
conidia. Similar to  
its growth in actual  
soil cultures.Dung Extract Agar.Good surface growth only.  
Oospores. Great septation.

In plate 8, are shown photographs of colonies of P. "atropae" and P. erythroseptica on Quaker Oat and Maize (Corn Meal) Agars. It is noticeable that they are similar on Maize Agar while dissimilar on Quaker Oat Agar, P. erythroseptica having no aerial growth in this case. Should comparison have been made on one set of cultures only, wrong conclusions might be arrived/

arrived at, for P.erythroseptica often makes a good aerial growth on Quaker Oat Agar. A similar variation was discovered in P."atropae" Two cultures, one on Malt Extract, and the other on Potato Dextrose, showed one side very densely aerial and the opposite side a very scanty aerial growth. It was wondered if these were mutants, and sub-cultures of these were taken to Quaker Oat Agar.

"A"		Dense	Area	Potato	Dextrose		gave	abundant	oospores.
"B"	"	Thin	"	"	"	"	no	"	
"C"	"	Dense	"	Malt	Extract	"	no	"	
"D"	"	Thin	"	"	"	"	abundant	"	

When these were sub-cultured in turn, it was found that the lines did not remain true, B2 and C2 also giving abundant oospores. It is supposed that some factor connected with the thickness or acidity of the media had caused the variation. Certainly it was not a case of true mutation. At no other time had any signs of mutations been noticed.



LIFE IN THE SOIL.

It is usually supposed that plants are infected by the mycelium produced by germinating oospores in the group to which Phytophthora "atropae" belongs. The actual details are not known; that is, whether a mycelium is produced directly by the germinating oospore or indirectly from zoospores first liberated by a sporangium formed by the germinating oospore. The oospores are supposed to be formed in the tissues of attacked plants from which they are liberated into the soil when the plants die and decay. In the case of fungi which, as far as could be determined, never formed their oospores in the host plant, such as Phytophthora infestans, these organs were considered unnecessary for infection. Murphy (37) has found that even P. infestans occasionally forms oospores on the surfaces of tubers in nature.

As Alcock (1) had said that nothing was known of the saprophytic life of P. "atropae" in the soil, and that it was a necessary piece of work, it was decided to attempt to grow P. "atropae" in soil. The first attempt was tried with sand and sand soaked with earth extract. Deep petri dishes, two inches deep, were half-filled with fine sand, soaked with water and sterilised. Others had the same sand to which was added the water extract of the same volume of earth and sterilised. Pieces of culture of P. "atropae" on Quaker Oat agar, about 3mm. square, were placed under the surface/



surface in some cases and half in and out in other cases. Pieces of aerial mycelium only were also placed in these sand cultures. They were carefully watched for signs of growth, sterile water being added when there was danger of the sand drying up. After four weeks as nothing was seen to grow, cultures on Quaker Oat Agar were taken from the sand near the pieces of culture and the pieces of aerial mycelium. None of these cultures produced P."atropae". It was therefore concluded that sand did not allow P."atropae" to grow saprophytically.

In the second attempt, petri dishes were half-filled with potting earth and with garden soil, sterilised and then inoculated in the centre with a piece of a Quaker Oat Agar culture of P."atropae". All soil cultures were damped before sterilising. In six days a visible surface growth had been made in each dish, about 1 cm. in the dishes with potting earth and 2 cms. in those with garden soil. In twelve days the fungus had grown 2.5 cms. in the potting earth culture which was almost dry, while in the same earth in another dish which was fairly humid, the fungus had reached the edge of the dish. The garden soil cultures showed a very vigorous growth and in Plate 9, Fig.18 is a microphotograph of the mycelium in these dishes. Before the fungus had covered the dish, sub-cultures were taken to fresh soil cultures, only/

only mycelium well away from the original inoculum being used. The idea was to test whether the growth of the fungus over the surface of the soil was only at the expense of the food in the piece of inoculum. In six days the sub-cultures had produced a growth of 1 cm. on potting soil and 2-2.5 cms. on garden soil. In a further month all dishes were covered with hyphae. In one dish oospores could be seen on the edge of the petri dish and also on a few lumps of soil. In the original garden soil culture the soil was covered with thousands of oospores in 39 days. Conidia were also formed on the surface of the soil. Every dish culture when sub-cultured to tubes of Quaker Oat Agar yielded the typical growth and oospores of P."atropae". There could be no doubt from these experiments that P."atropae" can and probably does form oospores in abundance in the soil naturally. It may therefore winter safely and infect young seedlings next Spring on germinating.

Other species of Phytophthora were also placed in soil cultures, this time in tubes. Procedure was the same as outlined in the case of P."atropae"; a control set of which was run at the same time and details need not be given again. In effect Phytophthora Cryptogea grew well on garden and potting soil, making its oospores in the soil; P.Richardiae grew well; while P.parasitica and P.Pini only grew slightly on fairly damp soil. As a check on P."atropae" P.erythroseptica was also grown on soil but the growth was poor.

Longevity/

LONGEVITY.

P.erythroseptica was proved to be alive in soil which had been allowed to dry up after four months by sub-culturing to Quaker Oat Agar and obtaining oospores. None of the other species, P.Cryptogea, P.parasitica, P.Pini, P.Richardiae and P.Syringae, were apparently alive at four months in thoroughly dry soil. In cultures on several media P."atropae" was not alive, as tested by sub-culturing when older than eight months; cultures up to twenty-two months in age were tested. P.erythroseptica was apparently dead after the seventh month. Conditions in artificial culture are, however, hardly comparable with soil conditions particularly in the field where there is always some moisture and where temperatures are not so constantly high as in a laboratory. It is probable that the longevity of P."atropae" would be as much as P.erythroseptica which lived four months at least in "dry" soil; therefore it may be expected to live two or even more years under natural field conditions, when P.erythroseptica was proved (see below) to live eighteen months in soil in the laboratory, by De Bruyn.

Review of other workers' results.

While these experiments were still in progress the writer was able to obtain De Bruyn's paper (11) on the saprophytic life of Phytophthora in the/

the soil, where the question of the formation of a vegetative life in various soils, the formation of oospores and the duration of life in the soil is thoroughly discussed. She quotes many investigators to prove that the various species (nine are considered), were transmitted to healthy plants via the soil. Experiments on the same lines as the writer's experiments were carried out, being continued longer in order to test longevity, with P.Syringae, P.erythro-septica and P.infestans. All of these were proved to have a saprophytic life in the soil. Cultures of P.erythroseptica on clay soil were vigorously alive at one and a half years, and on bog soil, leaf mould and sand at one year and nine months respectively. She also states that this species resists dessication well, as year old cultures on four different soils sub-cultured normally on to fresh media.

Morphology/

MORPHOLOGY.General Description.(a) Mycelium.

The mycelium is typical of the genus Phytophthora. When young it is much branched and non-septate, the protoplasm being very granular. When old it becomes more or less empty and cross walls are developed, their frequency varying with the medium on which it is growing. The width varies very much, though older mycelium is usually fairly wide. In plate 8 fig, 17 is shown a typical colony on Maize Agar and a colony of P.erythroseptica is also shown for comparison. In plate 9, fig. 19 is shown a micro-photograph of the advancing edge of a colony on Clear Maize Agar, where the type of branching and microscop-ical habit is demonstrated; the same of P.erythros-eptica is shown in Plate 9, fig 20, and it is seen that they are different. This colony edge habit is one character which helps to separate or affiliate several strains of Phytophthora species. In Plate 10, fig 21 is shown the septation developed when P."atropae" is grown on Dung Extract Agar, while Plate 10, fig. 22 shows the growth on Malt Extract Agar where septation is rare unless the colony is very old. The mycelium grows equally well submerged in the medium and aerially, the relative proportions varying according to the medium. In most respects the mycelium of P."atropae" agrees with P.erythroseptica, but this in itself cannot be taken as an argument for identification, as a number of/



of species of Phytophthora show similar characters as far as the mycelium is concerned.

(b) Sexual Organs.

The sexual organs are produced abundantly on a number of media and also in the decaying tissues of diseased plants. In their development they are similar to P.erythroseptica in several respects.

The Amphigynous Antheridium.

When Pethybridge (39) described for the first time the mode of development of the sexual organs of P.erythroseptica, a new method of fertilisation was recorded. The oogonial incept grew through the antheridium and developed into an oogonium proper on the further side. This type of development has since been called "amphigynous" in contra-distinction to the method known before for other species where the antheridium became attached to the oogonium and is now called "paragynous". Indeed the "amphigynous" method of fertilisation was new to the whole of the sub-class Oomycetes. Allusion has been made before to the presence of the "amphigynous" type of antheridium in P."atropae". The stages of the development in this fungus have been followed by growing it on a clear medium in hanging drop cultures. Quaker Oat Extract, Malt Extract, Potato Extract and Maize Extract Agars, have/

have been used for this purpose. A drop of the melted medium was placed on a clean cover glass which was inverted over a glass ring cemented to a slide. A small piece of mycelium was placed on the drop when set and the cover glass then cemented to the glass ring with vaseline. A drop or two of sterile water was placed in the chamber thus made and so a constant humidity was maintained.

The following account is similar to that by Pethybridge when he was describing P. erythroseptica. The antheridium is produced on a separate hypha to that of the oogonial incept, though the two hyphae may come from the same mycelium. The antheridium is produced terminally, laterally or intercalary, becoming shut off from the parent hyphae later by a cross wall, or in the case of the intercalary one, by two walls. The parent hypha and the mycelium for some distance back become empty while the antheridium remains highly granular. It is oval or spherical and a few times the width of its hypha. The oogonial incept is mistakable for an ordinary young branch but on meeting an antheridium displays its identity by commencing to force its way through the antheridium, and finally bursts through the opposite wall of the male organ. It now swells rapidly forming at first with the antheridium what has become to be called a "figure of eight." This stage is soon passed and the oogonium becomes more and more balloon-shaped and finally spherical. In living material/

material it is very difficult to see the stalk of the oogonium inside the antheridium. But in fixed and stained preparations or in those mounted in lactic acid without staining, this stalk is easily seen. At first the stalk is granular but it soon becomes empty but for a plug of refractive material in the centre, while the hypha and mycelium become empty also, like the antheridial mycelium. The oogonium is thin walled but becomes definitely thicker than the portion inside the antheridium. The antheridium becomes empty sometime during the formation of the oospore as will be more carefully described under the Cytology of the fungus. From now on it is easy to obtain the various stages, the prior stages not being so easy to procure. Pethybridge (39) states that the first stages were observed by watching the greater part of the night, but the writer found that by taking a sufficient number of cultures, they could be found during the daytime also, and Murphy (36) seems to have found the same. The contents of the oogonium begin to differentiate and the ooplasm separates to the centre, while the remainder of the periplasm with obvious degenerating nuclei shrinks to the periphery of the oogonium. The central ooplasm becomes spherical and sharply edged, though no wall can be seen and becomes the oosphere. A wall soon becomes formed round the oosphere and later on turns yellow with age. This is now the oospore. A large refractive body, probably oil, becomes/

becomes differentiated and the other contents of the oospores are distributed mainly round the periphery though the centre is not devoid of cytoplasm. Still later, other oil bodies are formed, and it is not easy to distinguish the true cytoplasm of the oospore. The various stages of the development are depicted in the drawings in plates 16-19 and in the microphotographs in plates 14-16 of which detailed annotations are given.

A point raised by Murphy (36) was regarding the formation of septa shutting off the antheridium from its hypha. This was formed at various periods according to Murphy, fairly late stages of development being found without any septa having been formed. His idea was that by not forming a septum the pressure caused by the in-growing oogonial incept was able to be lessened. He also recorded that antheridia sometimes formed a short tube to relieve the pressure of the ingrowing oogonial incept acting as a safety-valve. The writer has seen, a few times only, such short tubes which may be presumed to be similar to those Murphy (Plate 15 fig. 30) describes. / The ingrowing oogonial incept also causes the antheridium to be pushed in at that place and the invagination can be seen in most mature organs. (see <sup>Fig 41/</sup> Plate 19/). At the point of the formation of the oogonial incept too, is formed a swelling on the hypha and from which the incept grows. Murphy states that this swelling on the female hypha on touching another hypha stimulates the formation there of the antheridium.

The/

The presence of antheridia with no oogonial incept passing through them is explained by saying that the female hyphae had stimulated their formation but had for some reason failed to develop further themselves and had (apparently) broken away. Up to this point P."atropae" coincides in life history with P.erythroseptica.

#### The Paragynous Antheridia.

Neither Pethybridge nor Murphy ever saw the normal (the older) type of antheridium - the "paragynous" in P.erythroseptica, and Murphy is quite definite as to this. The writer has also examined numerous cultures of P.erythroseptica on various media and has never seen a paragynous antheridium. Yet the writer has seen this type in P."atropae" and although not common, indeed, it might be stated as being rare, it can be found by careful examination. It is shown in the drawing in plate 17, fig 36 and in the microphotographs in Plate 16, fig. 33.

One cannot state the proportion of paragynous to amphigynous antheridia as can be stated in some other species, and it seems that the paragynous type is more readily found on Filtered Oat Agar. Alcock, in an unpublished paper read to the Edinburgh Botanical Society, March, 1926, drew a distinction between P.erythroseptica, which never had paragynous antheridia, and P."atropae" which had both types. This is a fortunate confirmation of the writer finding the paragynous type, as there can be less doubt when two record such an occurrence than when only one does so. Delmer Cooper (19) states that he/



he could only find paragynous antheridia in a culture of Perythroseptica, but unfortunately his note is only an abstract, giving no details to permit judgment. It is an extraordinary record to make, as even if he found abundant "paragynous" antheridia there must have been some "amphigynous" ones too, as every worker on Phytophthoras can vouch for the amphigynous antheridium of P. erythroseptica. Although other workers in this group tacitly agree to disregard this record of Cooper the writer prefers to leave it in abeyance until further details are published by that worker.

It is not known what happens in the case of the paragynous type of fertilisation, as on the few occasions that they have been found, the oospores were already formed or the oogonium fully matured. Oogonia have been found with both types of antheridium attached, as is typical of some other species, particularly P. Pini. A microphotograph is shown in Plate 16, fig. 34 and a drawing in Plate 17, fig. 36.

### (c) Asexual Organs.

These are produced occasionally in P. "atropae" as already stated, on various media, such as Quaker Oat, Earth Extract and Potato Extract Agars. They are also formed on soil cultures but have not been seen on the living and infected host plant. They are produced abundantly when pieces of cultures or diseased tissue are placed in liquid cultures, such as sterile water, Potassium/

Potassium Nitrate solution (1:1000), or sterile bog water. It may be pointed out that although P."atropae" formed conidia on or in the solid medium in certain cultures which were more often dry than wet, P.erythro-septica on similar culture media did not produce conidia. This agrees with Pethybridge's statement that the latter species never ("or extremely rarely") develops conidia in the air, in solid or gelatinous media but "only in watery solutions" but emphasises another difference between that fungus and P."atropae". The conidia are produced terminally on long slender conidiophores and usually make it impossible to determine whether they are produced sympodially, - a diagnostic feature of Phytophthora, - or not. The conidia are inversely pear-shaped, the apex is blunt and usually flattened, with no papilla. They do not have a persistent pedicel at the basal end. The apical wall is thicker and more hyaline than elsewhere. A clear vacuole is present in the young conidium and later disappears. An oil vacuole is often seen in older conidia. Pethybridge did not describe proliferation of the conidia in P.erythro-septica, yet the writer has occasionally found this in P."atropae", as shown in Plate 18 fig. 40. The type of proliferation is somewhat different to the usual type where a second conidium is produced at the tip of the hypha which has continued to grow from the base of the conidium as shown in figures 7 and 8, Plate xlvii of Pethybridge's description/

description of P.cryptogea, ( 40 ). In this case a second conidium has rarely been seen. The conidium germinates by zoospores and when empty the basal plug, found in both P."atropae" and P.erythroseptica, continues to grow, but when it has reached anything from the length of the conidium to thrice its length it ceases to grow and makes a feeble attempt at producing a second conidium. Rarely more than a knob or slight swelling has been observed and often not even a swelling is produced. Nothing is more prone to form freak shapes than conidia of this fungus in water or solutions of various chemicals. It is usual for the conidia to germinate either by a germ tube at the apex or just below this point or by zoospores, hence it is often referred to as a sporangium. In freak cases more than one germination tube is produced and the result is often a chain of abnormal conidia with young tubes emerging in every direction. These have become known as "sphaero-conidia" and were first described by Lafferty and Pethybridge ( 30 ). Rosenbaum ( 44 ), Rose ( 43 ), and Blackwell et Waterhouse ( 7 ), have seen these in old cultures of P.Cactorum. Blackwell and Waterhouse also found them in old cultures of P.parasitica and P.cryptogea. The writer has not seen these in cultures of P."atropae" but only in water cultures, a drawing of which is seen in Plate 20 fig. 44 . The other two forms of spore referred to by Blackwell and Waterhouse the resting conidium and the chlamydospore, apparently are not produced by P."atropae".

The/



The process of germination by zoospores has been described very carefully by a number of writers, that by Pethybridge for P.erythroseptica being the most relevant considering its relationship with the Belladonna fungus. Pethybridge seemingly experienced the same difficulties as the writer in inducing production of zoospores, and he concluded that artificial stimulation of immature conidia is useless while it is unnecessary when they are mature. The writer has not had any difficulty in obtaining conidial production up to six months ago, when they were produced sparsely and seemingly unaffected by various changes in environment, such as temperature and aeration. It seems that the fungus from Belladonna loses its asexual vigour if grown repeatedly on one medium or two. The exact determining factor was not discernible. When the sporangium (zoospore producing conidium) is ripe, the contents become coarse and contract from the wall. The oil-drop breaks up and is scattered more or less evenly throughout the contents, which now begin to segregate into the zoospore units. These latter are not easily counted and for that purpose it is better to wait till they are liberated. The apex where the wall is thicker and hyaline, bulges out into a thin vesical and in the writer's observations does not attain such a size as Pethybridge describes for P.erythroseptica. The zoospores move in a mass into this vesical but rarely/

rarely do all of them pass out of the sporangium. The thin vesical disappears almost immediately and many times the zoospores have passed out and broken away before it was realised that the emerging stage had been reached. The zoospores do not remain in the mass longer than some seconds, and then disentangle themselves and swim away. Those that are left in the sporangium swim about there and soon find the exit. The broken apex seems sometimes to be too narrow for the mass emergence of the zoospores and these then squeeze out one by one, swimming away at once. The writer has noticed on rare occasions that one or two have not left the sporangium at all and did not seem active. Pethybridge found that these zoospores left in the sporangium of P.erythroseptica germinated there but this has not been observed in P."atropae". Pethybridge also saw cases where the zoospores were reluctant to leave the sporangium and the few that did so only swam to a little distance, germinating by tubes soon. Those left behind rounded off. The writer has not seen this in P."atropae", but has seen a clump of zoospores which have emerged only partly broken up and lying only a little distance from the mouth of the sporangium. These were not swimming nor were they showing any signs of germinating. It may be that certain unknown physiological factors influence their behaviour thereby producing these variations.



Measurement of the Fungus.(a) The Mycelium.

This varies between 2 and 10 microns, the aerial hyphae tending to be finer than the mycelium in the medium where it often becomes so irregular that only the maximum width can be measured. It tends also to be larger and coarser on rich media such as Quaker Oat and Maize Agars. The mycelium in the tissues of diseased plants is on the whole more stable, less branching, and measures between 4 and 7 microns. Pethybridge does not give measurements of the mycelium of P.erythroseptica, but this is not an important point, as the mycelium in all species of Phytophthora tends to be the same in its approximate range of width, although varying considerably in its mode of branching and vegetative habit. Rosenbaum ( 45 ) gave figures from approximately 2 to 17 microns for various species of Phytophthora, his range for P.erythroseptica being approximately 1.9 to 7.7 microns.

(b) The Sexual Organs.

In arriving at the average measurements of the sexual organs, only mature organs were considered as there is no guarantee that an immature organ at the time of measurement will not be larger when mature. Also a reasonable number of different media were taken to find the variation caused by artificial life and the relation to the organs produced naturally. The range determinations are entered only to one decimal point/

point as in a range of over 15 microns, the second decimal point is without meaning. They are collected in Table 9 while in Table 10 are given measurements recorded for P.erythroseptica.

Table 9. Sexual Organs.  
Measurements in microns of *Phytophthora "atropae"*

<u>M e d i u m.</u>	<u>No. measured.</u>	<u>Oogonia Mean.</u>	<u>R a n g e.</u>	<u>Oospores Mean.</u>	<u>R a n g e.</u>
Quaker Oat Agar.	100	33.21	43.2 - 25.5	30.40	38.7 - 24.4.
Filtered " "	25	34.51	40.0 - 30.9	28.84	32.2 - 23.8.
Dox " "	25	31.89	37.3 - 27.1	29.57	33.9 - 25.8.
Viola Extract " "	50	33.12	40.7 - 27.1	27.01	34.6 - 21.4.
Bean Pod " "	25	29.13	34.6 - 23.8	24.99	30.5 - 19.7.
Dung Extract " "	25	27.88	31.2 - 24.8	24.19	27.8 - 21.0.
Earth Extract " "	100	-	-	29.57	36.3 - 25.5.
Host Tissue	10	29.03	32.2 - 26.1	24.80	29.2 - 20.7.

**Alcock:** Tissue

40.0

Quaker Oat Agar

30.0.

Westerdijk & Van Luijk.

Oatmeal Agar 200.

Maize Meal Agar

35.23.

Oatmeal, Plum Juice

32.79.

& Peptone,

37.36.

Table 10.Measurements in microns of P.erythroseptica.

<u>Investigator.</u>	<u>Oogonia</u>	<u>Oospores.</u>
Pethybridge (Oat)	36	29-30.
Rosenbaum ( " )		35.5
Westerdijk & Van Luijk ( " )		32.61.

