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PHYTOPHTHORA VERRUCOSA IN ASSOCIATION WITH

TOE ROT OF TOMATOES

by

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PHYTOPHTHORA VERRUCOSA IN ASSOCIATION WITH  
TOE ROT OF TOMATOES.

INTRODUCTION.

In the chief Tomato growing areas of Scotland, considerable reduction in crop has been experienced in recent years as the result of widespread attacks of Toe Rot. This disease affecting the root system of Tomato plants, has been reported from many districts in Scotland. Howells (1936), who first recorded it, has found evidence of its presence in the counties of Aberdeen, Dunbarton, Dumfries, Lanark, Peebles, Perth, Renfrew, Stirling and Wigton. During the course of the present study the occurrence of the disease has been established in Ayrshire. It would appear therefore that the areas which provide most of the Tomato crop in Scotland are affected with the Toe Rot fungus Phytophthora verrucosa (Foister).

In England sporadic outbreaks of the disease have been reported from time to time, notably in Cumberland and Norfolk. It seems, however, that few English records exist, and there, it is not considered

a serious problem. It is possible that the colder northern climate provides conditions more favourable to the incidence and spread of the disease. In Scotland many growers have experienced heavy losses of plants both in the seedling stage and at half maturity.

In cases where the soil in greenhouses has not been sterilised or has received inadequate sterilisation, as much as 95% of the crop has been lost. A mild attack may reduce the crop by 25-30%. It would appear that steam sterilisation, when effectively carried out, gives reasonably good control of the disease, but in practice it is difficult to ensure uniform heating of the soil with the result that the resting stages of the fungus may escape destruction.

Although preliminary investigations on Toe Rot have been made by Howells (1936) and Alcock & Foister (1940), no detailed investigation of the problem has been undertaken. Accordingly it was considered desirable to make a careful study of the life history of the fungus in relation to the disease with the ultimate object of throwing further light on the problem of effective control.

REVIEW OF PREVIOUS WORK.

Toe Rot disease of Tomatoes was originally investigated by Alcock in Edinburgh in 1934, and ascribed to an unnamed species of Phytophthora. The name "Toe Rot" was given in order to distinguish it from Foot Rot caused by two distinct Phytophthora species, P. parasitica and P. cryptogea. Since the first record of the occurrence of the disease in Lanarkshire in 1929, Howells (1936) reported considerable damage to Tomato crops there and in other counties.

Alcock & Foister examined the structure of the Toe Rot fungus as it was found on diseased roots and Foister (1940) published the first description of the organism, naming it a new species, Phytophthora verrucosa. Attempts were made during a number of years to isolate the pathogen in pure culture, but these were unsuccessful and so artificial reinoculation to reproduce Toe Rot symptoms was not possible. These workers, however, consider that P. verrucosa is the fungus responsible for the disease now receiving attention.

SYMPTOMS OF THE DISEASE.

The presence of Toe Rot is commonly revealed at planting time, but the fungus may attack the plant at all stages of growth. The use of unsterilised soil in the seed pans is likely to lead to an early infection which will seriously prejudice the subsequent cropping of the plants.

The symptoms of the disease at this early stage are not very evident unless the roots are examined. The plant as a whole shows a starved appearance, the result of disturbed root action. The foliage is blue green and the veins are purple on the backs of the leaves. The stem, and growth generally, is hard. On the roots, symptoms of Toe Rot are unmistakable. The principal feature is that suggested by the name "Toe Rot", a constriction and rotting of the extremity of the main tap root, on which the lateral roots appear brown and decayed. Figure 7 illustrates two infected roots, cut longitudinally, displaying the brown shrivelled terminal portions, above which the tissue is of normal thickness. Later the production of strong adventitious roots is stimulated to replace

the root system reduced by disease.

Tomatoes attacked after planting out show similar symptoms of starvation on aerial parts of the plant, though in a more advanced degree. The plant as a whole is stunted and does not produce the luxuriant growth of a healthy specimen. During sunny periods it wilts readily but may recover to some degree in the cool of the evening. The growth is hard and slow, leaves are reduced in size and frequently the petioles are curved downwards with the leaflets hanging limp. Fruit trusses are produced, but the swelling of the fruit is impeded and Blossom-end Rot is prevalent. A considerably reduced crop of poor quality fruit is the result. The root systems of older diseased plants show, as before, the typical restrictions of the primary root system. Adventitious roots produced at the base of the stem in turn become infected from the diseased roots below, so that the plant barely survives, and is useless from an economic point of view.

Infected plants which have been allowed to grow and fruit are shown in Figs. 1 to 4, where the decrease in vigour as compared with the comparatively

healthy plants beside them is clearly evident. At this stage, the root system may be so drastically reduced that only a ball of matted brown roots remains. Fig. 6 shows all that is left of the root system of a heavily infected plant.

Essentially, therefore, the symptoms of Toe Rot involve a destruction of the primary root system with the consequent decrease in vigour and cropping of the whole plant.

#### MATERIAL AND METHODS.

Diseased roots used during the present investigation were obtained from plants grown in the Horticultural Department, Auchincruive, in unsterilised soil; otherwise they were obtained from commercial nurseries where the disease was present.

Material selected for microscopical examination consisted of small secondary rootlets, in which decay was not well advanced and the cortex and root tip were still intact. Oospores were found to be present on these pieces of tissue in abundance. The

method adopted was to pick off these small rootlets with fine forceps from the surface of the ball and shake them gently under water to free from adhering soil particles without disturbing the spores, which are produced superficially on the roots and are therefore easily rubbed off if roughly treated. The washed roots were transferred to a slide, crushed with a cover slip and examined microscopically. The oospores then showed quite clearly without staining, since their walls are a pale golden colour. Sporangia were sought on the region just behind the root tip. Tissue used for the supply of sporangia was treated with even greater care in order not to detach the sporangia borne on slender hyphae projecting some distance from the root.

On account of the superficial character of the reproductive organs of Phytophthora verrucosa, it was deemed unwise to use too strong a surface sterilising agent for material from which it was hoped to obtain a culture on artificial media. Acriflavine, 1 part in 2,000, was found successful in preventing bacterial growth whilst allowing free development of fungi.

The media employed in the plate cultures were as

follows:-

- Potato Agar - The extract from 200 gms. Potato Pulp in 1 litre with 15 gms. Agar.
- Beerwort Agar - 2% Beerwort, 1.5% Agar.
- Oatmeal Agar - 200 gms. Oatmeal, 15 gms. Agar per litre.
- Tomato Extract Agar - The sap was extracted from tomato roots by grinding in water with a pestle and mortar, filtered and made up to approx. 2% solution: 1.5% Agar was added.
- Maltose Agar - 2% solution, 1.5% Agar.
- Carrot Agar - The extract from 200 grams of pulp and 15 gms. Agar was made up to 1 litre.

All were sterilised by autoclave for 20 minutes at  $1\frac{1}{2}$  atmospheres.

During experiments involving the germination of oospores in hanging drops, the above solid media were tried, and also drops of the following Liquid Culture:-

Maltose                      2% Solution

Sucrose                      2% Solution

Liquid Tomato Extract, prepared by filtering a water extract from the crushed roots through the sterilising pad of a Seitz filter.

Potato Extract              2% Solution.

The germination of oospores referred to in a later section was carried out in moist cells, constructed of glass rings placed in a petri dish over moist filter paper in which holes were bored to coincide with each glass ring. A coverglass supported on the ring carried the hanging drop. At first difficulty was experienced in keeping the drop intact for any length of time as moisture from the filter paper was inclined to condense on the underside of the coverglass and add to the size of drop until it became too large and eventually ran to the edge of the ring, and so the spore which it carried was lost. Later it was found that by smearing a fairly wide band of vaseline round the coverglass, after it had been flamed, the drop could be confined to the central area of the glass. These petri plates so prepared, each carrying three moist cells, were kept during the course of the experiments in an incubator at 20° C.