The measurements demonstrate the great variation within a species and care has to be exercised when attempting to identify a strain such as P."atropae". It is noticed that the oospores measure approximately 30 microns according to both Alcock and the writer when produced on Quaker Oat Agar, and also coincide with Pethybridge's measurements of P.erythroseptica. In studying the writer's results it becomes apparent that the oospores become smaller the less nutritious is the medium on which it is grown. It is striking too that the oospores produced in the decayed tissue and also in the Bean Pod are almost the smallest found, in fact are the smallest, if the lower range is considered. This may be due to the slight inhibition of the plant juices, probably chiefly the acids, which may have very little food value. The oospores on the Dung Extract Agar have the lowest mean of all and here the acid inhibition is even more likely. It is at least noticeable that the oospores get larger and larger as the medium changes from one of plant extracts, as Viola and Oat, to one of synthetic chemicals, as Dox/

Dox, to one rich in starch and oils, as Quaker Oat. The high value on Earth Extract may be another confirmation of the readiness of the fungus to grow saprophytically in the soil. Westerdijk and Van Luijk's progressive increase in size of oospores is shown in passing from Maize Flour to Oatmeal, and then to Oatmeal plus certain useful foods as Plum juice and Peptone. It is obvious that all these figures cannot be compared, nor can they be averaged, since this would be mixing variations caused by laboratory and experimental differences and those caused by fundamental physiological differences. Metrical comparison can only be made between those figures obtained from some standard, or given, medium as used at different times, under different external conditions, in different parts of the world. In this case, therefore, comparison should be made between the measurements recorded on the most common medium, namely, Quaker Oat Agar. Studying these figures it is seen that Westerdijk and Van Luijk identified their Belladonna parasite as P.erythroseptica as the mean oospore diameter was only .22 microns less than that species as determined by Rosenbaum. Yet their own mean measurement of P.erythroseptica oospores is much less, nearly 3 microns, than Rosenbaum's figure. There is little evidence to support their diagnosis on that point. As both the writer's and Alcock's measurements practically coincide/



coincide with Pethybridge's figure for Quaker Oat cultures, there would be in this case more inducement to associate the Belladonna parasite with P.erythro-septica.

To make a definite decision as regards the writer's own measurements, the oogonia can be stated to average 33.2 microns and the oospores 30.4 microns: the standard medium to be Quaker Oat Agar. They coincide with measurements of Phytophthora erythrosep-tica.

The wall of the oospore measures between 2 and 3 microns, the average being about 2.4 microns. This is really three-fold, as described by Murphy (36). The outer layer is the original one laid down on the formation of the oosphere and is usually thin, appearing under ordinary high magnification as one dark line. It does not stain much, if at all, and under magnifications above six hundred times shows its two lines proving it to be a definite layer. It is quite regular and measures normally .45 microns, though occasionally thicker examples are found up to 1 micron. The second or middle layer is the thickest and is a secondary endospore according to Murphy. It measures usually about 2.4 microns, and is the part of the wall which/

which definitely absorbs stain. The third, inner layer, the primary endospore, according to Murphy, is very thin and measures .15 microns. Murphy did not find deposits of the nature of an exospore and considered this as an explanation of the smoothness of the spore, which he thought was not only a character of P.erythroseptica but also of the whole genus. That this cannot be taken as a character of the genus and even of a species is suggested by the fact that the writer found that the oospore wall of P."atropae" could be irregular, not only the inside but also the outside layer. These irregular spores were found very abundantly on Earth Extract Agar cultures, and had a very faint honey-yellow colour, the oily contents often becoming more yellowish than the wall. Earth Extract Agar cultures were the only ones on which these were found. This is not the same as the irregularly thickened wall of oogonia, which will be described for Phytophthora Porri, in the second portion of this thesis.

(c) Asexual Organs.

As these are not produced in or on tissue/

tissue, measurements were taken of those produced in liquid culture, Calcium Nitrate and Potassium Nitrate (1:1000 solutions) were used, as in these conidial production was abundant, and although abnormal or teratological specimens occurred, on the whole they were fairly constant. Those produced in pure water were not so constant, often being very small and decidedly immature. As far as possible only mature conidia were measured. In Table 11 are collected the measurements relative to the conidia.

Table 11.  
Measurements in microns of Conidia.

P."atropae"

	Ca NO <sub>3</sub>	:	KNO <sub>3</sub>	:	<u>B o t h.</u>
Mean	39.4 x 26.1	:	37.78 x 21.05.	:	38.59 x 23.58.
Range	52-28 x 37-16	:	54.5 -22 x 31-14:	:	54.5-22x37-14.
Mode	1.509	:	1.79	:	1.65

Mean

Range

Mode

<u>Alcock</u>	50 x 30	75 - 20	1.66.
<u>Westerdijk</u> & <u>Van Luijk</u>	43.99x26.46.		1.66.

P.erythroseptica.

<u>Rosenbaum</u>	44.85 x 27.65	1.57
<u>Pethybridge</u>	32 x 20.	1.60.

It is apparent from these figures that the proportion of length to width of the conidia of both P."atropae" and P.erythroseptica is approximately the same, particularly the three modes for the Belladonna fungus. The rest of the figures vary very much. Westerdijk and Van Luijk's results for their Belladonna parasite almost agree with Rosenbaum's figures for

P.erythroseptica, while the writer's figures are half-way between Pethybridge's and Rosenbaum's figures for P.erythroseptica. Alcock's results are the highest and far exceed Pethybridge's measurements. Pethybridge has since agreed that his published figures were based on too few conidia and therefore one can safely allow a margin in which case the writer's results with P. "atropae" are more likely to coincide with Pethybridge's extended values of P.erythroseptica. The value of the range is not much because with varying conditions of environment and of nutritive value of the culture medium, a certain proportion would be abnormal. Alcock found conidia as large as 75 microns, while the writer has found a few as large as 105 microns. These extra large conidia are not taken when computing the normal range. When the number of conidia of a certain measurement falls below one in one hundred, it is taken as being abnormal.

In round figures the writer's measurements of P. "atropae" conidia may be stated to be 39 x 24 microns, with a range of 55-22 x 37 -14 microns, the mode of length to width being 1.65. They are well within the measurements of other writer's results with both the Belladonna parasite and P.erythroseptica.

Relation to Leonian's Physiological Key.

With the object of testing the separation or the uniting of Phytophthora "atropae" and Phytophthora erythroseptica, certain experiments were made in addition to those carried out in ordinary cultural work, following the method described by Leonian ( 32 ). As there are many species and strains of Phytophthora in his identification key, only the peculiar characters pertaining to P.erythroseptica will be mentioned.

1. Both form colonies on Malt Extract Agar in six days.
2. Sporangia are not produced in Aspartic Acid by either species.
3. Oospores on Malt Extract of P."atropae" not of P.erythroseptica. Pethybridge found the latter made them on Wort Agar. Leonian did not find the latter made on Malt Extract Agar.
4. No sporangia of both in Ammonium Sulphate.
5. Sporangia of both in Potassium Nitrate.
6. No sporangia of both in Sodium Chloride or Glycine.

P."atropae" coincides in this key with P.erythroseptica except with regard to the production of oospores on Malt Extract Agar. If notice were taken of this point, it would be identified as Phytophthora Pini. But the sporangia of that species are much too large measuring 55.5 x 35 microns, with a range of 92.5-26 x 44.5-22, and the oogonia are too small, 29 microns. But there may be some difference in the Malt Extract Agar as made by the writer and that made by Leonian and this may account for the presence of oospores in the writer's cultures.



TEMPERATURE RELATIONS.

To find the relation of temperature to Phytophthora "atropae" a series of poured plates of Malt Extract Agar were inoculated in the centre with the fungus from a culture on Malt Extract Agar in plates 1 to 10, and from a culture on Quaker Oat Agar in plates 11 to 20. They were then left at a room temperature of 18 to 20°C. for two days to allow a measurable growth to take place, afterwards being distributed to different rooms of varying temperatures. They were measured from the second day onwards. Some cultures were discarded owing to slight contamination. The actual measurements need not be given but in Table 12 the rate of growth is expressed in millemetres for each temperature over several days. It will be noticed that the cultures kept in the cool glasshouse which averaged 8°C. (4.5 - 11.6) slowed down in their rate of growth after the first two days and again after the sixth day. Those kept in the Laboratory at 18-20°C. exhibited a fairly even rate of growth all the time, being actually greater after the first two days; while those at 23°C. increased to the third day, then slowly decreased <sup>in</sup> rate till the sixth day, falling greatly on the seventh day. The difference of growth at the various temperatures is shown in plate 11.

Another two cultures were made on Malt Extract Agar and kept at 30°C. in an incubator for two/

two days. After this they were returned to a temperature of 18-20°C. to allow growth if the fungus had not been killed. One never grew, while the other made some growth after 21 days. The incubator only varied 1°C, therefore the maximum temperature must be very close to 30°C. The results are expressed graphically in plate 12.

As the maximum temperature is not the death point a culture of P."atropae" on Quaker Oat Agar was placed in the incubator at 40°C. and kept there thirty hours. Sub-cultures of this treated culture made no growth and it is concluded that the death point lies between 30 and 40°C.

The minimum optimum and maximum temperatures are therefore found to be respectively below 4.5°C. 23°C, and 30°C.

The effect of alternating high and low temperatures is shown in plate 13 fig. 25 where it is seen that zones have been made by the vigorous growth at the high temperatures and the slow growth at the low temperatures.

It is interesting to compare the results obtained with P."atropae" with these thermal relations of other species of Phytophthora. Jones, Giddings and Lutman ( 28 ) found that P.infestans did not grow on Lima-bean agar at 5 & 30°C, grew only fairly at 10 to 11°C and at 25 to 27°C, but grew abundantly, fruiting also, at 16 to 21°C. Thermal death point was close to 40° C, but not below that temperature. This is /

is rather similar to P."atropae" Leonian ( 32 ) with an undetermined Phytophthora strain obtained an optimum growth at 20°C, and much less growth at 12° and 30° C. Kendrick ( 29 ) and Fawcett ( 21 ) made the minimum, optimum and maximum temperatures for Phytophthora terrestria respectively as 8°, 30° and above 35° C. and 8°, 32° and 37° C. The temperatures for Phytophthora (Pythiacystis) citrophthora were slightly lower according to Fawcett ( 21 ) , namely 8°, 26° and 32°C. The two latter species are typically tropical species while the P."atropae" and P.infestans are more temperate. The probable relation between the growth of P."atropae" at different temperatures and the period of the annual appearance of the disease in the field has already been discussed.

Table 12.

Showing Average Increase in Growth of *Phytophthora "atropae"*.

T (°C.)	<u>1 and 2</u> <u>Days.</u>	<u>(Mean)</u>	<u>3rd</u> <u>Day.</u>	<u>4th</u> <u>Day.</u>	<u>5th</u> <u>Day.</u>	<u>6th</u> <u>Day.</u>	<u>7th</u> <u>Day.</u>
4.5 - 11.6.	5	(2.5)	2 x 1	3 x 3	2 x 2	2 x 2	
	6 x 5	(3x2.5)	2 x 2	2 x 3	2 x 2	3 x 2	1 x 2
	6 x 4	(3 x 2)	2 x 3	2 x 2	2 x 3	2 x 1	2 x 2
	5	(2 x 5)	3 x 1	3 x 3	2 x 3	2 x 1	
	7 x 5	(3.5x2.5)	3 x 3	3 x 3	2 x 2	3 x 2	1 x 1
	5 x 4	(2.5x2)	3 x 4	3x1.5	2x2.5	2 x 2	2 x 2
	6 x 1	(3x0.5)	2 x 3	3 x 2	2 x 3	3 x 3	
	6 x 3	(3x1.5)	2 x 2	2 x 3	2 x 2	2 x 2	
	5	(2.5)	2	2	3 x 1	2 x 1	
	<u>5.6x4.1</u>	<u>(2.7x2.05)</u>	<u>2.3x2.3</u>	<u>2.5x2.5</u>	<u>2.1x2.28</u>	<u>2.3x1.8</u>	<u>1.5x1.75.</u>

Table 12 Continued.

<u>T<sup>o</sup>C.</u>	<u>1 and 2</u> <u>Days.</u>	<u>(Mean)</u>	<u>3rd</u> <u>Day</u>	<u>4th</u> <u>Day</u>	<u>5th</u> <u>Day</u>	<u>6th</u> <u>Day</u>	<u>7th</u> <u>Day</u>
18 - 20°	16 x 12	(8 x 6)					
	16 x 14	(8 x 7)	10 x 10	7 x 7	9 x 8	9 x 6	
	17 x 15	(8.5 x 7.5)	10 x 11	10 x 9	9 x 9	9 x 9.	9 x 10.
	21	(10.5)	8 x 5	10 x 13	10 x 9	10 x 4.	
		<u>(8.75x7.75)</u>	<u>9.3x8.6</u>	<u>9 x 9.</u>	<u>9.3x8.7</u>	<u>9.3x6.3</u>	<u>9 x 10.</u>
23	20	(10)					
	22 x 13	(11x6.5)					
	24 x 18	(12 x 9).	12 x 13.	11 x 9.	11 x 13.	8 x 3.	
	23 x 21	(11.5x10.5)	13 x 12	9 x 10.	10 x 5.	10 x 4.	
	22	(11)	11	13 x 11.	12 x 11.	10 x 12.	5 x 6.
		<u>(11, 1x9.4)</u>	<u>12 x 12</u>	<u>11 x 10.</u>	<u>11x9.7.</u>	<u>9.3x6.3.</u>	<u>5 x 6.</u>



DISCUSSION OF RESULTS.

In the experiments which were conducted to test pathogenicity of the isolated fungus, it was found that material inoculated into the stem causes a typical wilt and rot of the Belladonna plant. Any doubt that might arise as to the necessity of wounding was dispelled later in the control experiments in which the plants were not inoculated but sown in soil contaminated with cultures of the fungus. This confirmed the damping-off of seedlings of Belladonna in the field.

The fact that Phytophthora "atropae" causes a flushing of potato tubers and that it can infect growing potato plants via their stems is not direct evidence that it is identical with Phytophthora erythroseptica; especially as a survey of Phytophthora literature reveals the fact that species can be induced to attack the host plants of other species. For example, P. infestans attacks Tomatoes as well as the Potato, although the most frequent species to attack Tomatoes are P. cryptogea and P. parasitica.

It has been shown that P. "atropae" lies within the limits of experimental variation round the figures recorded for the asexual and sexual organs of P. erythroseptica, and for this reason might be identified as the latter species. But it has also been shown that P. "atropae" has the paragynous type of/  
of/

of antheridium in addition to the amphigynous. This might be considered reason enough for a separation of the Belladonna parasite from P.erythroseptica.

Pethybridge in 1922 made four groupings of the species of Phytophthora:- "A", entirely amphigynous antheridia, "B", preponderatingly paragynous but sometimes amphigynous, "C", entirely paragynous, and "D", sexual organs not found. P.atropae does not fall into any of these groupings; it would require a group "E", with preponderatingly amphigynous antheridia but occasionally paragynous. But it is believed that this method of grouping species in the genus may not be safe, as lately many species have been proved to have sexual organs where they were previously thought absent or to have the other type of antheridium not present before. Pethybridge(30) thought it possible that a renewed investigation of P.Nicotianae, the only species at the time he wrote which had entirely paragynous antheridia, might discover some medium on which sexual organs would be produced abundantly and amongst which a few amphigynous antheridia would be found. Also it is believed that the proportion of the two types to one another in culture and in nature varies according to certain conditions. This is demonstrated in the second disease described, caused by Phytophthora Porri n.sp., and this feature is discussed there more fully. Regarding its importance in/  
in/

in establishing the Belladonna parasite, it may be that the life of this fungus through many years on its present host has changed not only its natural ability to attack potatoes, but also its inheritable physiological characteristics, the dominance or the suppression of which may determine the amount of paragynous antheridia. In P.erythroseptica there may be a generic character which naturally suppresses the formation of paragynous antheridia and should this character have been altered by the probable life of this species on Belladonna, there may result a new strain, showing occasional paragynous antheridia, which may easily be erected as a new species.

Then it has also been shown that although there are differences between the two fungi in culture, these may be equally caused by the change induced by a protracted life on the Belladonna plant. The differences are rather outweighed by the similarities. The physiological characters of both fungi are also almost identical.

It is therefore decided that P."atropae" is similar to P.erythroseptica in its main characters, but that in its other characters it shows variations which are more or less constant, though influenced by external factors, and deserve a variety binomial. It is therefore named as Alcock had already suggested.  
It/

It should now read Phytophthora erythroseptica var.  
atropae n.v. mycelio ramoso quoad partes recentiores  
non septato quoad vetustiores multiseptato vacuatoque  
conidiis inversi-pyriformibus aut propemodum ovoideis  
sympodialiter genitis 39  $\mu$  x 24  $\mu$  (55-22 x 37-14  $\mu$ )  
antheridiis terminalibus aut lateralibus aut inter-  
-calariis rotundis aut ovoideis penetratis ad vel  
juxta bases suas oogoniis incipientibus quae deinde ex  
partibus summis emergunt oogoniis pyriformibus super  
hyphas quae origine distant ab illis hyphis quae illa  
antheridia gignunt partes inferiores intra antheridia  
superiores autem in summis antheridiis habentibus  
oosporis sphaericis crasso subfusculo episporio  
praeditis 30  $\mu$  sed infrequens oogoniis haud per  
antheridia penethantibus sed illa lateraliter copulant-  
-ibus vel oogonio singulo typis utrisque copulato.  
Radicibus partibus inferioribus Atropae Belladonnae L.  
parasiticus.

Hab. in Britannia et Hollandia.

and should be quoted:-

Phytophthora erythroseptica Pethybridge var.  
atropae Alcock.

Addendum on Cytology.

The Cytology of the Belladonna parasite was commenced and methods used similar to those described by Murphy (36). But beyond the later stages, after the formation of the oospore, a period of time necessary for a continued research such as this difficult field needs was never available, and much material which was in the process of embedding became spoilt owing to additional duties.

The presence of the male and female nuclei in the oospore, the male close to the wall in the young oospore, was noticed. The male joins the female nucleus at the centre and fusion takes place. These are drawn and shown in Plate 19 fig. 42.

The actual fertilisation tube described by Murphy has not been seen in microtome preparations, but a tube which has not been withdrawn is shown in the microphotograph in Plate 15 fig. 32. There it is seen passing from the top of the antheridium into the oogonium which has produced its oospore. This is, of course, abnormal, but Murphy also met instances of this nature.



The White Tip Disease of Leeks

caused by

Phytophthora Porri

nov. spec.

INTRODUCTION.

It is only during the last six years that any serious notice has been taken of the disease of Leeks which earned the common name of "White Tip" given to it by horticulturists and market gardeners.

As the first record of this disease and the naming of the causal fungus were made by the writer, this work is claimed as original.

The more practical side of the research, namely field control, has yet to be finished. As the Phytophthora responsible was only obtained in culture in October, 1930, there has been but little time to test out the fungus as the pathogen, to find out how it attacks and therefore the best method of controlling the disease in the field. This was delayed even more than it might be with other host plants, as Leeks grow very slowly.

For the supply of seeds, the use of ground for observations and for experimental plots, the writer is indebted to Robert Scarlett and Sons, Inveresk, and to David Lowe and Sons, Musselburgh.

Historical Review.

The disease upon which these researches have been conducted is not necessarily a new one, although it has only recently been recognised not to be an abnormal manifestation of another disease.

Growers of Leeks have noticed the symptoms of this disease for a generation, but particularly, during the last twenty years. They have given the common name of "White Tip Disease" for many years and supposed the disease to be due to many causes. Leek-sickness, or land upon which Leeks have been grown too long, was thought to be a cause. This is rather near the truth for quite another reason. Weather has also been blamed. Recently, it was connected with the "Downy Mildew" of Leeks caused by the fungus Peronospora Schleideni. This disease is not so common on Leeks as on Onions and indeed some writers state the attack on Leeks to be rare. However, it was thought that the "White Tip" symptoms might be caused by Peronospora Schleideni. It was in this connection that the writer, in conjunction with Mrs. N.L.Alcock, was brought into contact with the disease. Certain growers were anxious that trials should be initiated to find a means of controlling the disease. The writer and Mrs. Alcock therefore organised and supervised spraying experiments with this view, in September, 1928, choosing an area where the plants were so far not showing "White Tip" symptoms, and it was hoped therefore that/

that the plants were not infected. Close by, there were other plots of Leeks which had the White Tip symptoms and these were to act as infecting material on the trial plots, amongst which were controls. As a matter of interest, it may be said here that these trial Leeks may have already been infected as they developed the "White Tip" symptoms very soon after the spraying was finished.

Leeks already infected and brought in for examination under the microscope, provided the clue to the fact that a species of Phytophthora and not Peronospora Schleideni was partly, if not wholly, responsible for the disease. This clue was the finding of oospores which had the type of fertilisation known as "amphigynous" and it was already known that an important difference between Phytophthora and Peronospora was the fact that the latter genus never has the amphigynous type of fertilisation. Over-night the diseased Leek tissue produced great numbers of sporangia in a damp chamber, which dispersed any doubts that might have remained. They were typical of Phytophthora.

A search for records of a similar disease was not found, but an allied disease caused by Phytophthora allii Sawada had been recorded on the Japanese Welsh Onion, Allium fistulosum L. in 1913, by Sawada in Formosa. For various reasons the Phytophthora on the Leeks was not identified with P.allii, and therefore appeared to be a new record. For this reason

it/

it was decided to publish a record of this discovery and the writer did so in the Spring of 1929 (22), with the object of finding where else the disease was known, but as yet unrecorded.

Distribution/



DISTRIBUTION.

In Scotland, the disease is mainly confined to the eastern counties down to the Borders. Economically, it may be said to be absent elsewhere as Leeks are not grown on any scale outside of these districts. In this area, the disease is found on almost every field, although at certain times of the year and according to the weather, patches quite free may be found. Allotments and private gardens are also affected, and the fungus responsible must be thoroughly distributed. It would be purposeless to give a list of places where the disease is found, as they are very numerous; suffice it to say that the main observations and experiments have been and are being carried out in the Prestonpans - Musselburgh district.

As far as the writer has ascertained, the disease is not known outside of Scotland and England. In England, as a result of the publication alluded to (22 ), it was reported from many localities, but on sorting they proved to be mostly mistaken diagnoses, weather effects being taken for the genuine "White Tip" symptoms. Yet there is one area where the disease is now regularly reported as serious; this is the Cheltenham district near Bristol.

Economics/

ECONOMICS.

The effect of this disease, as will be seen from the symptoms, is considerable. In severe attacks, row after row of young plants will be checked and remain stunted, while older and larger plants rot away. In slight attacks the tips only will be attacked, but the growth cannot be the same and yield is necessarily reduced. The reduction in yield in the case of severe attacks may be as much as 50 per cent, a usual loss being 25 per cent. Of those that are not an absolute loss, many although sold, do not fetch the return commensurate with the cost of production. One effect of the disease is that leeks, although apparently good apart from the White Tip, when pulled, very quickly wilt and turn soft. The public will not buy such produce, as they do not keep in store, and the price, though lower than would be paid for good leeks, is still too expensive owing to this wastage in store.

Leeks which are slightly attacked will produce seed in the second year, but those that are severely attacked never progress the length of producing seed.

Pathology/

PATHOLOGY.Symptoms.