The measurements of oogonia and oospores are based on a count of 80 examples. Those of Atheridia on 10 examples of each type.

THE CAUSAL PARASITE.

Microscopically, the presence of P. verrucosa on Tomato roots can be determined, so far, only by the occurrence of reproductive structures. Mature <sup>oogonia</sup> ~~oospores~~, with their clearly rugose walls, are quite distinctive and large enough to be seen under the low power of the microscope. Fig. 8 illustrates <sup>oogonia</sup> ~~oospores~~ on a diseased Tomato root. Young <sup>oogonia</sup> ~~oospores~~ are more difficult to identify, as at this stage the rough walls are not well defined and it is possible to confuse young <sup>oogonia</sup> ~~oospores~~ of this species with the smooth walled <sup>oogonia</sup> ~~oospores~~ of P. cryptogea or P. parasitica. The <sup>oogonia</sup> ~~oospores~~ are to be found superficially on the root or in the outer cortex, and occur singly, or in groups of 2 or 3 spread along the root (Fig. 8). Other roots of the same plant, though rotted, may show no signs of the organism. In the case of potted plants, <sup>oogonia</sup> ~~oospores~~ have been found in greatest quantity on those roots which are on the outside of the soil ball and in contact with the pot.

Roots from Toe Rot plants, left for several hours in water have been induced to form true P. verrucosa

sporangia, by which the presence of the fungus can be confirmed, but, up to date, no distinctive vegetative mycelium has been determined within the host. By the time <sup>oogonia</sup> ~~oospores~~ are visible, thus giving confirmation of the disease, the roots have become invaded by mycelium of other organisms, e.g. Pythium sp. and other species of Phytophthora. As a result of the mixed infection, it is impossible to trace amongst the other coenocytic growth, the hyphae from which the <sup>oogonia</sup> ~~oospores~~ of Phytophthora verrucosa originate. The absence of a means of recognising the attack of this fungus in a vegetative condition has led to some difficulties. It is possible that the fungus has attacked the plant and is producing Toe Rot symptoms while still in a vegetative state, and therefore cannot be recognised microscopically. It was frequently found that, of a batch of Toe Rot plants received, all with evident symptoms of the disease, only 30-50% of these showed the presence of P. verrucosa <sup>oogonia</sup> ~~oospores~~. In the others it was probably present in a vegetative and unrecognisable condition. Moreover, there is reason to believe that the formation of <sup>oogonia</sup> ~~oospores~~ and sporangia has a seasonal sequence. A number of

plants growing in diseased soil were kept under observation from September until June. Samples of these plants were examined at intervals with a view to finding when attack takes place and to observe if P. verrucosa was present before the incidence of secondary parasites likely to obscure primary attack. From September till the end of January, no signs of verrucose <sup>oosporia</sup> ~~oospores~~ nor of sporangia could be found though large hyaline spores of Asterocystis were seen in quantity, sometimes almost filling the cells. Since these were apparent before the presence of P. verrucosa had been determined, the possibility of Asterocystis being a primary parasite had to be considered. Previous workers, Rives (1925) and Vanterpool (1930), however, have stated, with relation to Oats, that Asterocystis is not in itself sufficiently pathogenic to be of economic importance, unless under very moist conditions favourable to the fungus and unfavourable to the host plant. It may, however, in the opinion of Vanterpool (1930), pave the way for, or aggravate the damage caused by, more vigorous parasites. At the beginning of February <sup>oosporia</sup> ~~oospores~~ were found on the majority of plants which for the previous 4 months had shown no signs of

P. verrucosa. From February onwards till the end of July, <sup>oogonia</sup> oospores were found in quantity on 55% of the diseased plants showing Toe Rot symptoms. After this time, empty oogonial shells only could be found on diseased roots. This seems to indicate that the production of <sup>oogonia</sup> oospores under natural conditions does not take place in the late summer. During the whole period also, no trace of sporangial development was found. Roots were left lying in water and in dilute  $\text{KNO}_3$ , but again sporangial formation could not be induced by these means.