The first sign of this disease is the dying of the tips of leaves which turn white. This area varies from half an inch in early cases to six inches or even more in advanced cases. It is rare to find whole leaves turned white. The dead area falls over, and may remain limp or stiffen, cracking when touched. Upon this area grow saprophytic fungi such as Macrosporium commune and Botrytis allii. As the disease progresses the white area becomes larger, though rarely involving the whole leaf. The initial white area may also start on the side of the leaf any where, though usually about half-way down, and is shaped like a half oval. The tissue continuing to grow around this area causes a twist in the leaf. In these areas little is found of the causal fungus.

The second-stage is the appearance of water-logged areas below the white tip, often nearer the base of the leaf, which become very soft and split very readily. In these areas are found the causal fungus. In Plate 21 the white tip appearance is seen in five of the leaves, while in one is shown the side attack and in the middle one the soft, broken, water-logged area. In Plate 22 are three views of typical fields of diseased leeks, showing distant and close up views. The features that are noticeable are the/

the falling over of the leaves, the ability to see the ground between the rows and the general whiteness or light green colour as seen at a distance. In healthy leeks the leaves stand up well, produce sufficient leaf to almost hide the ground between the rows and at a distance have a blue-green colour.

Another sign of the disease, associated or not with white-tip and water-logged areas, is that when a leek is pulled up it gives way to the pressure of the fingers where it is gripped and breaks at the leaf bases leaving the long white stem and roots behind. Healthy leeks, even when anchored very firmly by a vigorous root system, pull out of the ground without any rupture .

#### Microscopical examination.

The fungus is not usually found in the white areas, though in old dried leaves oospores can be found in a resting stage. The active stage of the fungus is found in the water-logged areas or less readily in the immediate tissue surrounding the white edge where the tissue is sometimes yellowing, prior to turning white. The large non-septate mycelium is very obvious and ramifies the tissues in all directions being indifferently inter-and-intra-cellular. In these areas sexual organs in all stages of development can be found and provided the leaf is kept damp, non-sexual reproductive sporangia are also formed. The size and characteristics of the sporangia vary greatly, while both types/

types of sexual fertilisation are found with the oospores. The details of morphology will be given later. There are other fungi present which are secondary, and help the rot started by the Phytophthora species. These have been cultured from time to time and include Botrytis allii Munn, Macrosporium commune Rab. which yielded later the perfect stage Pleospora Herbarum (Pers.) Rab. Fusarium sp., yeasts (undetermined), and bacteria. When it was desired to find out how the disease commenced, the base of the shoot was sectioned to trace any mycelium of the Phytophthora that might be there. With naturally diseased material brought in from the field, it was with difficulty that this mycelium was proved to be present. It is present but very scarce.

#### ISOLATION AND CULTURE.

From October, 1928, thousands of cultures were made in attempting to isolate the causal Phytophthora; but all failed and it was only in October, 1930, that success was obtained. The method which had been used at first was to place as young material as could be found on to Quaker Oat Agar, taking tissue from white areas, and near the white areas; also from water-logged areas. If the secondary organisms had already gained entrance, (and this was the case mostly), the tissue was carefully teased out in sterile water till a piece was found which seemed to contain the non/



non-septate mycelium only and this was cultured. If not, the surface was sterilised with various liquids and the leaf then cut open aseptically and inside tissue only cultured. Mercuric Chloride solution (1 in 1,000) was used for sterilising, immersing the tissue for one minute or less and then washing in running water. Usually nothing grew at all following one minute in this solution and if the steeping was too short, the saprophytic fungi only grew in culture. It was thought that Mercuric Chloride was too strong for Phytophthoras although only a trace would penetrate inwards in the time of steeping. Therefore Methylated spirits was tried but proved no more successful. As the tissues, especially the water-logged areas, produced numerous sporangia when placed in water, bog water or Potassium Nitrate solution (1 in 1,000), small pieces in these liquid cultures were picked out on to a slide containing a drop of water and single sporangia teased off and placed in culture. No growth resulted. In a similar manner, oospores were isolated and placed in culture and again no growth resulted. Tissue was even teased out so much that small masses of hyphae of a non-septate nature, were picked away from everything else and cultured. This was also no use. The yeasts and bacteria always flourish in culture and it was thought that the Phytophthora may have actually started growing in these cultures but been killed by these organisms. Therefore a method was tried which aimed at eliminating contaminating yeasts/

yeasts and bacteria before culturing. After the usual precautions a number of pieces of infected leaves were placed in a series of petri dishes containing different liquids; - Tap water, sterile water, sterile bog water (or its equivalent) - solutions of Potassium Nitrate, Magnesium Nitrate and Magnesium Sulphate of 1/100th the Molecular weight in 1,000 ccs. of water and a nutrient solution used by the Centraal Bureau voor Schimmelcultures of which the formula is -

50 gms. Saccharose.  
 1 gm. Potassium Nitrate.  
 1 gm. Dihydrogen Potassium Phosphate.  
 0.5 gm. Magnesium Sulphate.  
 1,000 ccs. water.

Each day the solutions were decanted off and replaced with fresh solution, most of the bacteria and yeasts being carried away by this procedure. The yeasts were eliminated entirely and the amount of bacteria left was not sufficient to prevent a fair vegetative growth of the fungus in the liquid. Small pieces of this vegetative growth were removed and transferred to tubes of Quaker Oat Agar. Most of the cultures derived in this way were unsuccessful, but one taken from the petri dish containing tap water gave a good growth of the Phytophthora and on examination showed abundant asexual and sexual reproductive bodies. Sub-cultures were immediately taken. Still a great number of bacteria were present and many cultural methods were used in attempting to obtain pure cultures. The fungus now that it was isolated grew well on most media, the only one upon which its growth was exceedingly/

exceedingly slow being Malt Extract Agar. The extreme limits of vegetative growth were taken in sub-culturing but the bacterium also was taken, this keeping pace with the tips of growing hyphae. The hyphae seem to be surrounded with a gelatinous layer in which the bacteria multiply, and the latter are carried thus wherever the fungus is. Brown's method (10) is not successful in this case. Another method, used by mycologists who have encountered the same trouble depends upon the theory that when the hyphae of the Phytophthora grow deep into the medium the bacteria are left behind. So that by taking sub-cultures from the under-side of tube cultures, or by turning petri dish platings over, one should get the pure hyphae only. In this case no success attended this method and it must be assumed that the bacteria or some of them, were anaerobic and were not affected by the method tried. Another method tried consisted in raising the pH of the medium by adding lactic acid, one and two loopfuls (platinum wire) to the melted medium in the test tube. One loopful of lactic acid did not check the bacteria, while two loopfuls killed both bacteria and fungus. This was repeated, using 0.5, 1, 2 and 3% of lactic acid, but no growth was ever obtained on such media.

With the object of eliminating the gelatinous sheath and the accompanying bacteria, a narrow strip of the set medium in a petri dish was removed aseptically and the inoculation with the impure culture made/

made close to this gap. The fungus grew towards the gap and a few hyphae managed to struggle across the glass to the medium on the other side. Cultures were made from this opposite side when hyphae were seen to reach it. Success was not obtained at first, but later the technique was improved and resulted in pure cultures being obtained. Before pouring a plate with the medium, a glass strip was placed inside the petri dish on its edge and the whole moistened with methylated spirits, which was then flamed. The now sterile dish was quickly covered with its lid, sterilised in the same way, and the melted agar added in the usual way, pouring on both sides of the strip of glass. If the glass strip is ground flat and is fairly thick, it stands up and does not let any agar float under it. A good microscopic preparation slide cut in two lengthways was used. When the agar was set, the glass strip was removed aseptically and the plate inoculated with the impure Phytophthora. The glass of the petri dish between the two masses of medium was quite clear of medium and the hyphae grew over but left the bacteria behind. In the other method, removing a strip of medium, a thin film is left behind and this allows the bacteria to grow over with the hyphae.

The fact that a great abundance of bacterial contamination seemed to swamp out the Phytophthora was alluded to before. In this connection, it is interesting to note that live hyphae under microscopic examination/

examination were seen to be bombarded by hosts of bacteria and hyphae (dead?) were seen packed with active bacteria in place of cytoplasm. In many cases the walls seemed to have burst as a result of the increase of these bacteria. In mounted preparations the hyphae were often breaking down and disappearing, whether as a result of chemical or pathological causes is unknown. Petri (41) recorded Phytophthora (Blepharospora) cambivora, P. citrophthora and P. parasitica as being killed by bacteria, so the present case is not exceptional.

Pathogenicity/



PATHOGENICITY.

When the assumed causal fungus was at last obtained in pure culture, experiments were started to test its pathogenicity. The results are still being awaited and they cannot be included in this account. But the methods used so far are explained here.

To settle definitely whether infection could occur from the soil, 12" x 8" x 4" boxes were filled with sterilised earth and young leek seedlings planted in them. After a few days, during which time the seedlings recovered from the transplanting, two were infected with the leek fungus and the other kept as a control. In each box 4 rows of 32 seedlings were infected by placing a small piece of Quaker Oat culture against the base of the shoot where the soil had been carefully scraped away and then replaced after the inoculation. Wounding was carefully avoided. Two other rows of sixteen seedlings had a piece of inoculum placed between each seedling in the row and between rows. This was to test if the fungus spread sufficiently in the soil to reach and attack the seedlings. One box was kept in the cool glass-house with a minimum soil temperature ranging from 4-8°C. and a maximum of 18°C. the average mean being 12°C.. The other was kept in a laboratory room near a window and near a radiator, the mean soil temperature being 16°C.. Although every effort has been made to keep /

keep the plants under natural conditions as possible in the circumstances, no results from which conclusions can be made have been obtained. After three weeks in the warm room several seedlings with the inoculum on their base had collapsed and half-way down the slender primary leaf a white area had developed. These areas were examined carefully but no proof that they were due to Phytophthora was obtained. Certainly a small amount of non-septate mycelium was seen, but there is no proof that this was not a very young stage of some other fungus, as no fructifications could be found. In both boxes conidia were found to be produced by the fungus on the face of the soil. It is just possible that some of these were blown by draughts of air on to the leaves and started the white areas referred to. But apart from these no sign at all of infection can be seen and it is probable that natural infection of unwounded leeks at the base does not occur.

At the same time another set in pots was arranged, between 8 and 11 plants being sown in each, which were filled with sterilised earth. Two pots were not inoculated and six were inoculated with the fungus. Of these six, two were treated with Cheshunt Compound, and two with Mercuric Chloride (1:1000) solution. One in each set was kept in the cold glass-house and one in the warm room. But as the infection did not take, as in the previously described experiment, this preliminary control experiment was of no avail.

In/

In the latest experiment up to the present, leaf infection has been tried. 55 seedlings in another box were carefully staked up with thin slips of bamboo and then 23 were infected with small pieces of a culture on Quaker Oat Agar, the inoculum being placed in the loop of the loosely tied cotton thread, by which the slender seedlings were kept upright by tying to the stakes, and between the seedling and the stake. This ensured contact with the fungus. Nine of these seedlings were slightly wounded on the inside before the inoculum was placed on them. The whole box was then kept under a damp bell<sup>jar</sup>, four times the usual size, adequate aeration and light being arranged as best as possible, and placed in a warm room. The results are as follows: - Six uninfected out of 32 developed either a yellow or a white tip, rather uncertain, and less like the genuine symptoms. Four unwounded infected out of 14 developed similar symptoms, though not good and seven out of nine wounded and infected seedlings developed indifferent to really good symptoms. The percentages are: -

Uninfected	-	18%
Unwounded and infected	-	28%
Wounded and infected	-	77%

In most cases, though not all, a fair amount of non-septate mycelium was found in the yellow-watery areas or the white areas. These areas were not in the immediate vicinity of the inoculum but either well above or below this. It is unfortunate that the uninfected/

uninfected and the two types of infection had to be run in the same box, as there was no other way of keeping them under a humid atmosphere, the large bell jar being the only one available. The plants were finely syringed with tap water every day and it was noticed that usually a drop remained on the inoculum and the leaf at that place. This probably helped the infection. This is not proof but at least does hold out a promise that when this is repeated, and it is hoped under better conditions and using older and larger plants, definite proof may be forthcoming of the pathogenicity of the fungus. It does also suggest that infection is more likely to commence on the leaf. In that case, soil sterilisation will not be of value, commercially at any rate, and methods aiming at protecting the leaf will have to be worked out.

Reference may be made to the first field control experiments that were conducted at Musselburgh in September, 1928. At that time three common sprays were used, the usual control plots also being interspersed among the sprayed plots. Bordeaux and Burgundy mixtures at the usual strengths, with the addition of soft soap to aid adhesion were tried. Ammonium Polysulphide wash was also tried, Unfortunately the growers had notified the appearance of this disease too late, as apparently infection had already occurred. But it was noticeable that the plots sprayed with Ammonium Polysulphide were less severely attacked than the other plots. Further field work along the/

the lines of leaf-spraying or dusting is indicated.

As an additional method of controlling the disease, the growers have been warned that by ploughing in the diseased leeks they are replacing the infection in the soil. Whereas some have not taken the trouble of scrubbing up diseased material and burning, other good growers have and a diminution in the extent and severity of the disease on their farms is being noted. Whether this is really connected with the removal is impossible to prove but it is significant, and the practice should be persisted in.

Up to the present no immune or resistant varieties have been found, although in the diseased areas, observation has been made on the severity of the disease on the various varieties grown.

Progress/



PROGRESS AND SPREAD OF THE DISEASE.

The disease has now been under observation for three years and it has been demonstrated that the first signs are always those of the leaf infection. The white tips or the watery areas are the first indication in the field that the disease is present. It is not found that leeks die from the roots upwards but from the leaves downwards. Although it is therefore probable that the disease commences on the leaves it may be asked where the fungus has come from. The fact that the oospores are formed in the soil where the fungus can grow saprophytically may partially answer this question. It is also more probable that the oospores which are produced very abundantly in the diseased tissues are liberated into the soil when pieces of dead leeks are left in the soil. In fact it is quite customary for growers to plough or dig in patches of leeks which are too badly diseased for consumption. There is no fear but that the soil gets badly contaminated with oospores. These will germinate under natural conditions, it is supposed, although no laboratory conditions have allowed this to be verified. That is supposing that the ordinary mycelium of the fungus is killed by the winter conditions. The germination of the oospores in the Spring will re-infect the soil with the active stage of/  
of/

of the fungus. It might be wondered why the fungus does not grow into the base of the shoot or the roots and grow upwards, causing death more or less as the smuts do. The infection experiments seem to confirm the observations on the absence of basal infection.

It would have to be presumed that infection of the leaves was brought about by conidia produced elsewhere and blown to the leek leaves by the wind. That this is not only probable but almost certain is attested by the discovery that the conidia are produced on the surface of the soil, in large boxes used in the infection experiments as well as in petri dish soil cultures. That these were smaller than those produced in water cultures may be an indication of their conidial character, and not of a sporangial character, in which case they would more readily be blown off their conidiophores and fall on the leaves.

Presuming this explanation correct for the primary appearance of the disease, there is yet the secondary spread. It cannot be probable that every plant is infected by conidia produced on the soil in its immediate vicinity. But, on the other hand, it is not improbable that infected leaves produce a crop of conidia which are distributed by the wind and by splashing rain, and even by more mechanical agencies such as insects and man himself. If this is so, then the increasing area of infection would follow as a matter/

matter of course, as in such diseases as Potato Blight. This supposition is supported by the fact that conidia are produced in the leaves although these were not found on the outside but inside. It is likely that in nature a sufficient number of conidia are produced on the outside of the leaf to enable spread.

The spread is not rapid and has been reported by various growers as spreading down the rows. Some stated that spread occurred in any way, while others connected it with the direction of the wind. The writer's observations on this point are varied. In the majority of cases, spread seemed to follow the direction of the wind. In October 1928, when the first spraying experiments were conducted the disease was commencing at the east end of a large field on a hill. Very soon after it appeared on the leeks at the west end of the same field, 200 to 300 yards separating the two ends. Following the west end infection, a field of leeks in a valley below went down to the disease. This spread was almost due north east to south west, and this direction is also that of the prevailing wind. The connection in that case between the spread and the wind seemed fairly certain. Yet in other cases, particularly during this past winter, the spread was almost negligible, small or large scattered areas occurring in fields with a sharp demarcating line between healthy and diseased leeks. Often the areas were oddly shaped and no explanation seemed/

seemed to fit the case so well as one that the leeks were being infected directly or indirectly (by conidia from the soil to the leaves) at the same place by contaminated soil. The grower remembered that in several of these cases there had been bad patches of the disease the previous season and that the infected leeks had been ploughed in. In the other cases he could not remember what had preceded the crop.

Then there is the remarkable fact that this disease does not appear till late in the season. In the season 1928-1929, the disease began in September, in 1929 - 1931, in January, and in 1930 - 1931, in December. In the Cheltenham district, in England, the disease may commence in August, but chiefly spreads in October to December, according to Ogilvie who is keeping the disease under observation in that area. That the seasonal variation is connected in some way with temperature is quite likely; yet temperature cannot be entirely the cause. If the disease were connected with low temperatures why should it start in August in the south of England and in September in Scotland, for if anything, the low temperatures would be later in appearing in England than in Scotland. Higher temperatures might explain the difference between the two localities and to a less certain extent between the seasons. Infection might take place at the higher temperatures of July or early August in both localities, but the higher range in England might lessen the incubation period, and/

and so the first signs appear in August, while the lower range in Scotland, although high enough for infection, might increase the incubation period as compared with England. This may be quite likely. It is significant that the mean temperature for the Bristol area, Bath being the station, including Cheltenham, is  $61.5^{\circ}\text{F}$ . in July,  $60.7^{\circ}\text{F}$ . in August, and  $56.7^{\circ}\text{F}$ . in September, while for the Edinburgh district, Leith being the station, the mean is  $58.6^{\circ}\text{F}$ .  $58.3^{\circ}\text{F}$ . and  $54.7^{\circ}\text{F}$ . for July, August and September respectively. The optimum temperature for conidial production is  $15-18^{\circ}\text{C}$ . ( $59 - 64.4^{\circ}\text{F}$ ), and the fact that the Bath mean temperatures for July and August fall within the conidial optimum, while those of Leith do not, suggests that there may be some correlation there. As explained elsewhere, the experiments have not come on rapidly enough to give experimental data on those questions. So the question must be left at this point.

Morphology/



MORPHOLOGY AND LIFE HISTORY.Cultural Studies.

When Phytophthora Porri was obtained in culture, the question arose as to whether it was identical or related to some other species of the genus, although no other species had been recorded as attacking the Leek. Therefore it was grown on a series of media with the object of elucidating this point.

Quaker Oat Agar. It grows very well on this medium, producing a dense mass of white, aerial mycelium but not a great quantity of submerged mycelium. The sexual organs are produced abundantly on the aerial and submerged hyphae. Conidia are also formed on the aerial mycelium. In petri dish cultures, it is characterised by an edge of about 5mm., which is purely surface mycelium. This edge increases, as the colony increases, to about 12mm.. There is a tendency towards striation, that is, the centre is densely aerial and white, and from this white, aerial masses radiate to the edge in regularly spaced lines. The greatest growth is made upon this medium.

Maize Meal Agar. On this medium, made fairly coarse, the growth is rather similar to that on Quaker Oat Agar, but it is not so dense. The asexual and sexual organs are produced in this quite well.

Potato/

Potato Agar. A fairly dense, white, aerial mycelium is produced on this and again both asexual and sexual organs are found in abundance.

Potato Dextrose Agar. The growth is similar to that on Potato Agar cultures, but conidia are not produced, at least not in seventeen days, while oospores are fairly abundant.

Malt Extract Agar. Growth is very poor and exceedingly slow, aerial hyphae being comparatively absent. The colony has the appearance of solidity on the surface of the medium and radial striations are slightly noticeable. Sexual organs are produced but after a long time: four month cultures usually provide a few.

Clear Maize Agar. Growth is rapid and regular, but aerial mycelium is sparse compared with Quaker Oat and Maize Meal Agar cultures. Sexual organs are produced. As this medium is very transparent, it was used almost entirely for observing the formation and development of the sexual organs.

Nucleic Acid Agar. Growth is slow and almost entirely submerged. Sexual organs are not produced.

The various species which are comparable with Phytophthora Porri with regard to the size of spores have the following cultural features.

P.hibernalis: -

Quaker Oat Agar. Growth is mainly submerged and on the surface, but here and there sparse aerial mycelium is produced.

Potato/

Potato Agar. Aerial mycelium sparse.

The edge of the colony is more dense than in Phytophthora Porri, but the mycelium branches in the same manner.

Potato Dextrose Agar. Growth is not so rapid but is densely aerial. Carne( 17 ) reports conidia and oospores as being produced freely.

Grown side by side, there is no real similarity between this species and Phytophthora Porri.

Phytophthora Pini: -

Quaker Oat Agar. Growth is vigorous and a felted, grey-white, aerial mycelium, only about 1 to 2mm. high is produced, never a dense, white, cottony mass as in Phytophthora Porri. Sexual organs are abundant.

Malt Extract Agar. Growth is good and a felted, aerial mycelium is again produced. Sexual organs present.

Clear Maize Agar. Growth is very rapid, being about three times as vigorous as Phytophthora Porri. There is little aerial mycelium. The edge and branching is different to those of Phytophthora Porri.

Phytophthora Richardiae: -

Quaker Oat Agar. Growth is good and the aerial mycelium is white but not very dense. Sexual organs are produced.

Malt Extract Agar. Growth is good, a little aerial mycelium is produced, but no sexual organs.

Clear/

Clear Maize Agar. Growth is rapid and twice as rapid as Phytophthora Porri.

Comparison is made between the rates of growth of Phytophthora Porri, Phytophthora Pini and Phytophthora Richardiae, in Plate 23 and in the brief table below; while in Plate 24, fig. 52 is shown colonies of Phytophthora Porri on Quaker Oat Agar and Maize Meal Agars.

Table 13

Comparative Growths on Malt Extract Agar.  
(Mean Diameter).

<u>Age.</u>	<u>Phytophthora Porri</u>	<u>Phytophthora Pini</u>	<u>Phytophthora Richardiae.</u>
1st Day.	7 mm.	7 mm.	7 mm.
4th "	15 mm.	34.5 mm.	21.5 mm.
5th "	21 mm.	49.5 mm.	30 mm.
6th "	23.5 mm.	61.5 mm.	38 mm.
7th "	27 mm.	70.5 mm.	44 mm.

Influence of Depth of Medium.

To see if there was any effect on the diameter of colonies of Phytophthora Porri by varying the depth of the medium, petri dishes were poured with 10, 12.5, 25 and 50 ccs. of Quaker Oat Agar. The fungus was inoculated into the centre and the cultures kept at 25°C. The results are shown in the accompanying table 14.

Table /

Table 14

Diameter in mm. of Phytophthora Porri  
on Quaker Oat Cultures.