The reason for the paucity of sporangial development during 1943 is not understood, since in previous years Foister (private communication) found Sporangia in quantity in the spring. In mid July, however, Sporangia were produced in some quantity on root balls immersed overnight in tap water, but some doubt exists as to their identity. On roots which had been recently attacked these sporangia were found in the region immediately behind the tip.

Description of *Phytophthora verrucosa*.

The structure of the fungus has been studied as it grows in situ on the Tomato root. The configuration of the <sup>oogonia</sup> oospores is the most strikingly characteristic feature of this species of *Phytophthora* by which it merits classification by Alcock & Foister (1940) as a distinct species. <sup>oogonia</sup> Oospores are, when mature, thick walled, rough coated, spherical bodies borne superficially on the root, or within the outer cortex. They vary in size from 21 to 46 $\mu$  in diameter, with an average of 36.4 $\mu$ . The wall is 2.5-5.5 $\mu$  in thickness, irregularly thickened both externally and inwardly. The outer surface of the thickened wall presents a warted appearance and the inner an irregularly folded or dented contour (Fig. 9). When the spore is immature, the oogonial wall shows little or no irregular thickening and at this stage it is difficult to distinguish the oogonium of *Phytophthora verrucosa* from those of other species which also attack Tomato roots. Fig. 9 shows drawings of young and mature <sup>oogonia</sup> oospores.

The antheridia are amphigynous 12.2-20.3 x 8.12-12.2 $\mu$ , average 15.43 x 11 $\mu$ , or paragynous

10.2-18.3 x 8.12-12.2 $\mu$  , average 14 x 10.2 $\mu$  . Those examined showed predominantly the former type. Figs. 10 and 10a show oogonia with antheridia of both types attached.

Oospores lie free within the oogonial cavity and measure 14.5-32.5 $\mu$  in diameter with an average of 21.4 $\mu$  , the smooth hyaline wall being approximately 3.4 $\mu$  thick. The contents of the oospore usually are hyaline but occasionally a granular oospore can be detected amongst the larger ones. In oospores which had been stored for some time, a change in appearance of the contents occurred, large colourless globules, illustrated in Figs. 22 and 23, being formed. On one occasion, an oospore with contents resolved into globules was observed released from the oogonial wall (Fig. 24).

Structures which appear to be empty oogonial shells, sometimes having fully formed antheridia attached to them, were found among normal <sup>oogonia</sup> ~~oospores~~ on diseased roots. It seems possible that these structures may be <sup>oogonia</sup> ~~oospores~~ from which the <sup>of the oospore</sup> contents <sub>^</sub> have been liberated as motile zoospores. Fig. 11 compares one such oogonial shell with a mature <sup>oogonium</sup> ~~oospore~~.

Mycelium associated with P. verrucosa cannot easily be distinguished since it has been examined only within root tissue in which several other fungi were also present. It has not been possible to detect hyphae undoubtedly attached to the <sup>oogonia</sup> ~~oospores~~ of this species but, in regions where they are present in abundance, a rather coarse coenocytic hypha of an average thickness of  $4\mu$  can also be found branching at frequent intervals within the outer cortical cells. The lateral branches arise at a fairly wide angle from the main continuing hyphae. This mycelium may be that of the fungus under examination.

In addition, it has been observed that short, much branched hyphal knots having the appearance of haustoria are present in the cortical cells of diseased roots. They are a rather constant feature of infected tissue, therefore worthy of record although no attachment has been traced between <sup>oogonia</sup> ~~oospores~~ and these 'hyphal knots' (Fig. 30).