<u>Age.</u>	<u>10ccs.</u>	<u>12.5 ccs.</u>	<u>25 ccs.</u>	<u>50 ccs.</u>
5 days.	39	36	23	23
7 "	48	44	38	35
11 "	80	77	?	50

It is therefore shown that the diameter of a colony of this fungus is influenced by the depth of the medium up to approximately two and a half times the depth of the usual poured plate, i.e. 2-3mm., after which the diameter remains the same. This may be interpreted as meaning that the mycelium cannot grow in medium which is removed from reasonable aeration. In other words, the fungus is aerobic.

LIFE/



LIFE IN THE SOIL.

As with the Phytophthora from Belladonna, this fungus was inoculated into petri dishes containing ordinary garden soil which had been sterilised. The soil was kept just damp in two dishes and very damp in another. The fungus did not grow in the very damp soil but grew well in the moderately damp soils. One of these dishes was kept in the dark and the growth in this was much more than in the dish exposed to light. Transfers were taken to fresh dishes of soil from the edges of the growing soil colony. The fungus continued to grow and proved thereby that it grows independently of the culture medium with which the soil was inoculated. Therefore it is capable of a saprophytic life in the soil.

It was found that the fungus produced conidia abundantly on the surface of the soil. Oospores are also produced in the soil and, no doubt, serve to carry the fungus through adverse periods. The number of Phytophthoras which are known to live saprophytically in the soil, as well as parasitically, has increased particularly as a result of De Bruyn's work which was discussed before.

MORPHOLOGY.General Description.(a) Mycelium.

The mycelium of this fungus is typical of the genus but exhibits the characteristics to an extreme degree. It is very granular and branches only moderately. Septa are rare in young cultures, but fairly frequent in old cultures, particularly those on rich media. The species has a characteristic which tends to differentiate it from other species; this is its great tendency to coil into one or more spirals and this is more noticeable in the region of sexual activity. In Plate 24 fig. 53 is shown a microphotograph of the mycelium on Clear Maize Agar illustrating its mode of branching and in Plate 24 fig. 53 and Plate 25 fig. 55-56 is the same of the coiling. For comparison a microphotograph of the mycelium of Phytophthora hydrophila is given in Plate 24 fig. 54 and the dense branching and knotting illustrates a type of the many variations in the genus. As far as the writer is aware there is no other species that shows coiling as Phytophthora Porri. In none of the cultures of twenty-eight species in the writer's possession has this feature been noticed. It may be present in some species or strains which the writer has not been able to obtain, but no photograph or drawings seem to have been published which show the coiling referred to. The mycelium is also rather unequal/

unequal in width and often swells out into large, long vesicals<sup>le</sup> which are certainly not any form of spore. This is shown in Plate 27 fig. 62. It is often swollen considerably where it branches. The submerged hyphae seem very rich in fats, for when they are broken, immense quantities of fat globules escape. When fairly old the mycelium becomes empty as in most species in the genus.

(b) Sexual Organs.

These are produced quite abundantly in several media, such as Quaker Oat, Maize Meal and Clear Maize Agars. They are also produced very readily in the diseased tissues of Leeks. Sterile pieces of mycelium, produced in the method used by Leonian (32), formed them in certain liquid cultures as well. In this species both types of antheridia are produced abundantly, but the proportion of the one to the other varies with the medium or substratum in which they are formed. On Quaker Oat Agar the paragynous type is very dominant, but the amphigynous type is still common. On Maize Meal Agar the proportion is practically 50-50, while on Clear Maize Agar the type is almost entirely amphigynous.

There was a chance that the amphigynous and the paragynous antheridia were produced by two different species obtained in culture, one or other or both responsible for the disease. It would no doubt have been difficult to have separated these had this been correct. By very careful tracing of the two types of/

of antheridia, the hyphae which produced them were seen to arise from a common mycelium. But it is exceedingly difficult to demonstrate; in fact, so far as any other observers are concerned, they will have to decide this point on their own, for apart from verbal statement there is no photographic or even drawing proof of this point. This will be realised when it is stated that the several hyphae of the two types were traced back to their common origin over a distance of five to seven millimetres. Cultures which had been taken from the edges of colonies in petri dishes, pieces having a quantity of branching mycelium being used, gave both types of antheridia. The small mass of mycelium taken was seen to arise from a single hyphae. Therefore this should dispel not only any doubts as to whether two species were present but also any doubts as to the presence of heterothallism in the genus. Narasimhan ( 38 ) found that certain stains isolated from various plants in Mysore were non-sexual but that when two were paired, sexual organs were formed, while when one of these and still another strain were paired, no sexual organs were formed. He argued from his results that one strain was female and the other two male, and only when one or other of the male strains were paired with the female strain could sexual organs be produced. But although heterothallism occurs in some species it does not follow that every species will be heterothallic.

In/

In the case of Phytophthora Porri and other homothallic species, there may be internal physiological characters which determine the production of the two types of antheridium, and their proportion may be further influenced by various external factors.

The Amphigynous antheridium is produced in the same manner as already described for Phytophthora erythroseptica var atropae. This account need not be repeated.

The Paragynous antheridium is produced and attaches itself to almost any part of the oogonium, sometimes being so close to the stalk of the oogonium that it appears to be amphigynous. More than one may be attached to the one oogonium sometimes, and once or twice an oogonium had both an amphigynous, and a paragynous antheridium, an example of which is shown in Plate 25 fig. 58 only the stalk of the amphigynous antheridium is not in focus in the photograph. Multi-paragynous fertilisation is shown in Plate 25 fig. 57 . The various stages of both types of fertilisation are shown in Plate 27-28.

The oogonial wall is rather thicker than in some species and is hyaline. After the oospore is mature, it tends to collapse and presents a very irregular shape. The oospore is spherical, usually light yellow in colour, with a wall which is thicker than usual. It usually does not fill the oogonium. Not only is the oogonial wall liable to collapse, but it is often irregularly thickened. This is also found in/



in the oogonia of Phytophthora Lepironiae.

(c) Asexual Organs.

Conidia are the only asexual form so far found in this species. They are produced in the diseased leaves as well as in artificial culture, but in the latter, they are not very abundant. They are produced more readily if pieces of pure cultures are placed in water and certain liquid cultures. They are inversely pyriform, with or without an apical papilla. When this is present it is broad more often, though a beaked kind is also sometimes formed. The hyaline thickening at the apex is not very prominent and might be said to be generally shallow. The non-papillate form is far the more common. They may germinate either by germ tube or by zoospores. The formation of the zoospores and their emission is similar to that already described for P. erythroseptica var. atropae. The zoospores germinate by a germ tube which develops into a mycelium. But in this species repetitional diplanetism has been found, though only once, nor could the phenomenon be reproduced. When the zoospores are emitted, they swim only a short distance, then settle down, form a wall and germinate by a short tube from the end of which another zoospore is emitted. In other words, the primary zoospore acts as a sporangium. All the contents of the first zoospore pass into the new zoospore and this breaks away, swims normally and ends up with germinating in the usual/

usual way to produce a mycelium. Drechsler ( 20 ) found another type of diplanetism in which the zoospore after encystment produces a long, slender germ sporangio-  
-iophore, at the tip of which a small sporangium is formed. This may also be formed from an unsuccessful evacuation tube. The miniature sporangium forms one zoospore from its contents and this is liberated. The latter method has not been found in this fungus so far. It often happens that the zoospores are not liberated from the sporangium, and these encyst and germinate by a tube, which may grow through the sporangium wall. The numbers of zoospores produced in the sporangium depends upon the size of the latter. Over twenty have been counted from one sporangium, while in others, a few were left inside the sporangium and others were bunched outside; the numbers could not be determined with certainty, but they were between fifteen and twenty. A small kind of sporangium has been found in this fungus which is more spherical than the normal type, and forms only a few zoospores, perhaps three to five. These were not the miniature sporangia produced from zoospores referred to before. They have not been found again. They were produced in a hanging-drop culture on Clear Maize Agar, the same in which the diplanetetic zoospores were produced. Neither have been seen in liquid cultures as described by Dreschler. According to him, the direct type of repetitional development of the zoospore was observed in *Phytophth-*  
-ora/

Phytophthora Fagi, the indirect type in Phytophthora hibernalis and both types in Phytophthora parasitica and some strains referable to it, and Phytophthora cactorum, Phytophthora citrophthora, Phytophthora melongenae and a strain from Honey-dew Melon.

The direct germination of the sporangium by a tube may take place either at the apex or just below it. The sporangia are produced at the tips and also intercalarily. The sporangiophore may be inserted in the usual place, at the centre of the broader end, or on one side. No plano-convex sporangia have been noticed, as was found by **Leonian** in Phytophthora Pini. A few sporangia, both in water culture and in tissue, have a pedicel. Most of these stages, described above, are figured in Plate 28.

Measurement/

MEASUREMENT OF THE FUNGUS.(a) The mycelium.

This varies rather considerably according to whether it is aerial, when it is thin, or in the substratum, when it is thick. Rich media tend to produce very variable hyphae which swell out into odd shapes. There is no stability in the mycelium in the tissue either. The average width approximates 6-8 microns and the range is about 2.4 to 12 microns.

(b) The Sexual Organs.

The same precautions were taken with regards measuring only mature organs as were described for Phytophthora "atropae"

Table 15Measurements in microns of the Sexual Organs.

<u>Sub-Stratum</u>	<u>Oogonia</u>		<u>Oospores</u>	
	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range</u>
Quaker Oat Agar.	38.367	46-29	32.369	39-23
Water	37.864	44-33	31.679	39-22
Bog Water	38.966	44-32	33.144	39-27
Mg.NO <sub>3</sub> (1:1000)	39.123	46-34	32.272	39-27
Tissue	34.596	44-29	29.022	36-19

The writer's figures which were given in the first record of the disease ( 22 ) are reproduced for comparison. It is seen that enough oogonia were not measured to give a representative value. The figures according/

according to **Ashby**, who examined cultures sent to him by the writer are also given.

Table 16

Other Measurements in microns of the sexual organs.

	<u>Oogonia.</u>		<u>Oospores.</u>	
	<u>Mean</u>	<u>Range</u>	<u>Mean</u>	<u>Range.</u>
Writer(1929) Tissue	---	39-27	27.0	31-20.
Ashby(1931)Culture	42.7	46-37	35.0	40-32.

Although **Ashby** also made his measurements of the fungus on Quaker Oat Agar cultures, it is seen that his mean for oogonia is approximately 4.3 microns and for oospores 2.6 microns higher than the writer's results. His upper range for both oogonia and oospores is almost identical but his lower range is higher than the writer's ranges. It is not known but it is probable that Ashby did not measure as many of the organs as the writer, who measured on the average 100 but in some cases, such as Quaker Oat cultures, 200 were measured. A great mass of statistics are not being given here, but the results of analysing these can be stated. It is believed that an approximate mean, within the limits of experimental error, can be obtained if when measuring spores small groups be taken well scattered. The usual method of measuring all the spores in a preparation takes a great time and great care, if a repetition of measured spores is to/



to be avoided. The writer usually takes small bunches of spores in fairly well separated areas in a slide and measures about ten, sometimes twenty spores. In one slide one hundred to two hundred can be measured without much bother. If then this is repeated in more slides, just a few here and there, it appears that <sup>a</sup> true average is obtained just as well as if 500 or more were laboriously measured on one slide. In fact if the measurements are very scattered and mature organs always taken, an average still within the experimental error limits can be found with only 50 to 100 measurements. In the figures presented it is noticed that a variation between the means as determined for different substrata covers about 2 microns, for both oogonia and oospores, if the host results are excluded. Considering the variation recorded by workers on other species in the genus, this 2 micron variation is very reasonable. The range variations, of course, are greater, but they are not sufficient to prevent a decision as to the probable true range. Not that the range is of great value in identifying any species, the mean being of far more value. Ashby may have measured all the large spores and so obtained higher values. But although the large organs are abundant, so are the medium and smaller ones, and so these should not be excluded. There is as much reason for measuring only the small organs, provided they are abundant, as for measuring the large. There is a certain amount of danger in excluding certain spores, just because one has observed another size as /

as being abundant. It is quite probable that on careful analysis just as many of the excluded spores are present as those taken. Rosenbaum ( 45 ), also argued on these lines and presented graphs which proved this theory. The only difference is that Rosenbaum measured great numbers and did not measure an ordinary number which were very scattered. The above discussion was considered necessary to explain the omission of fuller statistics.

It is to be noticed that the oogonia and <sup>in the host</sup> the oospores/are much smaller than those in solid and liquid culture. This is not unusual, for several species in the genus also have this characteristic, for example Phytophthora cryptogea and Phytophthora Richardiae. These can not therefore be compared with the recorded measurements of other species, unless they also have produced their sexual organs on the host too.

The mean of the organs may therefore be stated to vary between 38 and 39 microns for the oogonia and between 32 and 33 microns for the oospores.

The antheridia average 12.5 microns but range from 7.3 to 19.4 microns. One abnormal one was found in Magnesium Nitrate solution and measured 24.2 microns. The amphigynous antheridia are usually larger than the paragynous, roughly being twice the size.

The/

The oospore wall which has three layers as described for P. "atropae", varies between 3 and 4.5 microns thick, the average being about 4 microns. The outer layer is about .4 microns and the inner .2 microns, the rest, 3.4 microns, being the middle layer. It is rather a thick wall when compared with some other species.

(c) The Asexual Organs.

The conidia also vary in size according to the conditions of production. On the leaf they tend to be larger than when they are produced in solid culture or in liquid culture. Only normal, mature spores were measured.

Table 17.

Measurements in microns of Conidia.

<u>Sub-</u> <u>-stratum.</u>	<u>No.</u>	<u>Mean</u>	<u>Range</u>	<u>Mode.</u>
Host	100	58 x 42	75-37x48-31.	1.38.
Water	200	51 x 35	82-31x52-23.	1.46.
Soil	10	46 x 32	55-31x42-28.	1.44.
<u>Ashby: -</u>				
Water	?	47.9x32.9.	62-33x45-25.	1.46.

The question arises as to what measurements are to be taken for comparative purposes. These  
of  
conidia are not like those/Phytophthora infestans  
which are produced naturally and abundantly aeri-ally  
on the leaf, or on diseased tubers when cut and kept  
in a humid atmosphere. They are produced in culture  
but not enough to use them for metrical purposes, part-  
-icularly/

particularly when they are produced abundantly in liquid culture. For this purpose a survey of the species thought to be nearest to this one must be made. This will be deferred for the moment and discussed under the subject of taxonomy.

The wall of the conidia is approximately 2.4 microns thick except at the apex. There is a plug at the point of production of the conidium on the conidiophore. The proportion of the width to the length of the conidia is rather constant and is 1.46. A few only show a variation which gives a mode up to 2 and down to 1.1. This means that these are either twice as long as they are broad or that they are almost spherical.

The papillae when they are present are about 5 microns high and the width of the apex is about 10 to 12 microns. The beaked form of papillae are usually 12 microns long.

The zoospores vary in size. The usual size is about 10 to 15 microns, that is when they have stopped swimming and rounded off. But the kind which produced secondary zoospores were larger averaging, when they had rounded and produced the emission tube, 18.7 microns, and ranging 17.4 to 20.4 microns. The tube that is formed is about 5 microns long but longer ones, up to 10 microns are also produced.

PHYSIOLOGY.The Relation of Temperature.

Petri dishes of Maize Meal Agar were inoculated with a 3mm square of <sup>a</sup> culture of Phytophthora Porri and grown at three different temperatures, in an incubator at 25° in the laboratory at 18-20°C. and in the glasshouse at 8-10°C. The rates of growth are very different.

Table 18.Diameter in mm. of P. Porri.

<u>Day</u>	<u>8 - 10°C.</u>	<u>18-20°C.</u>	<u>25°C.</u>
1st	3	3	3
3rd	7	-	-
4th	13	-	-
5th	-	25	39
7th	-	37	48
8th	20	-	-
9th	25	-	-
11th	-	65	80

It is obvious from this that the fungus grows best, vegetatively, at 25°C. and the minimum must be below 8°C.. Cultures kept in an incubator running between 30 and 33°C. hardly grew at all, while cultures at 40°C. were killed.

It may be concluded that, within the ordinary limits of variation, the minimum, optimum and maximum temperatures for the vegetative growth of Phytophthora Porri/



Phytophthora Porri are below  $8^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$  and  $33^{\circ}\text{C}$ , while the death point is at or slightly below  $40^{\circ}\text{C}$ .. It will be seen from this preliminary experiment that this fungus corresponds with Phytophthora "atropae" in its thermal relations. This is rather peculiar as the temperatures at which the disease progresses are much lower and has been discussed before.

Temperature also influences the production of conidia in liquid cultures, though not entirely. Conidia are produced at  $23$  to  $25^{\circ}\text{C}$ . but are very few and are very small and slender. They are really abnormal. At room temperature,  $18-20^{\circ}\text{C}$ , they are produced fairly well, but abundantly at  $15$  to  $18^{\circ}\text{C}$ . At  $10^{\circ}\text{C}$ . no conidia are produced. These relations seem to place the leek Phytophthora in the temperate group. Temperate species chiefly produce conidia at temperatures between  $16$  and  $25^{\circ}\text{C}$ , while tropical species require temperatures above  $20^{\circ}\text{C}$ , maximum production occurring at  $27$  to  $30^{\circ}\text{C}$ . For example, Jones, Giddings and Lutman ( 28 ) found the maximum number of conidia of Phytophthora infestans at  $16-18^{\circ}\text{C}$ , but not below  $10^{\circ}$  or above  $23^{\circ}\text{C}$ .. With the same fungus other workers obtained higher temperatures for the best conidial production; Vowinckel ( 56 ) obtained  $19-22^{\circ}\text{C}$ . and Melhus ( 35 )  $22-25^{\circ}\text{C}$ . Rose ( 43 ) found the optimum temperature for Phytophthora Cactorum was between  $10^{\circ}$  and  $20^{\circ}\text{C}$ . Uppal ( 54 ) found  $22-23^{\circ}\text{C}$ . was optimum for Phytophthora Colocasiae. Waterhouse ( 57 ) found that her cultures of Phytophthora

Phytophthora Fagi produced conidia in three days at 15-20°C. but none at 22°C. Ashby ( 5 ) found that the more tropical species Phytophthora parasitica had an optimum conidial temperature of 27-30°C. and Reinking ( 42 ) with Phytophthora Faberi (= Phytophthora palmivora) found an optimum of 27-30°C. also but no conidia were produced below 20°C.

Although conidia of Phytophthora Fagi were produced best at 15-20°C. according to Waterhouse, the best vegetative growth of the same fungus was at 22°C. This is the same as Phytophthora Porri, although the temperatures are not exactly the same.

#### The Relation of Light.

Experiments have been carried out by many workers to test the relation of light to the growth of fungi in culture. But the results in the genus Phytophthora are negative. The writer also compared the rate of growth of cultures kept in the dark and those in ordinary light. There was a difference, but so little that it could be caused by experimental variation.

As far as could be determined, light had no influence on the production of conidia either. Waterhouse ( 57 ) found the same although she thought once formed, light might have an effect on the abundance.

The relation to Leonian's Physiological Key.

The method used by Leonian ( 32 ) for determining the effect of chemicals on the production of conidia and oospores in culture was used by the writer. The procedure was as follows. A small portion of a Quaker Oat culture of the fungus was placed in a petri dish containing a nutrient solution made up according to Leonian's formula. As no growth was made in this culture, it was taken out and washed in running water for an hour and then transferred to a petri dish with fresh nutrient solution. After three days good vegetative growth had been made but as there were a few bacteria, the colony was again washed in running water and replaced in fresh solution. The growth was now very good, and two small pieces were teased off the large colony with sterile forceps and one each placed in a dish (4 and 4a). A set of petri dishes were now poured with solutions containing 1/100th the molecular weight of various chemicals and each inoculated with a piece of colony from dishes 4 and 4a. The results are given in Table 19 .

Table/

Table 19.

Effect of Chemicals on Spore Production in P.Porri.

Aspartic Acid	None.
Potassium Nitrate	Sporangia.
" Carbonate	None
" Acid Phosphate	None
Calcium Nitrate	Sporangia
Sodium Chloride	Sporangia
Ammonium Nitrate	None
" Sulphate	None
Magnesium Sulphate	Sporangia
Dextrose	None
Glycine	Sporangia. Oogonia.
Levulose	Sporangia
Mannose	None
Leucine	None
Nutrient Solution	None.

Oogonia are produced on Malt Extract Agar and no oogonia and no sporangia are found in Nucleic Acid Agar.

By the colonies produced on Malt Extract Agar and the absence of sporangia in Aspartic acid group 6 of Leonian's key is reached and the formation of oogonia in Malt Extract Agar places the fungus in group 7. The absence of sporangia in leucine or Ammonium Sulphate would identify the fungus as Phytophthora Pini. If it were conceded that the number of oogonia on Malt Extract Agar were too few and could be taken as equalling Class I of Leonian's analysis this would take the fungus in to group 9 of the key. No sporangia are produced in nutrient solution and no chlamydo spores are produced in any medium. The finding of sporangia in Potassium Nitrate and in Sodium Chloride solution but not in Mannose solution, brings the fungus down to group 15. As sporangia are produced liberally, at room temperature, in/

in water although not in leucine, would identify the fungus as Phytophthora Nicotianae. No other than the two species mentioned can be considered from the results of the physiological experiments and using Leonian's key. Whether there are reasons otherwise for accepting or refuting one or both of these two species will be discussed next.

TAXONOMY/



TAXONOMY.

There are five main headings under which this subject can be discussed, and each of which have been used as a means for classifying the species of Phytophthora. These can be reviewed separately for purposes of clarity.

A. Parasitology. When the first members of the genus Phytophthora were erected, there was a belief that each species was a specific parasite, attacking only the host plant on which it was recorded. With other groups of fungi, the tendency arose to identify a species by its host till the situation became impossible. Stevens expresses the position very well when he quotes Cobb as writing: - "Is a fungus species newly found on a peach? Call it new and name it pruni. Same genus on the grape, name it ampelinum. On the apple? New, call it mali. On banana? christen it musae. What next? Sparrow in a pear tree, Passer pyri?" (p. 478). Although the situation has never been so hopeless in the genus Phytophthora as in many genera in the Imperfecti, there has nevertheless been a tendency to name new species on host qualifications. There is P. infestans on the Potato. A slight variation of the same fungus was found on Phaseolus lunatus in America and named P. Phaseoli (59). Investigations have since shown this to deserve a variety name only, P. infestans var. phaseoli. Villada erected /

erected a new species P. Agaves on Agaves sp., yet gave no details of the fungus to support his action (55.). Nothing further has been heard of it and it is probable that a connection might have been found between his fungus and the other tropical species. It should, no doubt, have been left as an undetermined species. Coleman (18), on finding a Phytophthora on Theobroma erected P. Theobromae, and yet it has been found to agree with P. palmivora. Maublanc (34) erected P. Faberi on the same host and Coleman afterwards withdrew his binomial. Butler (16) and Ashby (5) identify P. Faberi as P. palmivora, although they are distinct strains. A number of species have a very wide range of host plants. For example P. parasitica is found on Tomato, Tobacco, Ricinus communis, Hibiscus sabdariffa, Citrus, Rubber, Banana, Cotton. P. crypto-ogea not only attacks Tomato and Petunia, but also Tulips, Asters and other plants.