In previous seasons, Sporangia were found without difficulty by Foister (1940), but during 1942-43, in the course of the present investigation, on roots transferred to distilled water and various solutions

following the technique found successful by Foister, no sporangial formation was observed.

Attempts to induce Sporangia formation.

From March to August, plants on which the incidence of P. verrucosa had been confirmed, by true Toe Rot symptoms and the presence of verrucose <sup>osonia</sup> ~~oesperes~~, were placed, with minimum disturbance of the soil ball, in beakers of distilled water.

The roots were allowed to remain immersed for periods of from 1 to 4 nights. This method was carried out according to recommendations made by Foister. After treatment, rootlets that could be picked off from the outside of the ball, preferably those retaining the root tip, were selected for examination, but the distinctive sporangia described by Foister (1940) were not observed. On several occasions nonpapillate sporangia (Figs. 12, 13, 14) were found whose proliferation occurred more frequently within than beyond the previous sporangium, and measured from 29.5 to 56.8  $\mu$  in length and 22.3 to 26.5  $\mu$  in width: average 38.98 and 28.42  $\mu$ . These features conform to the description

of sporangia of P. verrucosa given by Foister (1940); the broadly rounded or rather flattened sporangial tips were, however, absent. It is probable that the sporangia found occasionally during the present work are those of P. cryptogea, but it is worthy of note in connection with possible confusion of species that young sporangia of P. verrucosa, as illustrated by Foister (1940), in which the flattened tip is less evident, might easily be confused with P. cryptogea. Further, Sporangia found during this study, when kept under observation until the actual release of zoospores, were found to display a distinct retraction of contents from the region at the apex (Fig. 12). This is but a short lived phase, lasting about 1 minute before the zoospores are discharged, but it gives an impression of flattened tips which might be confused with those of sporangia of P. verrucosa.

Further endeavours to promote sporangial development were carried out with seedlings grown in infected soil. Most of them showed little signs of actual Toe Rot though they had been given ample time - over five weeks - in which to contract the disease. With a view to promoting sporangial formation, plants were

gently shaken free of soil and their roots immersed in 0.5% solutions of Potassium Nitrate, Urea, Ammonium Sulphate and Asparagine for periods varying from 20-70 hours, after which they were immersed in vessels of Distilled Water for 72 hours before microscopical examination as before. These treatments yielded no Sporangia of any kind.

ATTEMPTS TO ISOLATE THE FUNGUS  
IN PURE CULTURE.

In the course of their investigations Alcock and Foister attempted to induce Phytophthora verrucosa to grow on a variety of artificial media, but without success. Further endeavours were made during this work.

A. ROOT FRAGMENTS ON AGAR PLATES.

Roots, seen to be carrying <sup>oogonia</sup> ~~oospores~~ of P. verrucosa were plated on the following media:- Potato Agar, Oatmeal Agar, 2% Beerwort Agar, Carrot Agar, Malt Agar, Tomato Root Extract Agar, 2% Maltose Agar.

At first Mercuric chloride was used as a surface sterilising agent, but later a 1 in 2,000 solution of Acriflavine was substituted. Considering the superficial development of reproductive structures, there seemed a possibility that the difficulty experienced in culturing this fungus may have been due to its having been injured during surface sterilisation. Acriflavine in the above concentration possesses a strong bactericidal but weak fungicidal action.

As a result of these platings a number of cultures were obtained of Phycomycetous fungi of the Phytophthora type, but these remained vegetative in spite of numerous attempts to encourage the development of reproductive structures by which they might be recognised. Transference of pieces of the agar culture to tubes of sterile water or  $\text{KNO}_3$  produced in one case abundant intercalary sporangia, some of which were empty, thus suggesting the release of zoospores, and in others some of the spores had germinated and the germ tubes had perforated the sporangial wall. Since intercalary sporangia have not been associated with P. verrucosa, this fungus was not considered to be of that species. No response to these treatments

by sporangial or oogonial development was obtained with the other isolates. On agar plates containing cultures of each of these isolations, sterilised Tomato seed was sown and allowed to germinate. Within a few days the tip of the radicle and lateral rootlets penetrating the culture became brown, but after 4 weeks no indication of P. verrucosa spores could be found. Subsequent transfer of parts of the vegetative culture to sterile water resulted in the formation of round intercalary sporangia in no way resembling P. verrucosa.