It is thus seen that because no species of Phytophthora have been found on a host, that the newly discovered one is new and worthy of a specific name. Thus the Leek Phytophthora has no right to a name such as Phytophthora Porri just because no Phytophthora had been found before on that host. It must stand specifically on all the five main characters before such a course would be justified.

As to the records of Phytophthora on Leeks, it has already been noted that no species had been recorded before the writer's record was published. In/

In the genus Allium, the only Phytophthora record is that of Sawada's ( 46 ) on Allium fistulosum, the Japanese Welsh Onion, P.Allii. Sawada attempted to infect other plants. The ordinary onion (Allium Ceba) could not be infected, but Epiphyllum truncatum (Cactaceae) was successfully infected. The injury on the Japanese Welsh Onion was not serious nor widely distributed. Comparison, under other heads, will be made later between Phytophthora Allii, and the Leek fungus. Again, even although Phytophthora Allii is on a related species of Allium, there is no reason, just because of that alone, to connect it with the Leek fungus.

#### B. The Type of Sexual Organs.

When Lafferty and Pethybridge ( 30, ) divided the species of Phytophthora into four groups, they used the sexual organs as the factor, these groups being

1. Species in which so far as is known at present the antheridia when present are always amphigynous.
2. Species in which the antheridia are preponderatingly paragynous but are sometimes amphigynous.
3. Species in which so far as is known at present the antheridia are always paragynous.

4. Species in which the mode of development of these organs is not known or in which these organs have not yet been found.

The following species are placed in these groups, according to the facts known up to the present with Group 5 with dominant amphigynous and sometimes paragynous antheridia.

Group 1.

Arecae,	palmivora = Faberi and
Cambivora (Ashby)	Theobromae.
Capsici,	parasitica emend.
Cinnamomi,	= Allii,
Colocasiae,	Melongenae,
Cryptogea,	Nicotianae,
" v.richardiae,	parasitica,
erythroseptica,	Terrestris,
hydrophila	Heveae ?
infestans	
" v.phaseoli,	
Meadii	

Group 2.

Group 3.

Group 4.

Group 5.

Cactorum	Cambivora (Petri)	(Agaves)	"atropae"
=Omnivora	Lepironiae	Boehmeria	hibernalis
Cyperi-	Paeoniae	Caricae	Mexicana.
rotundati		(citri)	
Fagi =		citricola	
Sempervivi		citrophthora	
Pini		(fici)	
" v.antirrhini		Jatrophae.	
Syringae.		Tabaci	
		Thalictri	

NOTE: - "atropae" is now P.erythroseptica v.atropae.

Those in brackets are species of which nothing further has been heard, and were possibly identical with, or strains of, other species noted here.

The/

The Leek Phytophthora has been shown to produce the two types of antheridia, the proportion varying with the medium. In the main the paragynous is dominant on standard culture media, but on other media, such as Clear Maize, and in the host tissue, the amphigynous type is dominant. In the case of Phytophthora Nicotianae, paragynous antheridia were only found by Breda de Haan ( 8 ) on cultures of infected tissue in water, while Ashby( 4 ) found only amphigynous antheridia on Corn-meal and Quaker Oat agars, using paired cultures. It therefore seems best to refer any species to its sexual grouping according to the type produced on standard media and not to less normal occurrences in nature or unusual media. Ashby ( 4 ) has already suggested this in discussing Leonian's ( 32 ) separation of Phytophthora parasitica var. Rhei into stable and unstable morphological mutations when cultivated on Malt Agar, a saccharine medium. Therefore referring to its growth on Quaker Oat and Maize Meal Agars, the Leek Phytophthora should be placed in Group 2.

It is obvious that the groups are rather artificial, for when it comes to deciding between two bona fide results which are opposite, such as Phytophthora Cambivora which Ashby puts in Group 1, and Petri in Group 3, which is to be taken? It is rather like the Imperfecti again, where a genus or species may be in more than one group. It is believed, too, that the whole truth concerning production and type of sexual/



sexual organs is yet to be known. For example, it was noted with Phytophthora "atropae" that oospores were formed on Malt Extract Agar at 18-20°C. but not at 23-25°C. Is this a true and stable character? Could this be duplicated by other workers? This is not known and it would be interesting to see if this could be confirmed. When variations occur even with a standard medium, it is going to be difficult to know when results are to be relied on. On the whole the writer considers the presence or proportion of the two types of sexual organs to be an unreliable diagnostic character. But this characteristic of a Phytophthora can be held in reserve to help in weighing up its position when other diagnostic features also have been enquired into.

The three other main characteristics, vegetative life (cultural characters), physiology and measurements can be reduced considerably when discussing their relation to the taxonomy of the Leek fungus, as those species which have characteristics far removed from this fungus need not be considered.

C. Measurements.

It was seen that the Leek fungus has the following measurements in microns: -

Oogonia:	mean, 38-39;	range, 29-46.	Culture.
Oospores:	mean, 32-33;	range, 22-39.	Wall, 3-4.5 do.
"	29 ;	" 19-36.	Host
Conidia:	" 59x42;	" 75-37x48-31	Host.
"	51x35;	" 82-31x52-23	Water.
	mode, 1.38-1.46.		

(Antheridia; mean 12.5; range 7.3-19.4).

Other species can now be compared with the above figures.

P. Allii Sawada. On Allium fistulosum only. Formosa.

Oogonia:	Mean, 20.7;	range 17-26.	Host only.
Oospores:	" 16.9;	" 14-23.	Wall. 0.5-1.5. Host only.
Conidia:	" 49.4x36.5,	range 74-40x50-30.	do.
	mode 1.38.		

(Antheridia: 8-18 x 10-14) amphigynous only.

Taking into consideration the fact that the measurements are of the fungus on the host only, there is still a great deal of difference between the sexual organs of this fungus and the Leek parasite. The conidia have almost the same range as those on the Leek leaves, although the mean is much lower. The shape of the conidia and the mode are the same in both, and both have hemi-spherical papillae, almost the same size. The Leek fungus has in addition the beaked form of papillae. But conidia are the most variable part of the /

the fungus and many species overlap. This has been included by Ashby in the "microspora" group of Phytophthora parasitica emend. This does not approach the Leek fungus.

P.cyperi - rotundati Sawada ( 49 ) On Cyperus rotundatus, Formosa.

Oogonia: mean,-----;range, 31-47x25-45. Host only.

Oospores: " 30.8; " 22-39.Wall:1-3." "

Conidia: " 45.4x25.1; " 22-67x14-34; " "

mode 1.81.

Antheridia: Para:- 13-20x11-13; Amphi:- 13-16x19-22.

It is seen that the range of oogonia approximates the Leek fungus, while the oospores have exactly the same range in both. In the translated description (from the Japanese) sent by Ashby to the writer, is "Oospores.....22-39, mostly 32; average (150) 30.8." It is peculiar that the 'mostly 32' is the same as the mean for the Leek oospores. The conidia are smaller and narrower than the Leek conidia. A short, thickened pedicel is present. These are all on the host though, and the oospores on the host in the case of the Leek fungus are 3 microns smaller and have a lower range. This fungus approaches the Leek fungus but has not been adequately described from the host alone. The disease is prevalent at 25 to 28°C. much higher than the Leek disease.

P.Lepironiae/

P. Lepironiae Sawada ( 48 ). On Lepironia mucronata,  
Formosa and South China.

Oogonia: mean: -----; range, 40-52x40-48; Wall: 4(!) Host only.

Oospores: " -----; " 30-38. Wall: 3-4 " "

Conidia: " 54x44.6 " 40-64x36-52.

Mode 1.21.

Antheridia: Paragynous, 20-24x14-16.

Here the oospores and oogonia on the host are larger than those on the Leek fungus, on the host. The conidia are smaller and broader than the Leek conidia, though similar in being mainly non-papillate. The oogonial wall is unequally thickened but thicker than in the Leek fungus. Only paragynous antheridia were found in the host tissues. This is less like the Leek Phytophthora than P. cyperi-rotundati.

Phytophthora Pini Leonian. On Pinus resinosa ( 32 ).

Oogonia: mean, 29; range 18.5-37.

Conidia: " 55.5x35. " 92.5-26x44.5-22.

mode 1.59

Antheridia: Paragynous mostly, also amphigynous.

The size of the conidia is not the same as the Leek fungus, but is near enough if only the oospores were the same size. These latter are much too small, being less than 29 microns in mean. The antheridia are mostly like the Leek Phytophthora, especially in the habit of multi-fertilisation. The cultural characters are not similar. The narrower conidia, with/

with definite or blunt papillae, of this fungus are not quite similar to the Leek conidia.

Phytophthora hibernalis Carne. On Citrus ( 17, ),

Oospores: 45.6 - 22.

Conidia: 56-17 x 28 - 10.

Antheridia: Mainly amphigynous,  
rarely paragynous.

The conidia are too small, although the average oospore size may be near to the Leek fungus. Fuller figures are not given. The cultural characters are different from the Leek fungus. It seems, in addition, a far cry from a citrus to a leek.

Phytophthora Richardiae Buisman. On Richardia ( 13 ).

Oospores: mean, 29.

Conidia: " 52 x 33.

The antheridia are entirely amphigynous, and its affinity is nearest Phytophthora cryptogea, a variety of which Ashby has made this fungus. Its growth on media is not similar to the Leek fungus.

Phytophthora Capsici Leonian ( 31 ). On Capsicum annuum.

Oospores: range, 25-35.

Conidia: " 85-35x56-21.

Antheridia: Amphigynous.

The measurements are not helpful, as they are only ranges but taking into consideration the antheridium, the host, the physiology (Leonian's key), and/



and its cultural peculiarities, none of which are similar to those in the Leek fungus, it is almost certainly not the same.

P. citrophthora (Smith & Smith) Leonian. On Citrus ( 50 , 51 , 32 ).

Oospores: range, 30 - 40.

Conidia: mean, 50 x 35, range 90-30 x 60 - 20.

The conidia are very close to those of the Leek fungus, but as it does not form oospores on Oatmeal Agar and forms sporangia and aerial hyphae on Nucleinic-acid Agar, while the Leek fungus does just the opposite, need not be considered. The cultural characters are also different.

It seems, therefore, that the species that approach in any measurement to those of the Leek fungus vary in these respects and more often in others, such as antheridial type, cultural and physiological characters. The nearest are Phytophthora cyperi-rotundati and Phytophthora Pini, but the conidia in the former and the oospores in the latter are rather stumbling blocks to associating them with the Leek parasite. The similarity is not prominent enough to grant a varietal binomial. For instance in P.Pini, the cultural characters, the oospores and partly the conidia are too divergent to connect the Leek Phytophthora with it; if only one of these had been divergent/

divergent the variety would have been justified. In the case of Phytophthora cyperi-rotundati, the size and shape of the conidia, the pedicel, the slightly larger (host) oospore and the high temperature of the disease deny a reasonable connection with the Leek fungus also.

It seems, therefore, that it is a new species. In naming it, confusion with already existing species had to be avoided, and conformity with its characteristics to be made. There has been danger in naming species from their host plants in the past, owing to the fact that very soon afterwards, the same fungus has been found on other host plants. The erection of an adjectival name has been considered, but on the whole there seemed as much objection as to the host-name. For example, in giving Phytophthora albifasciens to indicate the White Tip symptom caused by the infection, or Phytophthora albigerens for the same meaning, objection might be made on the grounds that the name did not distinguish the fungus from the host. In fact, some thirty Phytophthora species have been named after their host plants and no confusion has been made, except that made by incomplete knowledge of species, and the great variations in the various species. The fact that a species had already been erected on 'another' species of Allium - Phytophthora Allii Sawada on Allium fistulosum - was adjudged sufficient to prevent /

prevent the erection of Phytophthora Porri on Allium Porrum by Ashby. He thought Phytophthora Allii-porri would be more consonant with usage. But to the writer's knowledge, and also to others of more experience, this hardly seems correct. If Phytophthora Allii had been parasitic on Allium Porrum, or if Phytophthora Porri was somewhat similar to Phytophthora Allii, then there might be grounds for the erection of Phytophthora Allii-porri, although even then, the writer thinks a variety name would be best, as Phytophthora Allii var. porri. But the writer has decided that it deserves a distinguishing name which will definitely show that it is not Phytophthora Allii and Phytophthora Porri seems to do this. It does not seem likely at present, but should it ever be considered identical with another species already described, then it will merely take that name. But it is felt that the writer is justified in erecting Phytophthora Porri n. sp.. The diagnosis will be: -

Mycelium branched, non-septate when young, septate and empty when old, coiled in spirals, in region of sexual activity. Conidia inversely pyriform or oval, 51 x 35 (82-31 x 52-23) microns, produced at the end of conidiophores or intercalarily, usually without a papilla, occasionally with a papilla which is broad, 5 x 10-12 microns, sometimes with a beaked papilla, 12 x 10-12 microns; apical thickening shallow and hyaline, mouth of discharge, broad, germinating by a/

a tube or by zoospores, 10-15 microns, which may germinate in turn by zoospores (repetitional diplanetism) or by a germ-tube. Antheridia oval or a flattened sphere, terminal or intercalary, not on same hypha as oogonium, 12.5 (7.3-19.4) microns; oogonium spherical when mature, with an unevenly thickened wall, 38-39 (46-29) microns, fertilised mostly by a paragynous antheridium, sometimes by an amphigynous, sometimes by more than one paragynous, and even by both a paragynous and an amphigynous antheridium. Oospore spherical, honey-yellow when old, 32-33 (39-22) microns, with a thick wall 3-4.5 microns. Conidia produced abundantly in water and in the host tissue, less often in solid media; sexual organs formed abundantly in solid media, in water and in the host tissue. Parasitic on the leaves and stems of Allium Porrum L., causing watery areas, followed by a whitening of the tips of leaves. Hab. in Great Britain.

## SUMMARY/

S U M M A R Y.

Two diseases of economic plants which cause considerable loss and the fungi which cause them are described.

Atropa belladonna L. is attacked by a species of Phytophthora which causes a wilt and root rot. It is known only in Great Britain and Holland. The fungus was grown in artificial culture and its life history and vegetative characters are described. It differs from Phytophthora erythroseptica in the production of paragynous antheridia, in mixed fertilisation, and in slight mycelial characters. The measurements are almost identical. It is considered to be a variety of Phytophthora erythroseptica and its full diagnosis, based on the original of Pethybridge is given.

It is proved that it is really responsible for the disease by inoculation experiments. The best method of control was found to be Mercuric Chloride solution (1:1000). It was found to be capable of infecting potato plants and also capable of causing a rot of potato tubers, the pink flush being produced. The pink flush of infected potatoes is proved not to be a diagnostic characteristic of Phytophthora erythroseptica and a list is given of those species of Phytophthora that can also give a pink flush to infected tubers. The spread of the disease is discussed and it is considered that the conidia/



conidia which are produced on the surface of the soil help in distributing the fungus by the emission of zoospores which swim in the water present, being carried further distances by draining water. The spread is slow and is connected with the absence of aerial fructifications on the shoot system which would be distributed by wind currents. The fungus has been proved to live saprophytically in the soil and forms its oospores there, which are able to resist the winter conditions. The spread is also considered to be helped by the distribution of infected debris and by contaminated soil on cultural implements and boots. No other natural host plant has been found.

Climate is shown to have a relation to the disease both by its relation to the over-wintering, temperatures being too low for the survival of the fungus except by the oospores and by its relation to the progress of the disease, there being a connection between the summer temperatures and the temperatures at which the fungus grows best. For the fungus in culture the minimum, optimum, maximum and death point temperatures are  $4.5^{\circ}\text{C}.$ ,  $23^{\circ}\text{C}.$ ,  $30^{\circ}\text{C}.$  and below  $40^{\circ}\text{C}.$

A "White Tip" disease of Leeks is described and the economic effect discussed. It is shown not to be a new disease but an old one which had been considered to be an abnormal manifestation of quite another disease, namely, Mildew caused by Peronospora Schleideni. It is not known except in Great Britain and/

and the fungus is stated to be a new record of a Phytophthora species. The fungus has been isolated in pure culture, the procedure being fully described, as great difficulty was experienced. Its life history and cultural characters are described and its characteristics compared with other species. It is decided that it is a new species and is named Phytophthora Porri n. sp., an English diagnosis being given. Repetitional diplanetism has been demonstrated in this fungus.

The importance of the type of sexual organs is discussed and it is decided, for both diseases, that they are not a main diagnostic feature, but that they may be used as confirming evidence in decisions regarding the specificity or variety of a Phytophthora.

The fungus was proved capable of living saprophytically in the soil, where oospores and conidia were produced. The progress and spread of the disease are discussed and infection from the soil via the roots or stems is not supported, but evidence is brought forward to support the theory of leaf infection as the first stage of the disease. Wind is put forward as a contributory agent in the spread of the disease. Climate is also related to the seasonal appearance and the earliest dates at which the disease appears seems to be connected with the difference in the incubation period caused by the different temperatures.

Infection/

Infection may be connected with natural mean temperatures approximating the temperature optimum for conidial production.

Control measures have not been tested owing to the fungus having only been recently isolated and used in controlled experiments.

REFERENCES.

1. ALCOCK , N.L.,      A preliminary Note on a *Phytophthora* on *Atropa belladonna*.  
Pharmaceutical Journal cxvi,  
3254, p. 232, 1926.
2.        "        "        "      A *Phytophthora* on Belladonna.  
Read only to the Edinburgh  
Botanical Society, March, 1926.
3. ALCOCK, N.L., HOWELLS, D.V., & FOISTER, C.E., -  
Strawberry Disease in Lanark-  
shire. Scottish Journal  
Agric. xiii, 3, July, 1930.
4. ASHBY, S.F. -      The oospores of *Phytophthora*  
*Nicotianae* Br.de Haan, with  
notes on the taxonomy of *P.*  
*parasitica* Dastur. Trans.Brit.  
Myc. Soc. 13: 86-95, 1928.
5.        "        "        "      Strains and Taxonomy of *Phytoph-*  
*thora palmivora* Butler (*P.Faberi*  
Maubl.)  
Trans. Brit. Myc. Soc. xiv.  
18-38, 1929.
6. BEWLEY, W.F. -      Diseases of Glasshouse Plants.  
1923. p. 61.
7. BLACKWELL, E.M. & WATERHOUSE, G.M. - Spores and Spore  
Germination in the genus  
*Phytophthora*.  
Trans. Brit. Myc. Soc. XV:294-  
310, 1931.
8. BREDA de HAAN, J. van - De bibitziekte in de Deli -  
tabak veroorzaakt door *Phytoph-*  
*thora Nicotianae*.  
Meded. Lands. Plantentuin, 15:  
107 pp. 1896.
9. BROOKS, F.T. -      Plant Diseases. 1928. p. 81.
10. BROWN, W. -      Two Mycological Methods. Annals of  
Botany 38: 401-404, 1924.
11. BRUYN, H.L.G. de - The Saprophytic Life of *Phyto-*  
*phthora* in the soil.  
Meded. van de Landbouwh, 24:4,  
1-38, 1922.
12.        "        "        "      - The Over-wintering of *Phytoph-*  
*thora infestans* (Mont) de By.  
*Phytopathology* 16:121-140, 1926.

13. BUISMANN, C.J. - Phytophthora Richardiae.  
Meded. u. h. Phytopath. Labor.  
'Will. Comm. Schol' Baarn,  
xi, 1927.
14. " " - Een wortelziekte van Calla,  
veroorzaakt door een Phytoph-  
thora -soort.  
Tijdschr. over Plantenz., 33:  
2, 17-22, 1927.
15. BUTLER, E.J., - An account of the genus Pythium  
and some Chytridiaceae.  
Mem. Dept. Agric. India, Bot.  
Ser. 1: 82-83, 1907.
16. " " - Sci. Rept. Agric. Res. Inst.  
Pusa, 82; 1918-1919.
17. CARNE, W.M. - A Brown Rot of Citrus in Australia  
(Phytophthora hibernalis n.sp.)  
Journ. Roy. Soc. West-Australia.  
12:13-41, 1925.
18. COLEMAN, L.C. - Diseases of the Areca Palm.  
Ann. Mycologici 8:591-626, 1910.  
Also in Dept. Agric. Mysore, Myc.  
Bul. 11: 1-92, 1910.
19. COOPER, D. - Paragynous antheridia of Phytophthora  
spp. Phytopathology 18: 149-150,  
1928.
20. DRECHSLER, C. - Repetitional Diplanetism in the  
genus Phytophthora. Journ. Agric.  
Res. 40: 6, 557-573, 1930.
21. FAWCETT, H.S. - The temperature relations of growth  
in certain parasitic fungi.  
Univ. Calif. Publ. in Agric. Sci.  
4:8, 183-232, 1921.
22. FOISTER, C.E. - White Tip Disease of Leeks.  
Gardener's Chronicle, lxxxv, 2198,  
p.106, 1929.
23. " " - The Relation of Weather to Plant  
Diseases. Agricultural Section.  
Conference of Empire Meteorologists,  
London, August, 1929.
24. " " - Blossom Blight and Shankng of Tulips.  
Gardeners' Chronicle lxxxvii, 2253,  
171-2, 1930.
25. GADD, C.H. - Phytophthora Faberi Maubl..  
Ann. Roy. Bot. Gard. Peradeniya,  
9:47-89, 1924.



26. GADD, C.H. - The relationship between the Phytophthoreae associated with the Bud-rot Disease of Palms.  
Ann. Bot. 41: 253-280, 1927.
27. JONES, F.R. & DRECHSLER, C. - Root Rot of Peas in the United States caused by Aphanomyces euteiches.  
Journ. Agric. Res. 30: 293-325, 1925.
28. JONES, L.R., GIDDINGS, N.J. & LUTMAN, B.F. - Investigations of the Potato Fungus Phytophthora infestans.  
U.S. Dept. Agric., Bur. Plant Industry, Bul. 245, 1912.
29. KENDRICK, J.B. - Phytophthora rot of Tomato, Egg-plant and Pepper.  
Proc. Indiana Acad. Science for year 1922; 299-306, 1923.
30. LAFFERTY, H.A. & 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. Proc. Roy. Dublin Soc. 17: 29-43, 1922.
31. LEONIAN, L.H., - Stem and Fruit Blight of Peppers caused by Phytophthora capsici, sp. nov.  
Phytopathology, 12: 401-408, 1922.
32. LEONIAN, L.H. - Physiological Studies on the genus Phytophthora.  
Amer. Journ. Bot. 12: 444-498, 1925.
33. LESTER - SMITH, W.C. - Some observations on the oospores of Phytophthora species.  
Ann. Roy. Bot. Gard., Peradeniya, 10: 3, 243-257, 1927.
34. MAUBLANC, A. - Les maladies des plantes cultivées dans les pays chauds.  
L'Agric. prat. des Pays Chauds 1X, p. 314, 1909.
35. MELHUS, I. E. - Germination and Infection with the fungus of the Late Blight of Potato (Phytophthora infestans).  
Wis. Agric. Exp. Sta. Res. Bull 37: 1915.
36. MURPHY, P.A. - The Morphology and Cytology of the Sexual Organs of Phytophthora erythro-septica.  
Ann. Bot. 32: 115 - 153, 1918.

37. MURPHY, P.A. - The production of the resting spores of Phytophthora infestans on Potato tubers. Scien. Proc. Roy. Dublin Soc. 18 (N.S.): 407 - 412, 1927.
38. NARASIMHAN, M.J. - Studies in the genus Phytophthora in Mysore.  
I. Heterothallic strains of Phytophthora. Phytopathology 20:3, 201-214, 1930.
39. PETHYBRIDGE, G.H. - On the Rotting of Potato Tubers caused by a new species of Phytophthora having a method of sexual reproduction hitherto undescribed. Sci. Proc. Roy. Dublin Soc. 13 (N.S.) 35, 529-565, 1913.
40. PETHYBRIDGE, G.H. & LAFFERTY, H.A., - A Disease of Tomatoes and other plants caused by a new species of Phytophthora. Sci. Proc. Roy. Dublin Soc. XV.(N.S.) 487 - 505, 1919.
41. PETRI, L. - Un batterio parassita di alcune. Phytophthoreae. Boll. T. Staz. Pat. Veg. 7 (N.S.): 4, 457-464, 1927.
42. REINKING, O.A. - Comparative study of Phytophthora Faberi on Coconut and Cacao in the Philippine Islands. Journ. Agric. Res. 25: 267 - 284, 1923.
43. ROSE, D.H. - Leather Rot of Strawberries. Journ. Agric. Res. 28: 357-376, 1924.
44. ROSENBAUM, J. - Phytophthora Disease of Ginseng. New York (Cornell) Agric. Exp. Sta. Bull. 363: 61-106, 1915.
45. ROSENBAUM, J. - Studies of the genus Phytophthora.
46. SAWADA, K: - Phytophthora allii K. Sawada sp. nov. in Noji Shikenjō Tokubetsu Hokoku (Special Report Agric. Exper. Sta.) Taiwan, (Formosa). No. 11, 59-60, March, 1915. (Abstracted by T. Tanaka in Mycologia IX: 249).
47. SAWADA, K. - A preliminary Report of a New Phytophthora disease of Lepironia mucronata Rich. Agric. Exp. Sta. Govt. Formosa Bull. 131, 1919.

48. SAWADA, K. - Nozemia lepironiae Saw. - in Descriptive Cat. of the Formosan Fungi, pt. 1. Agric. Exp. Sta., Govt. Formosa Spec. Bull. 19, 1919.
49. SAWADA, K. - Phytophthora cyperi-rotundati Saw. in Descriptive Cat. of the Formosan Fungi, Dept. Agric., Govt. Research Inst., Formosa Rept. 27: 26-29, 1927.
50. SMITH, R. E. & SMITH, E. H. - A new fungus of economic importance. Bot. Gaz. 42: 215 - 221, 1906.
51. SMITH, R.E. & SMITH, E.H. - Further studies of Pythiaceous infection of Deciduous Fruit Trees in California. Phytopathology 15: 389 - 404, 1925.
52. STEVENS, F.L. - The Fungi which cause Plant Disease. 1913. p. 478.
53. TUCKER, C.M. - Sabal causiarum (Cook) Becc: a new host of the coconut bud-rot fungus. Journ. Agric. Res. 34: 879-888, 1927.
54. UPPAL, B.N., - Relation of oxygen to spore germination in some species of the Peronosporales. Phytopathology 16: 4, 285-292, 1926.
55. VILLADA, - Quoted by Gandara for P. Agaves - Mem. y. Rev. Soc. Cient. Antonio Alzale, 25: p. 293, 1908 - 9.
56. VOWINCKEL, O. - Die Anfalligkeit deutscher Kartoffel-sorten gegenüber Phytophthora infestans (Mont.) de Bary, unter besonderer Berücksichtigung der Untersuchungsmethoden. Arb. Biol. Reichs. für Land - u. Forstwirtsch. xlv. 1926.
57. WATERHOUSE, G.M. - The production of conidia in the genus Phytophthora. Trans. Brit. Myc. Soc. xv. 311-321. 1931.
58. WESTERDIJK, J. & LUIJK, A. van - Phytophthora erythroseptica Peth. als parasit von Atropa belladonna. Meded. u. h. Phytopath. Lab. "W.C. Scholten" Amsterdam, iv, 31-32, May, 1920.

59. THAXTER, R. - A new American Phytophthora.  
Bot. Gaz. 14: 273-274, 1889.

PLATES.



Plate 1.

Fig.1. Young shoot of Atropa belladonna showing wilting of the leaves and browning of the conducting vessels and pith at the base where it has been cut

x  $\frac{1}{5}$  natural size.

Fig.2. Older plant of Atropa belladonna showing general appearance of disease in the first stages in the field

x  $\frac{1}{10}$  natural size.

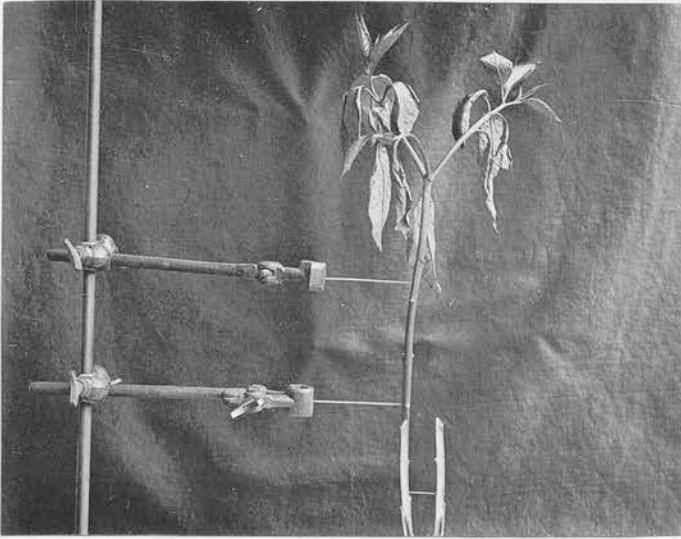


Fig. 1.



Fig. 2.

Plate 2.

Fig. 3. Normal and healthy root system of Atropa belladonna, although not maximum size.

x  $\frac{1}{5}$  natural size.

Fig. 4. Slightly diseased Atropa belladonna; same scale as Fig. 3.

x  $\frac{1}{5}$  natural size.

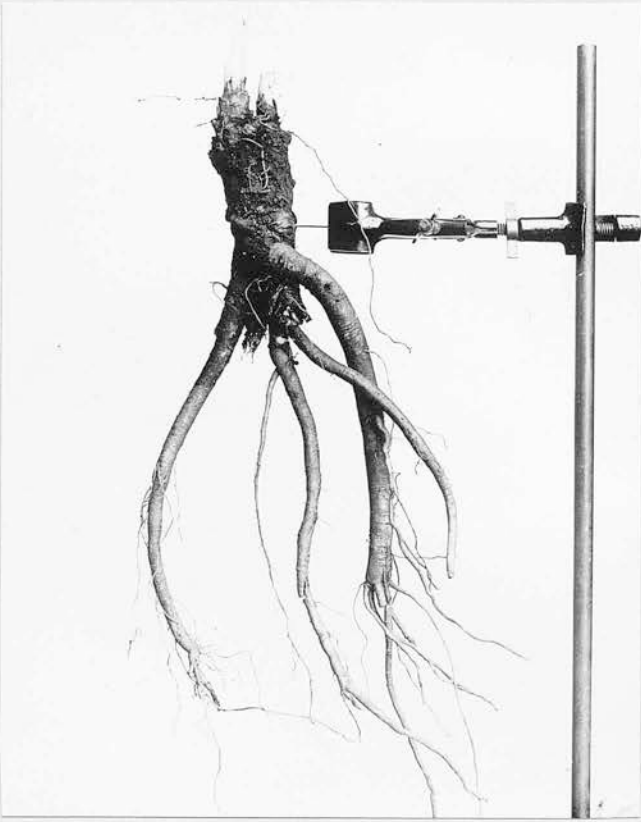


Fig. 3.

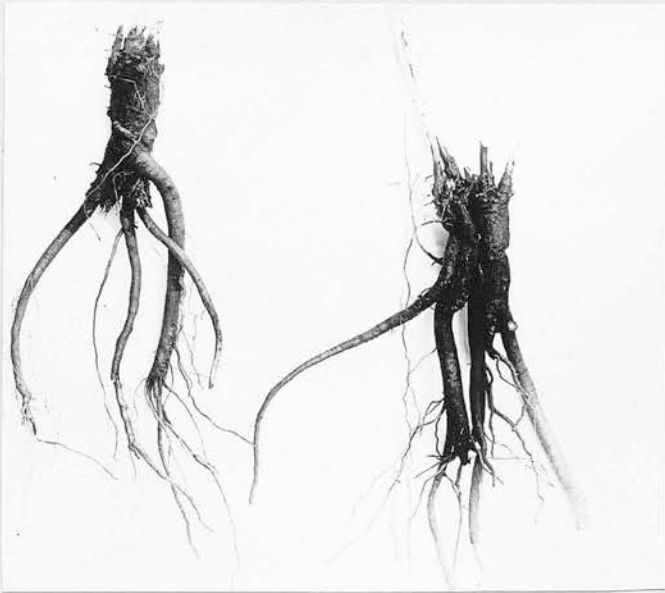


Fig. 4.

Plate 3.

Fig. 5. Badly diseased Atropa belladonna. Whereas two fair sized roots have been produced, the rest is either atrophied or rotted. Same scale as Figs. 3 and 4.

x  $\frac{1}{5}$  natural size.

Fig. 6. Micro-photograph of a hand-section of a diseased stem of Atropa belladonna, showing the broad, non-septate, branching mycelium of Phytophthora "atropae".

x 240 times.





Fig. 5.

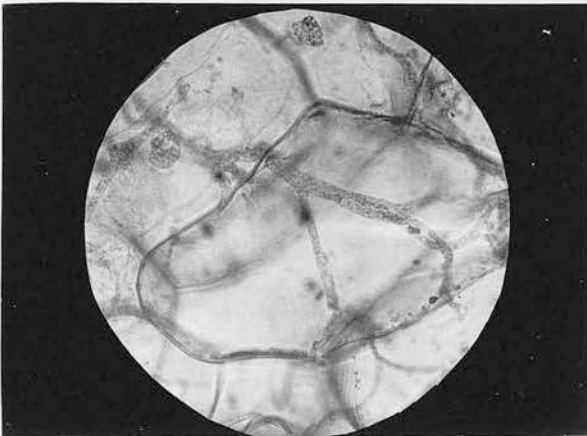


Fig. 6.

Plate 3.

Plate 4.

Fig. 7. Conidia of Phytophthora "atropae" produced from a piece of culture placed in  $\text{KNO}_3$  solution (1:1000).

Fig. 8. Infection Experiment No.1. Note that the young seedlings in the infected pot (left) have withered away, while those in the uninfected pot(right) are quite healthy.

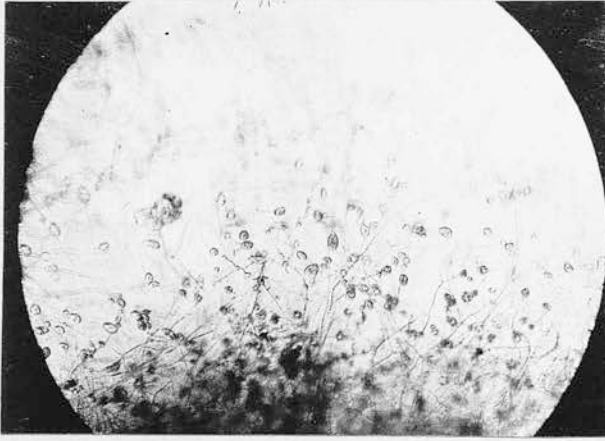


Fig. 7.



Fig. 8.

Plate 4.

Plate 5.

Fig. 9. Infection Experiment No. 2. Photographed before infection.

Fig. 10. Same as Fig. 9. Results after 14 days. Note that numbers 3, 5 and 6 are still healthy.

Treatment.

No. 1	<u>P. "atropae"</u>	in to stem below soil
No. 2	do.	" " " " "
No. 3	do.	on " " " "
No. 4	do.	in " " above "
No. 5	No infection, control.	
No. 6	" "	" "
No. 7	<u>P. erythroseptica</u>	in to stem below soil.



Fig. 9.



Fig. 10.



Plate 6.

Fig. 11. Water painting of "Pinking" of Sharpe's Express tuber infected by Phytophthora cryptogea.

Fig. 12. The same, but caused by Phytophthora erythroseptica.

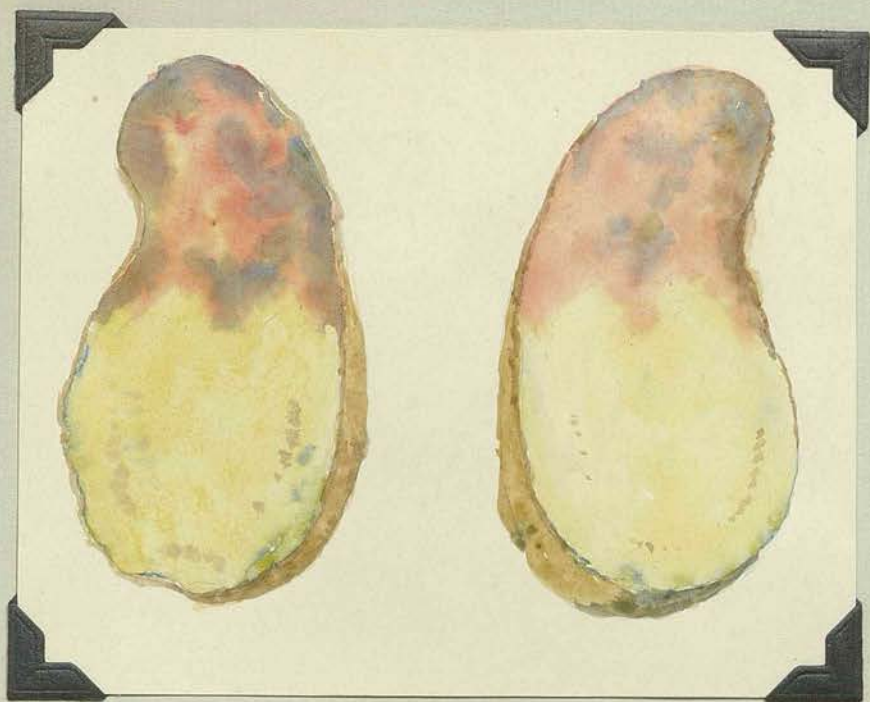


Fig.11.



Fig.12.

Plate 6.



Plate 7.

Fig. 13. Water painting of "Pinking" of Sharpe's Express tuber infected by Phytophthora "atropae!"

Fig. 14. Same of Sharpe's Express (left), and Epicure (right), caused by Phytophthora hydrophila.

Fig. 15. Same of Epicure caused by Phytophthora parasitica.



Fig.13.



Fig.14.



Fig.15.  
Plate 7.



Plate 8.

Fig. 16.

Colonies of P. "atropae" (A) and P. erythroseptica (B), on Quaker Oat Agar. This shows a variation of B. in which aerial mycelium is almost absent. The difference from A. is striking but, being inconstant, is not a diagnostic feature.

Fig. 17.

The same as in Fig. 16 but on Maize Meal Agar. The two colonies are almost the same in appearance.



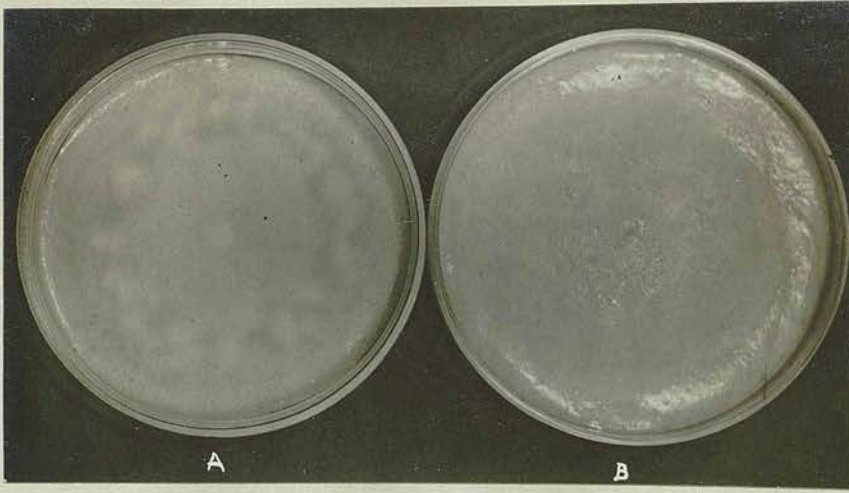


Fig. 16.



Fig. 17.

Plate 9.

Fig. 18.

Microphotograph of the mycelium of P."atropae" growing on and in a fine garden soil in a petri dish.

(Reflected light) x 12.8

Fig. 19.

Microphotograph of the edge of a colony of P."atropae" on clear Maize Agar, showing the branching of the mycelium x 35.

Fig. 20.

The same as Fig. 19 of P.erythroseptica showing the slightly different habit of the mycelium x 35.



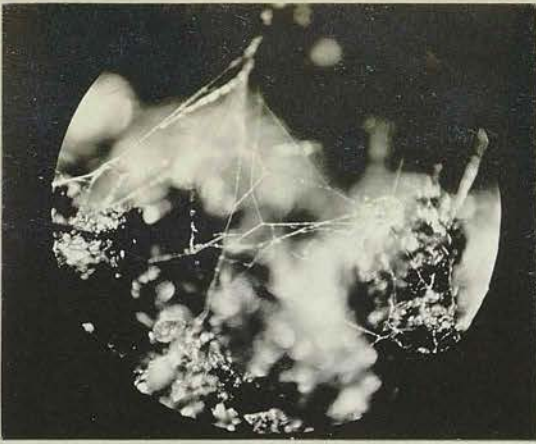


Fig. 18.



Fig. 19.



Fig. 20.

Plate 10.

Fig. 21. Microphotograph of the mycelium of P."atropae" on Dung Extract Agar showing the irregularity and the septation. This is not an old culture.

x 240.

Fig. 22. The same as Fig. 21, but on Malt Extract Agar, showing the simpler branching and non-septation.

x 240.

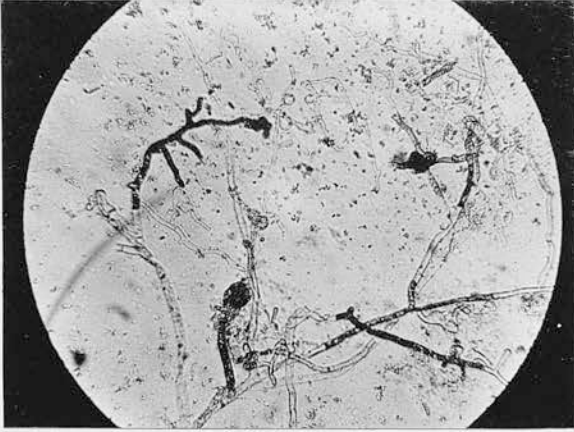


Fig. 21.

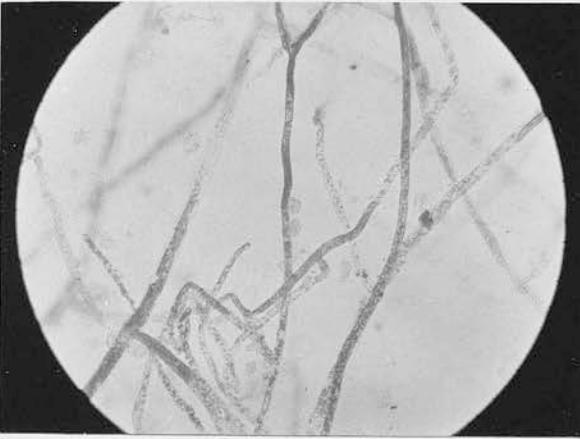


Fig. 22.

Plate 10.

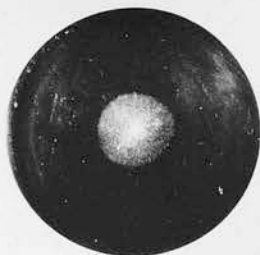


Plate 11.

Fig. 23.

Photographs of colonies of P."atropae"  
on Malt Extract Agar at different  
temperatures.

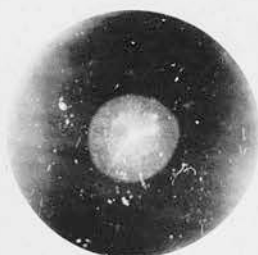
Two Days.



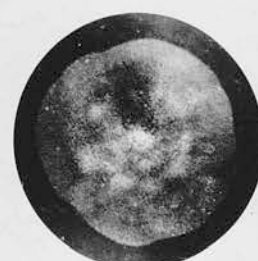
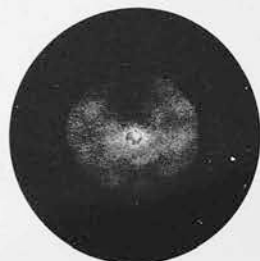
Three Days.



Four Days.



AT 8° C.



AT 18° C.



AT 23° C.

Fig. 23.

Plate 12.

Fig. 24.

The actual rate of increase of colonies of P."atropae" on Malt Extract Agar represented graphically.