Since plated roots yielded such a varied fungal flora, even among those of a coenocytic habit, and since, as a result of many trials, a culture of P. verrucosa could not be isolated, it was decided to try a more selective technique, using the reproductive bodies of the fungus as the starting point from which to derive a pure culture of the fungus, and simultaneously to obtain information regarding the behaviour of oospores on germination.

B. GERMINATION OF OOSPORES.

A supply of oospores was available on diseased roots from February to July. Prior to that period none could be found, suggesting that their formation was governed by seasonal influence. The germination tests were carried out intermittently from February onwards, using oospores chiefly from one batch of plants on which they were first seen at the end of January. The oospores employed during the tests were not older than 6 months and in some cases, for example in the early trials with untreated oospores, they were only recently formed.

The technique employed in detaching the spores and transferring them to hanging drops of liquid in moist cells has been described previously.

(a) Germination of oospores attached to host root  
in water.

Selected pieces of root, on which verrucose <sup>oosporic</sup> oospores were fairly plentiful, were kept in Sterile Water under observation during early February. These spores must therefore have been recently matured.

After 4 days the appearance of some of the oospores had changed. The oospores could no longer be distinguished and an apparently empty oogonial wall remained, associated with some of these oogonial shells, thin walled sub-spherical vesicular structures with granular contents were observed (Figs. 25 and 28). Movement of the coverslip failed to release the vesicle from the spore, suggesting that the structures were attached to the <sup>oogonia</sup> oospores. Some such structures were found in which the vesicles were burst at one point and their contents released. A few motile bodies remained swimming within one evacuated bladder and the spherical oospore could no longer be determined within the verrucose oogonial wall. A drawing was made at this stage and is reproduced in Fig. 15. This suggests that these vesicles behave as sporangia, but since they were formed on a rotted tomato root infected with numerous secondary parasites it was not considered desirable at this point to relate these vesicles to P. verrucosa. They might be sporangia of another fungus or an alga adhering superficially to <sup>oogonia</sup> oospores of P. verrucosa.

Again in June and during the following spring many more vesicles were found attached to <sup>oospores</sup> ~~oospores~~ on diseased roots kept under moist conditions. Full vesicles were observed and some from which the contents had escaped. Figs. 17 and 18 represent vesicles drawn from permanent preparations.

On two subsequent occasions the changes taking place during the release of zoospores through a perforation in the side of the vesicle were actually seen. Figs. 16 and 27 show empty vesicles and Fig. 28 a vesicle full of granular contents. In Fig. 28 the contents are seen to be dividing prior to the formation of zoospores. Fig. 29 shows a vesicle from which the evacuation of zoospores was witnessed. The photograph was taken immediately afterwards.

(b) Detached oospores germinated in Liquid Media.

In order to avoid confusion caused by the presence of other fungi associated with P. verrucosa on the root, oospores were detached and attempts made to induce germination in isolation from their normal environment. During the spring of 1942, when supplies of oospores were available, germination trials were carried out in

a number of different media. <sup>Oogonia</sup> Oospores were scraped from the root surface into a drop of sterile water and transferred unsterilised, by means of a capillary tube, to sterile tubes of culture media. The following media were employed, and in some cases slightly acidified in order to inhibit bacterial development.

Tomato Root Extract (Heat sterilised) pH 5.2

1.5% 'Yeastrel' acidified pH 3.8

Asparagine Medium pH 3.6

Formula	K H <sub>2</sub> PO <sub>4</sub>	0.05 gm.
	NH <sub>4</sub> NO <sub>3</sub>	0.05
	MgSO <sub>4</sub>	0.02
	KNO <sub>3</sub>	0.02
	Asparagine	0.1
	Glucose	0.5
	Distilled Water	100 cc.
	Acidified with	10% HCl.