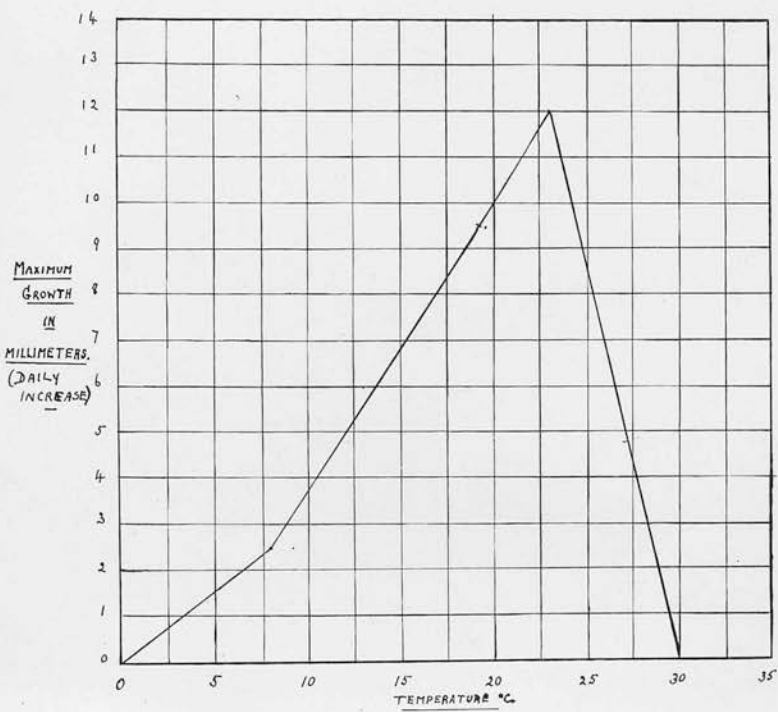
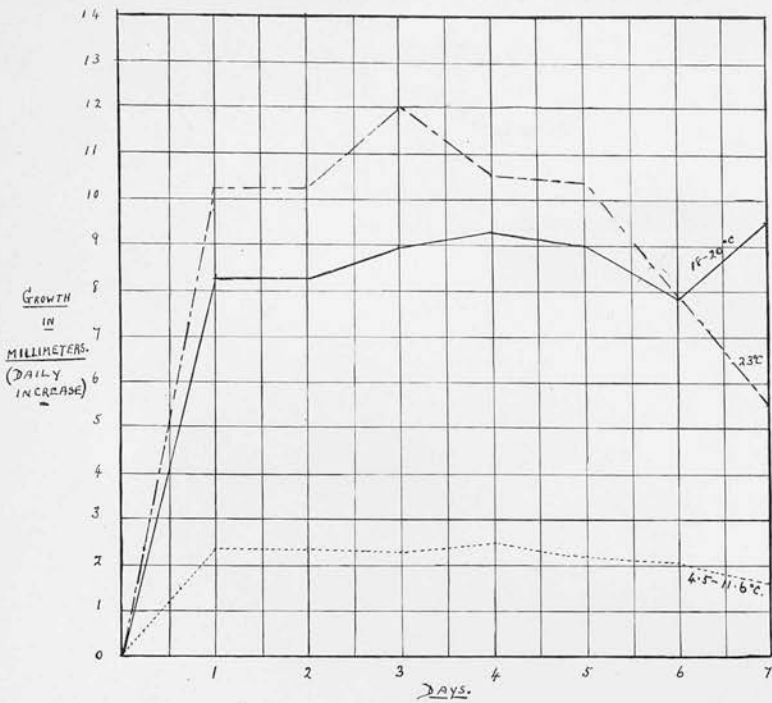


Fig. 24.

Plate 12.

Plate 13.

Fig. 25.

A colony of P."atropae" on Malt Extract Agar at alternating high and low temperatures.

Fig. 26.

Microphotograph of "sphaero-conidia" in a water culture of P."atropae"

x 240





Fig. 25.



Fig. 26.

Plate 14.

Fig. 27. Microphotograph of oospores of P."atropae" on Viola Extract Agar. This gives an impression of the deep natural yellow-honey colouring of old oospores.

x 56.

Fig. 28. Same as Fig. 27.

x 240

Fig. 29. Microphotograph of the "Figure of Eight" stage of an amphigynous arrangement of the sexual organs of P."atropae". The oogonium has nearly finished swelling out. On Malt Extract Agar.

x 240

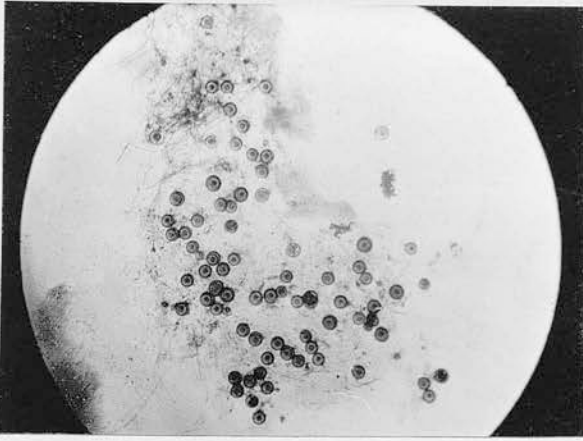


Fig. 27.

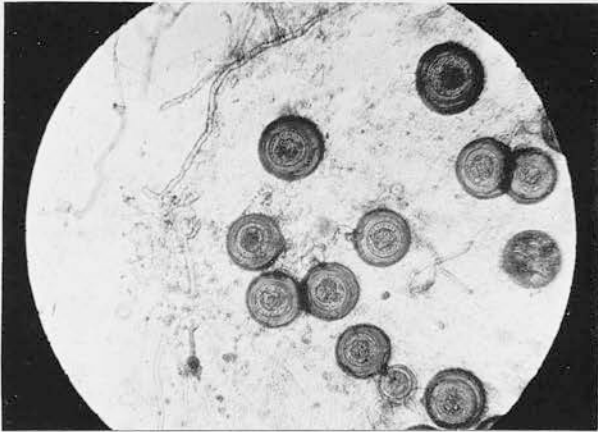


Fig. 28.

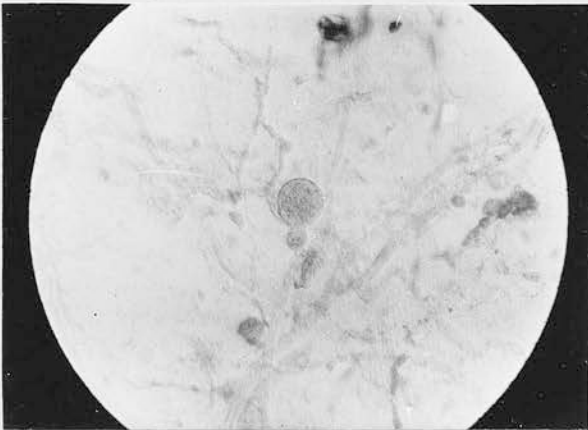


Fig. 29.

Plate 15.

Fig. 30. Microphotograph of an amphigynous antheridium of P. "atropae" with a "safety valve" which is presumed to be formed to relieve the pressure caused by the oogonial incept growing through the antheridium (marked)

x 480

Fig. 31. Microphotograph of a young oospore of P. "atropae" showing traces of residual nuclei between the oospore wall and the oogonium (marked).

x 240

Fig. 32. Microphotograph of an amphigynous antheridium of P. "atropae" showing an abnormal fertilisation tube running from the antheridium to where the oosphere was before the oospore wall was formed. This usually disappears. Oil Immersion.

x 610.

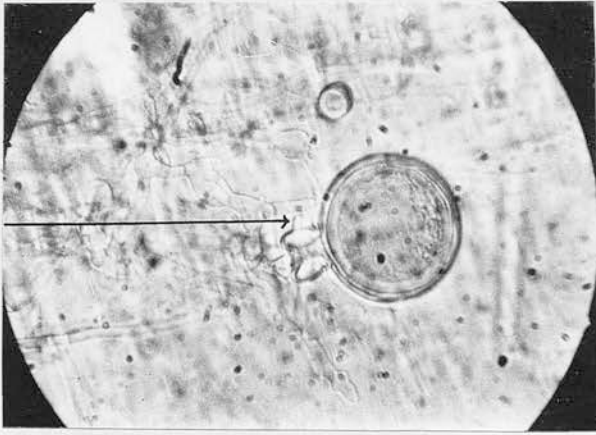


Fig. 30.

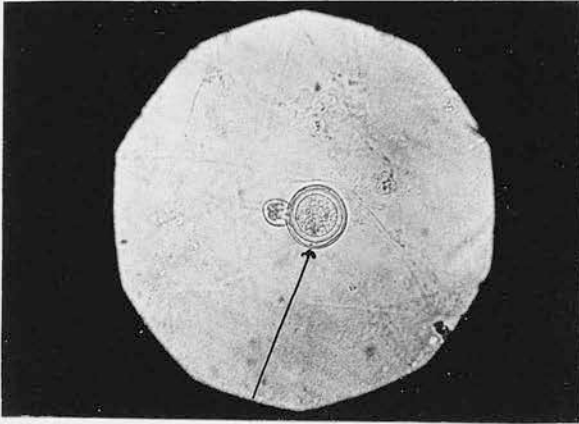


Fig. 31.

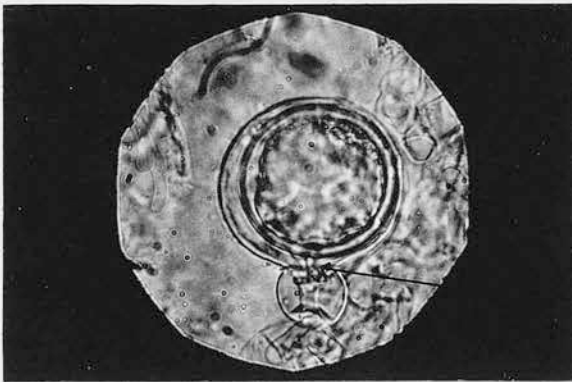


Fig. 32.



Plate 16.

Fig.33. Microphotograph of a paragynous antheridium of P."atropae" on Filtered Oat Agar.

x 240.

Fig.34. Microphotograph of double fertilisation of an oogonium of P."atropae" Amphigynous antheridium to the right and paragynous to the left.

x 240.

Fig.35. Drawings of amphigynous antheridia and oogonia of P."atropae" To the left is the oogonium seen when the section does not cut through the stalk of the oogonium. The plug is shown in the oogonial stalk. To the right the section has gone through the oogonial stalk which is also shown outside of the antheridium. In both the oosphere is forming and the residual nuclei are approaching the oogonial wall and degenerating.

x 750 approximately.

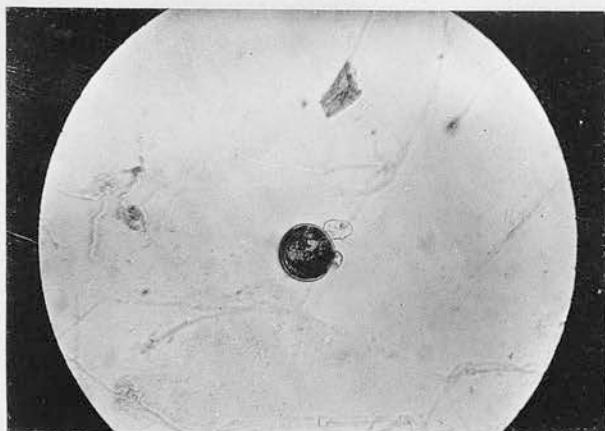


Fig. 33.

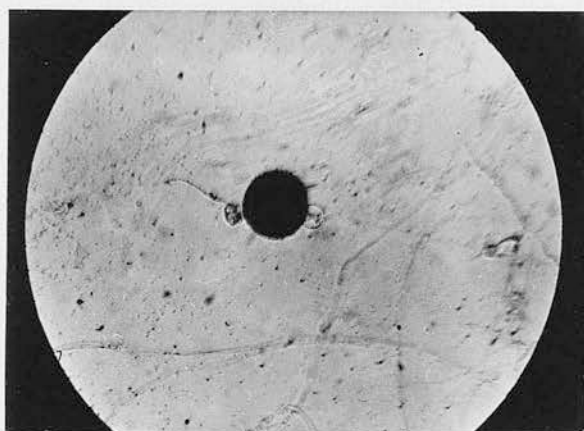


Fig. 34.

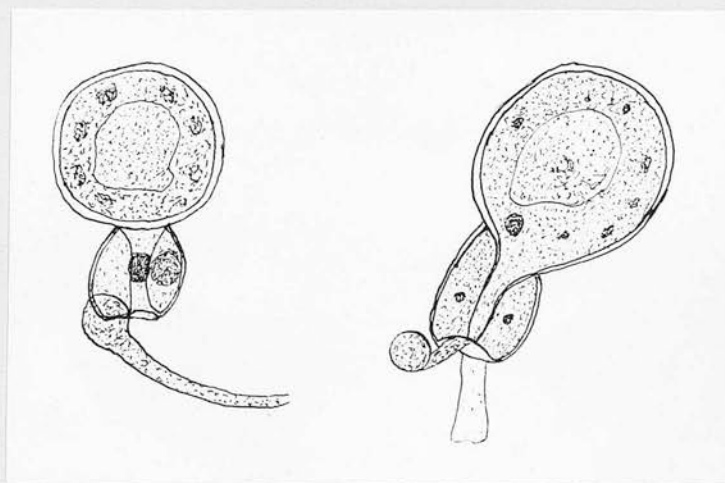


Fig. 35.

Plate 17.

Fig. 36. Drawing of the sexual organs of P."atropae" showing one definite paragynous antheridium, one possible paragynous and one hidden amphigynous antheridium. The antheridial stalk and the oogonial stalk were seen by focussing.

Fig. 37. Drawings of amphigynous antheridium of P."atropae" cut in different planes. To the right, the cytoplasm in the oogonium has commenced to form the oosphere and the residual nuclei are degenerating. In the centre, the oosphere is forming its first wall. To the left, young oogonium not fully expanded.

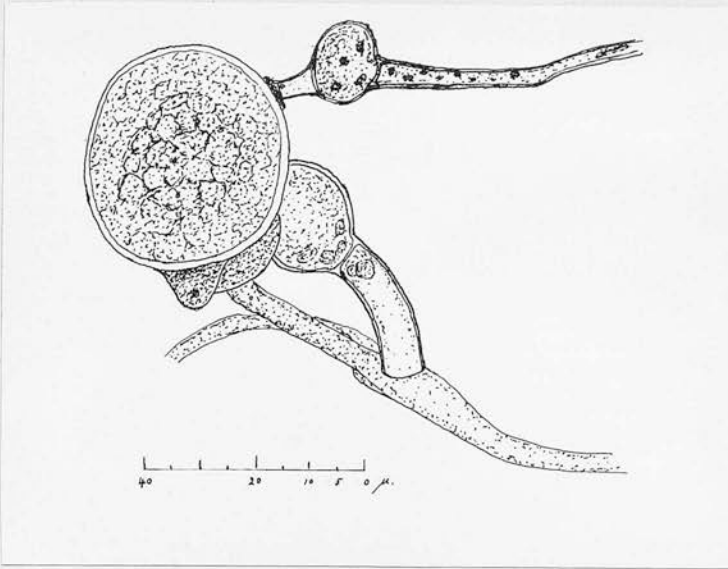


Fig. 36.

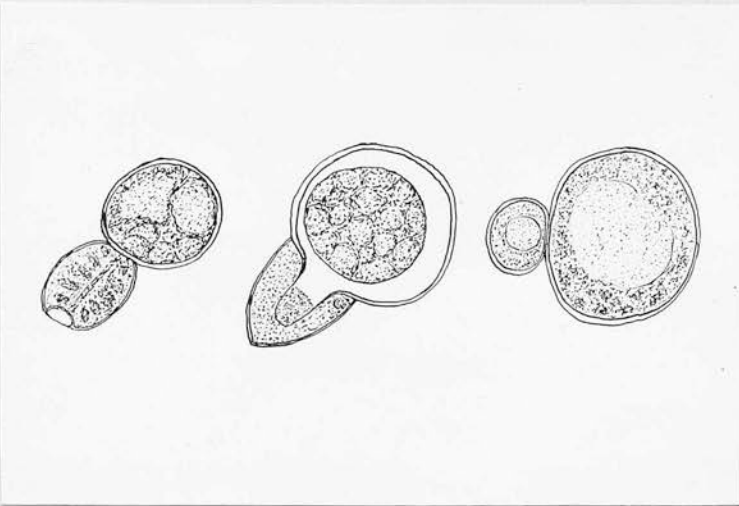


Fig. 37.

Plate 17.

Plate 18.

Fig. 38. Drawings of variously shaped conidia of P. "atropae"

Fig. 39. Drawings of a conidium of P. "atropae" extruding its contents (left) and germinating by zoospores followed by proliferation which has not been completed (right).

Fig. 40. Drawing of a proliferating conidium of P. "atropae". This is the only one found.



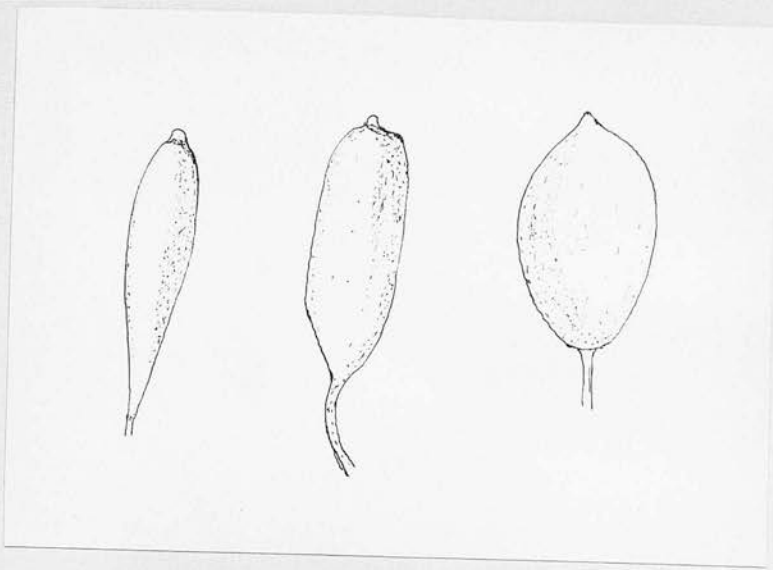


Fig. 38.

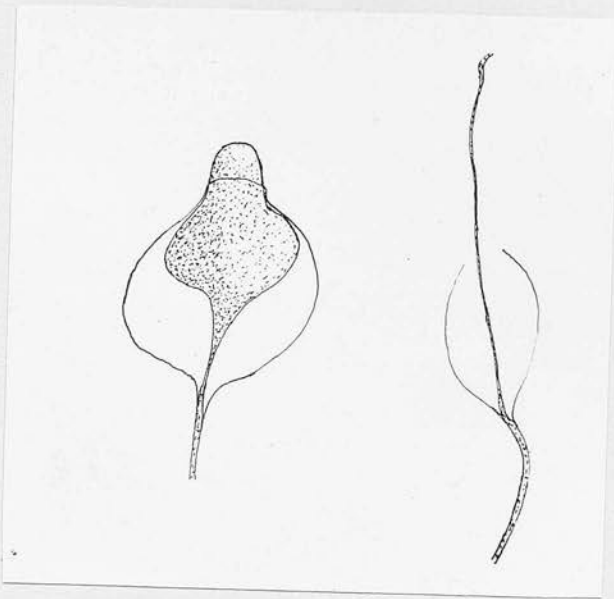


Fig. 39.

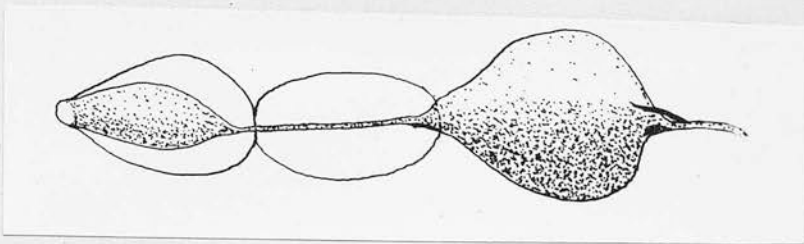


Fig. 40.

Fig. 41. Diagram of the amphigynous antheridium shown in Plate 15, Fig. 30.

1. Antheridial hypha.
2. Antheridial hypha tip showing that antheridium is intercalary.
3. Antheridial wall.
4. Antheridial wall invaginated by
5. Oogonial hypha which has grown <sup>through</sup> the antheridium.
6. Plug in oogonial stalk.
7. Oogonium wall.
8. Oospore; outside layer of wall.
9. Oospore; middle layer of wall.
10. Oospore; inner layer of wall.
11. Oospore; contents.
12. Safety Valve formed by antheridium.

Fig. 42. Various types of sexual organs of P"atropae".

1. Young type of oogonium, before zonation stage, which has grown through the side of antheridium.
2. Abnormal, young oogonium with paragynous antheridium.
3. Young oogonium, also before zonation, with both an amphigynous and a paragynous antheridium.
4. Oospore after wall formation but before the fusion of male and female nuclei, which have met towards the side, which is less usual.

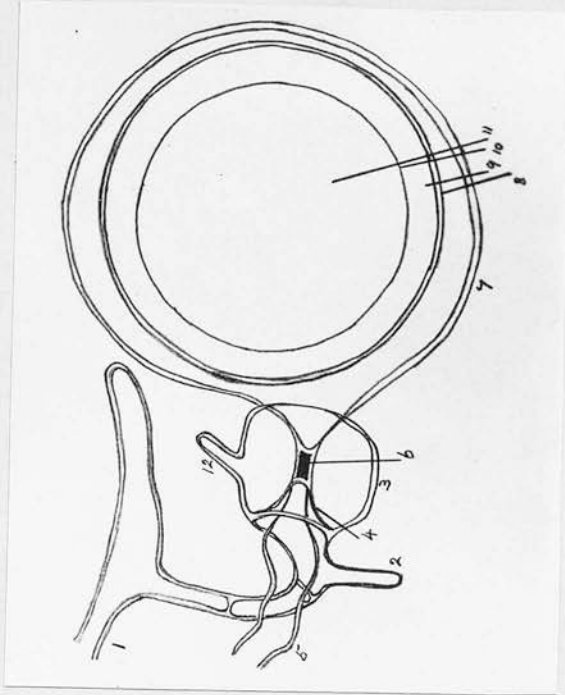


Fig. 41.

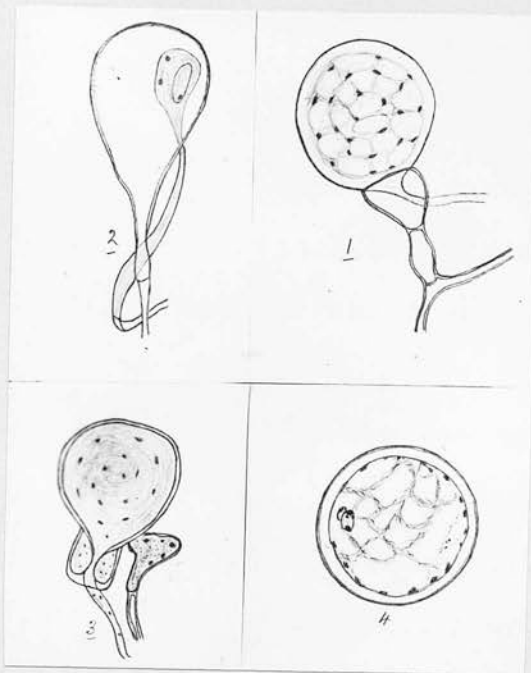


Fig. 42.