Adaption of Leonian's (1934) Peptone Medium pH 4.6  
 substituting Witte Peptone for Proteose Peptone.

A number of apparently coenocytic cultures were obtained in the Asparagine medium and a few in Yeast Extract. The Root Extract yielded no growth whatsoever and the cultures in Leonian's medium were nearly all clouded with Bacteria. The cultures which were obtained in Asparagine medium showed no indication of

fructifications, which would aid in their identification. Transfers to Bog Soil Extract induced in most cases round sporangia suggestive of Pythium sp. One growth remained vegetative.

As a result of these attempts, it was obvious that this method did not allow of sufficiently close observation of the outcome of placing oospores in the culture media. It was impossible totally to exclude the spores of other organisms more vigorous in growth than P. verrucose, which has up to the present resisted all efforts to isolate in culture. Should germination of the oospore take place, therefore, the hyphae would be overgrown by some other fungus introduced accidentally.

Future cultures were, on that account, confined to hanging drops or agar films, in which the oospore used as inoculum could be kept under observation from the start, any slight sign of germination appreciated, and any contaminants recognised as such before over-running the culture.

(c) Detached oospores germinated in Hanging Drops.

Oospores detached from the host root were mounted

during February and March of 1943 and during April and May of 1944 in hanging drops of sterile water, Potato Extract, Tomato Root Extract and 2% Maltose Solution, also on inverted films of Potato Agar, 'Difco' Agar and Tomato Root Extract Agar.

The great majority of oospores so treated failed to germinate at all. A single one did germinate, however, in a Potato Extract Drop and produced in 20 days a distinct vesicle closely attached to the outer wall. The structure corresponded to those previously seen associated with <sup>oosonia</sup> oospores on roots immersed in sterile water in February.

Since no other fungal growth was present in the hanging drop, and, in view of the close contact between vesicle and <sup>oosonia</sup> oospore together with the apparent disappearance of the <sup>oosonia</sup> oospore contents, it may be assumed that the vesicle is produced by the oospore in the process of germination.

DETAILED DESCRIPTION OF GERMINATING  
OOSPORES AND VESICLES.

<sup>Oogonia</sup>  
Oospores on which vesicles had formed resembled those described on the root as empty "oogonial shells". They were pale golden in colour and the distinctly rounded thick walled oospore, visible inside mature oogonia, was no longer present. Their walls, in places, seemed to have collapsed inwards. No connecting channel was observed between the empty cavity of the <sup>oogonium</sup> oospore and the vesicle, but the side of the vesicle adhering to the <sup>oogonium</sup> oospore was flattened, giving it a subspherical outline. Vesicles when full of granular contents, and prior to discharge, measured 16.3-25 x 25-27 $\mu$  approx. Their walls appeared as thin membranes distended by the contents which occupied the whole of the space. Shortly before discharge the contents of the vesicle became sub-divided (Fig. 28) and later a large number of very active zoospores could be seen within the vesicle wall. These zoospores were very nearly round and measured 3.5 $\mu$  in diameter. Their release took place not by the bursting of the containing wall but gradually through

a perforation in the side of the vesicle. The evacuation of the vesicle was completed within one minute. After the escape of the contents the colourless membrane remained for some time at least attached to the side of the empty oogonial shell (Fig. 16). In some cases it collapsed and became folded (Fig. 27). Some empty shells were seen to which there was no remnant of vesicle attached; presumably this had later become detached.

#### GERMINATION OF PRETREATED OOSPORES.

Blackwell (1943) studying P. cactorum found that a period of refrigeration at 2-3° above freezing point can speed up germination of oospores by one or two months. McKay (1939), working with Peronospora oospores, advanced the theory that additional oxygen supplied in the form of KMnO<sub>4</sub> or H<sub>2</sub>O<sub>2</sub> increased or hastened oospore germination.