Fig. 43. Abnormal conidial growth of P."atropae" in water culture. The central conidium has germinated with tube hyphae.

Fig. 44. Types of conidia in water culture of P."atropae".

5 & 6 : Papillate and terminal.

7 : Non-papillate and terminal.

8 : Non-papillate and intercalary.

9 & 10 : Germination by tube just below  
and through the apex.

11 & 12 : Forms of "sphaero-conidia".

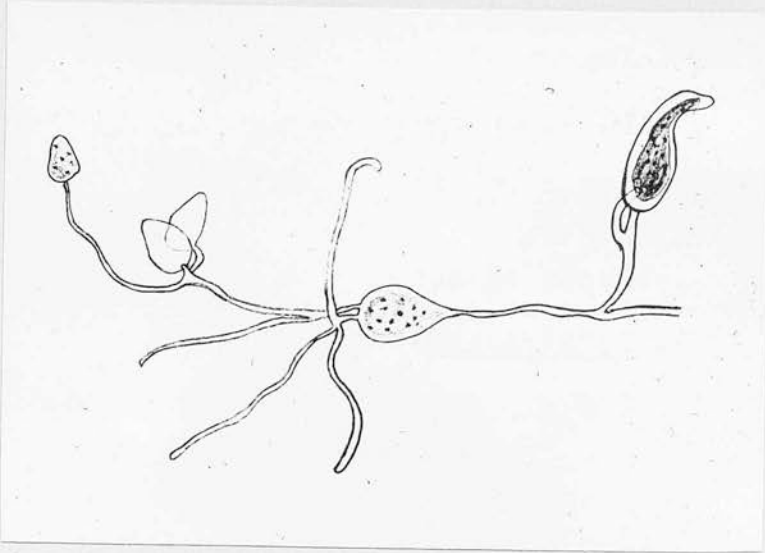


Fig. 43.

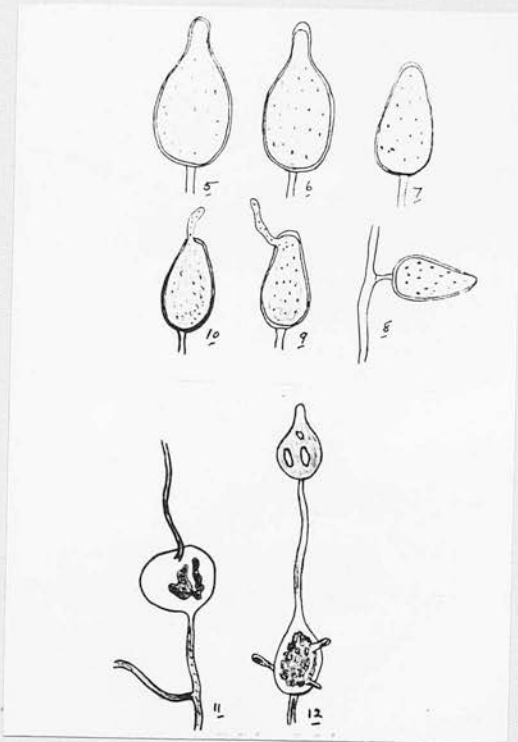


Fig. 44.  
Plate 20.



Plate 21.

Fig. 45.

Photograph of Leek leaves affected by White Tip Disease. The third from the right shows a marginal attack and the central one shows the watery areas that develop.

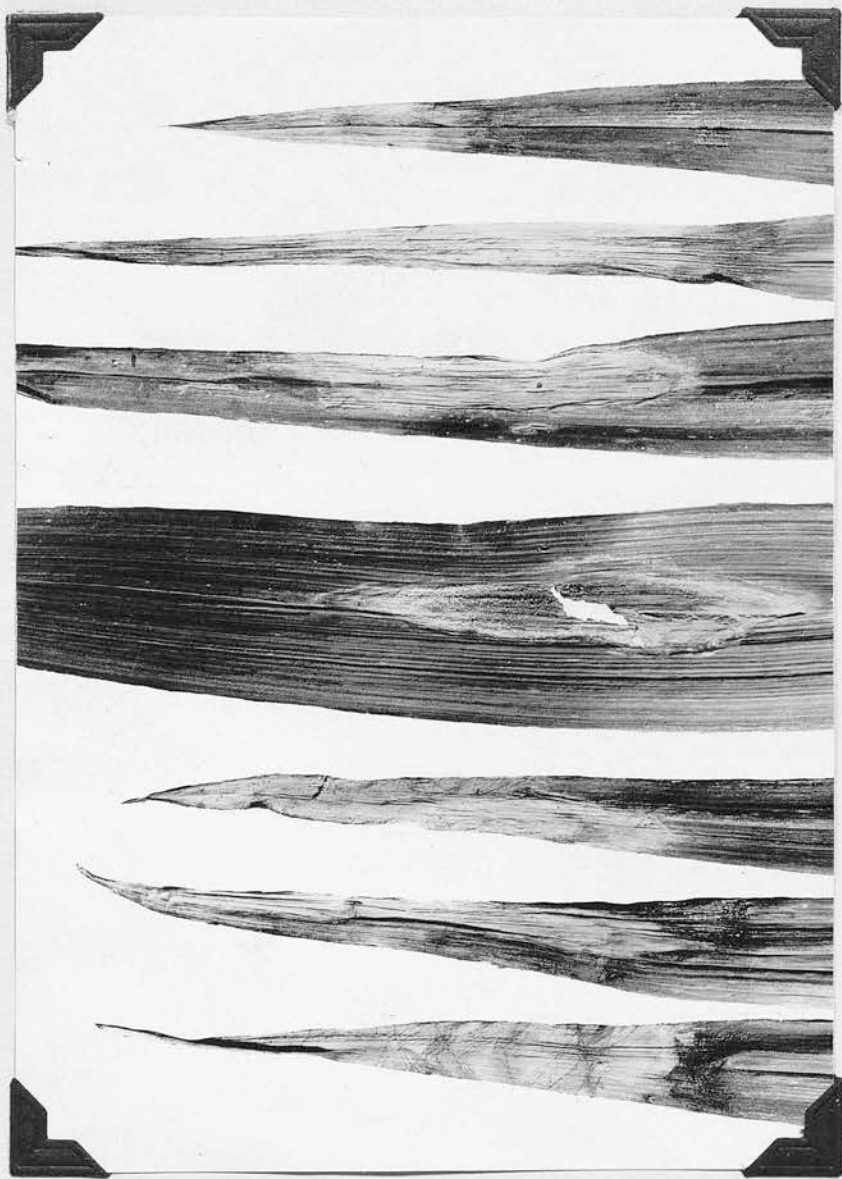


Fig. 45.

Plate 21.

Plate 22.

Fig. 46. General view of a field of leeks affected with White Tip Disease. Note the general whiteness, instead of the dark colour of healthy leeks.

Fig. 47  
and  
48. Close-up views of diseased leeks showing the collapsed whitened leaves and the relatively small amount of soil covered by the leeks.



Fig. 46.



Fig. 47.



Fig. 48.

Plate 23.

Figs. 49,      Photographs of colonies of respectively  
50 & 51.      Phytophthora Porri, Phytophthora Richardiae  
and Phytophthora Pini on Malt Extract  
Agar.      All same scale.



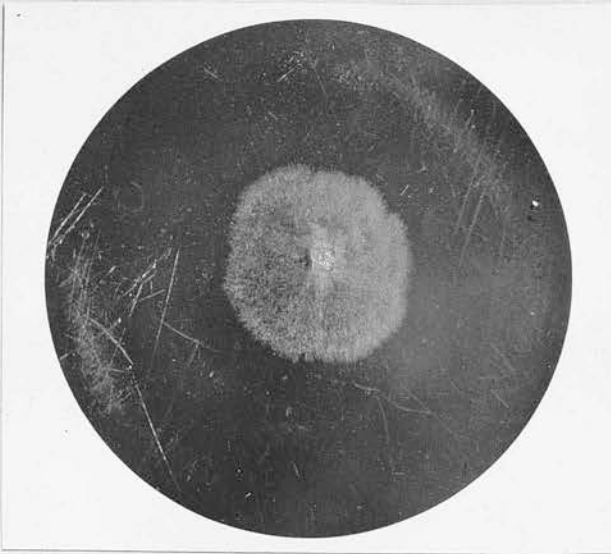


Fig. 49.

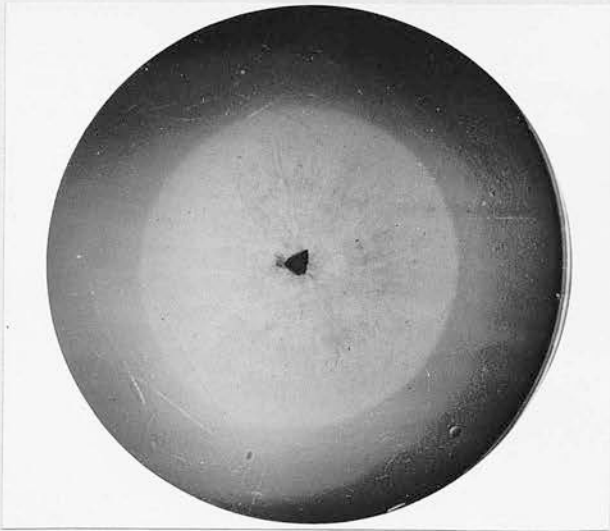


Fig. 50.



Fig. 51.

Plate 24.

Fig. 52. Photographs of colonies of Phytophthora Porri on Quaker Oat Agar (right) and Maize Meal Agar (left).

Fig. 53. Microphotograph of the mycelium of Phytophthora Porri on Clear Maize Agar.  
x 56.

Fig. 54. Same as Fig. 53 but of Phytophthora hydrophila for comparison and showing one variation of mycelium in the genus.  
x 56.



Fig. 52.

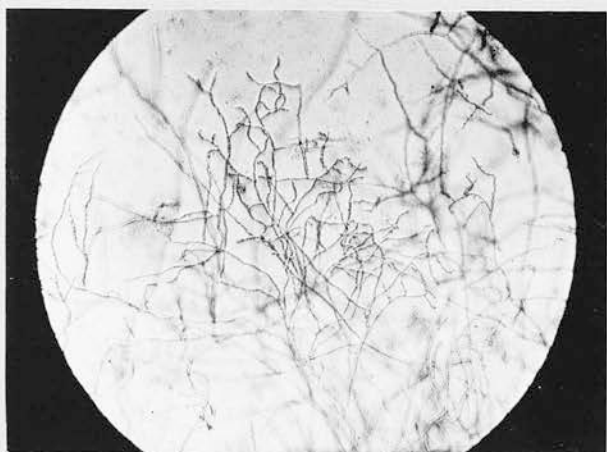


Fig. 53.

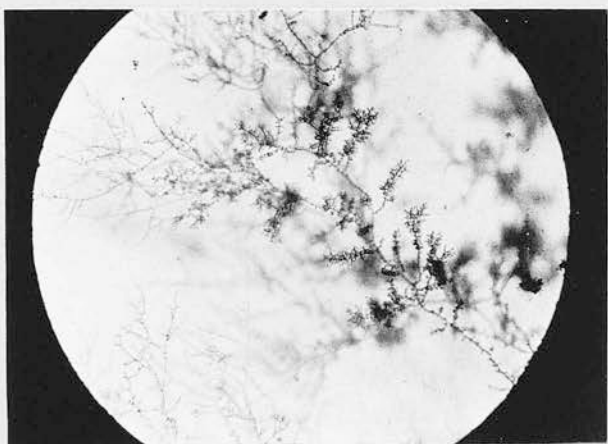


Fig. 54.

Plate 25.

Fig. 55. Microphotograph of a culture of Phytophthora Porri on Clear Maize Agar as taken through the bottom of a petri dish, showing the oospores and the coiled hyphae.

x 56.

Fig. 56. Same as Fig. 55 showing the coiling and a "figure of eight" stage of the young sexual organs.

x 240.

Fig. 57. Same as Fig. 55 showing two paragynous antheridia, their hyphae only being in focus.

x 240.

Fig. 58. Same as Fig. 55 showing a paragynous antheridium to the right and an amphigynous antheridium to the left, the stalk of the oogonium in this case not being clearly seen.

x 380.

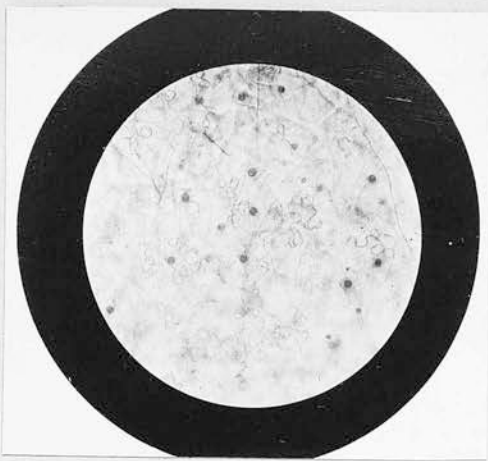


Fig. 55.



Fig. 56.

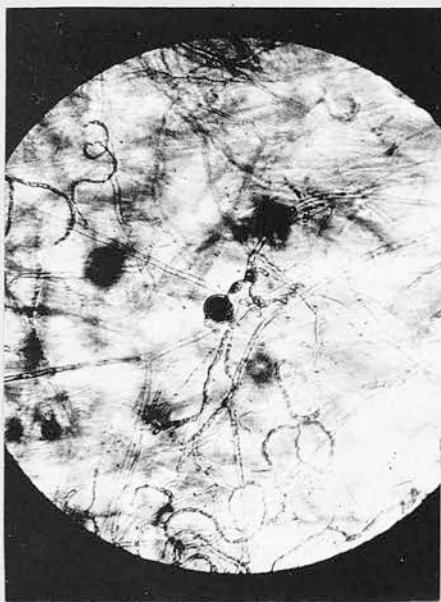


Fig. 57.



Fig. 58.



Plate 26.

- Fig. 59. Microphotograph of the conidia of Phytophthora Porri in sterile water.  
x 12.8.
- Fig. 60. Same as Fig. 59 larger, showing the non-papillate inversely-pyriform conidia.  
x 56.
- Fig. 61. Microphotograph of a sporangium of Phytophthora Porri with emergent zoospores and some left in the sporangium. The one marked has a tube from which a secondary zoospore had been liberated (see drawing). Clear Maize Agar Hanging drop culture.  
x 180.



Fig. 59.

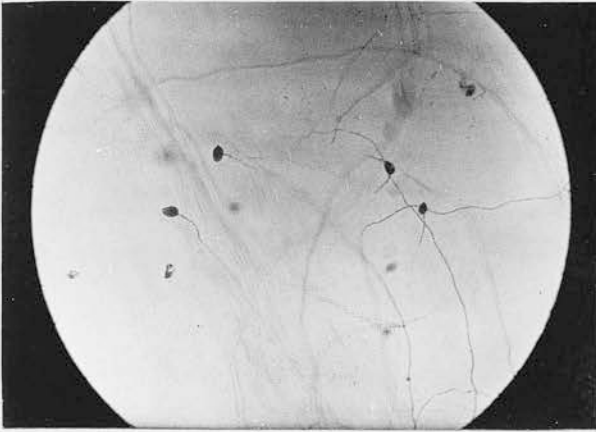


Fig. 60.

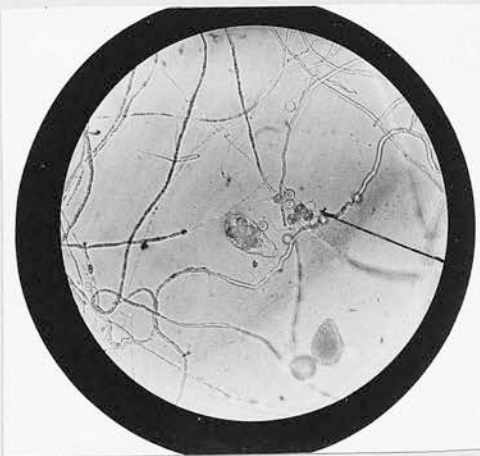


Fig. 61.

Plate 27.

Fig. 62. Drawings of the abnormal mycelium of P.Porri in Quaker Oat Agar culture(1 & 2), and of an amphigynous antheridium with a young oogonium just finishing expanding(3).

Fig. 63. Drawings of various stages of the sexual organs of P.Porri.

1. Paragynous antheridium attached to an oogonium showing the unequally thickened wall, before the oosphere has been formed.
2. An unfertilised oogonium.
3. An intercalary oogonium with a parthogenetically formed oospore(?).
4. Paragynous antheridium of a large oogonium, the oospore of which fills only half.
5. Oogonial incept passing through antheridium.
6. Paragynous antheridium attached to the oogonium at 12 noon one day, when zonation had not commenced and
7. the same twenty-four hours later, when the oosphere has been formed, and the residual nuclei have been left outside and are being pushed to the periphery.

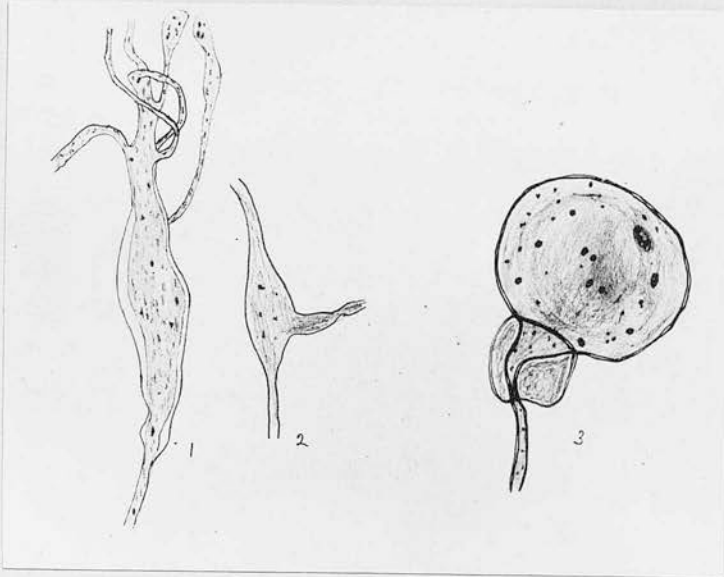


Fig. 62.

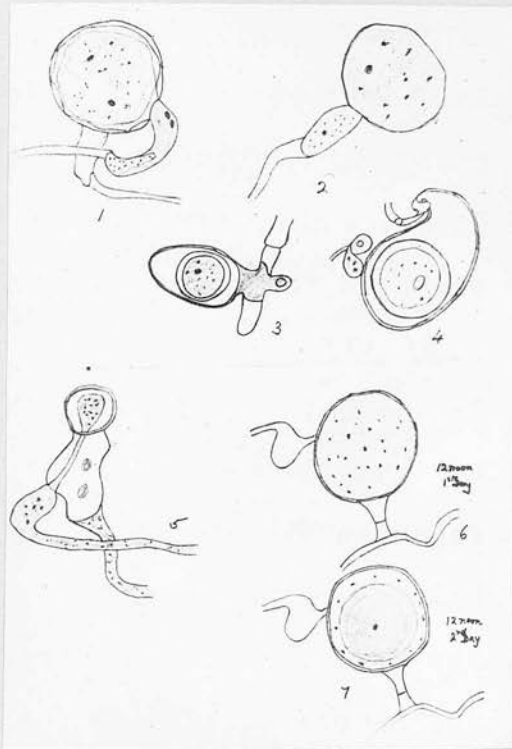


Fig. 63.

Plate 28.

Fig. 64. Various stages of the sexual organs of P.Porri.

1. Amphigynous antheridium.
2. & 5. Paragynous antheridia.
3. "Figure of Eight" stage of amphigynous antheridium.
6. An oospore formed partho-genetically(?).
- 6, 7 & 8. Teratological sexual organs.

Fig. 65. Asexual organs of P.Porri.

1. A broad mouthed, non-papillate sporangium, from which ten zoospores have not emerged.
2. a, b & c. Stages in the germination of a normal zoospore.
3. Zoospores germinating by tube and also forming secondary zoospores, two of which are emitting a single zoospore and two are shown empty, with their germination tube still showing.
4. A typical conidium of the fungus.
5. A bunch of conidia in water.



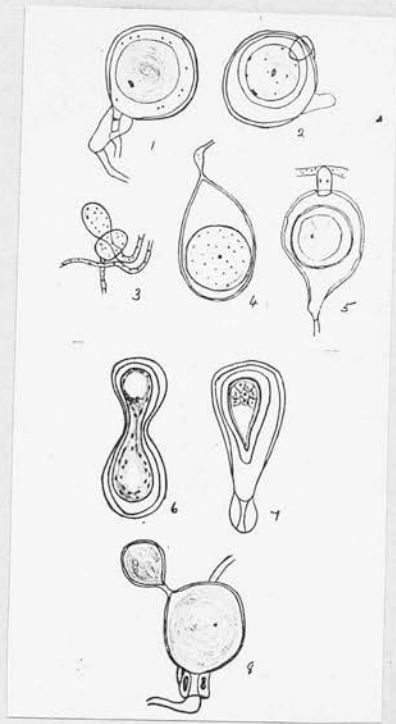


Fig. 64.

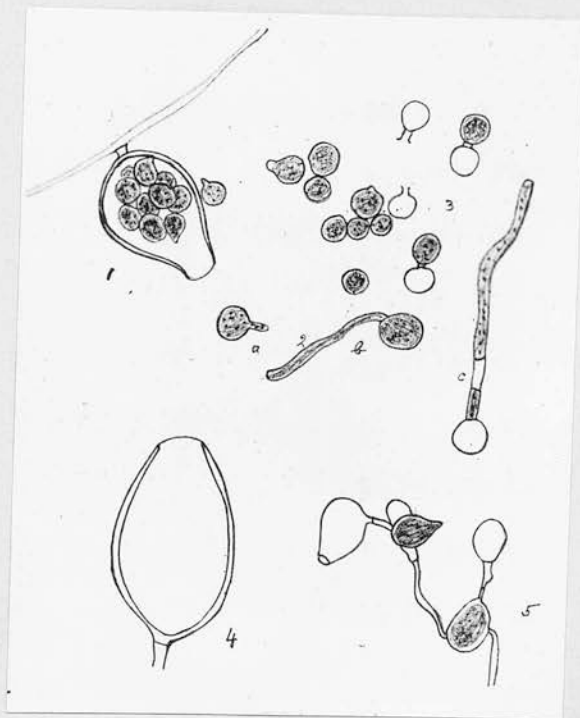


Fig. 65.  
Plate 28.