Infection of Tomato plants has occurred after steam sterilisation of the soil. It may be, therefore, that if sterilisation is inadequately carried out

oospores survive and the alternation in temperature may stimulate germination.

These forms of pretreatment were carried out in an attempt artificially to hasten germination of the oospores.

A. EFFECT OF INCREASED AERATION IN OOSPORE GERMINATION.

(a) Aeration.

Roots bearing verrucose <sup>oosonia</sup> oospores were placed in a tube of water fitted to a suction pump. Air was drawn through the water intermittently for 8 hours, the tube was left over night and aeration resumed for another hour next day. In spite of the considerable agitation set up, the majority of the <sup>oosonia</sup> oospores remained attached to the roots on which they had been formed. After aeration had ceased the <sup>oosonia</sup> oospores were scraped free and individually transferred by a capillary pipette, as already described, to hanging drops of sterile water.

After 14 days, of the 16 oospores so treated, 2 showed signs of germination but this time produced a germ tube directly which branched to form a mycelium.

Fig. 19 reproduces one of these germinating oospores at this stage. No internal change of the oospore could be determined as a result of germination by this method. The length of germ tube produced during this period of 2 weeks was about five times the diameter of the oospore. No further development took place from one spore; the other grew very slowly for another 12 days, after which growth ceased. It was hoped that once germination had been induced a culture might be obtained by removing the germinating spore from the water drop to a more nutritious medium which would support continued growth. The addition of 2% sucrose solution to the germinating spore caused no increase of growth and neither did subsequent removal to a Maltose Agar Plate, on which a sterilised Tomato seedling was growing.

No development was observed from the remaining 14 spores even after 8 weeks.

Aeration may have contributed towards the promotion of germination but since so few of the spores employed successfully germinated, the presence of Oxygen does not seem to be the ruling factor.

The important point emerging from this experiment

is the demonstration of a second form of germination which can occur under conditions of adequate aeration. "Direct" and "indirect" germination were recorded by Uppal (1926) for Sporangia of Phytophthora species according to the presence or absence of oxygen. It would appear from the present work that the same condition may hold for germination of oospores of the species under investigation.

(b) Oxygenation by Chemical Means.

Roots bearing verrucose <sup>oospores</sup> ~~oospores~~ were soaked for three hours in H<sub>2</sub>O<sub>2</sub> (4 vol.), after which the <sup>oospores</sup> ~~oospores~~ were detached and mounted individually in hanging drops. Six were mounted in sterile water and six in films of Tomato Extract Agar.

In twelve days' time, one oospore in sterile water produced a short germ tube, apparently arising from the base of the antheridium, as shown in Fig. 20. None of the other oospores showed any development, though those in water were later transferred to Petri Solution. Probably the presence of a nutritive ingredient in the germinating medium is of little importance in assisting the actual germination,

sufficient nutriment for the initial stages being derived from the spore itself, but for subsequent growth, energy will be required from an external source. So far a suitable medium for continued growth has not been found.

B. ALTERNATION OF TEMPERATURE AS A MEANS OF INDUCING OOSPORE GERMINATION.

An alternation of temperature between 50° C. and 10° C. was achieved by heating the <sup>oosporia</sup> ~~oospore~~-bearing roots in a tube of water held in a water-bath, which was maintained at 50° C. for 10 minutes. The tube was then rapidly cooled to 10° C. and held at that temperature for 30 minutes, after which the heating was repeated to subject the roots to a further 10 minutes at 50° C. before cooling to room temperature. The <sup>oosporia</sup> ~~oospores~~ were separated from roots and mounted, as before, in hanging drops of a variety of Media - 2% Asparagine, Tomato Extract and Sterile Water.

A total of 30 oospores were mounted and of these, one in water produced an unbranched germ tube in twelve days. This oospore is shown in Fig. 21.

After the initial growth no further development could be induced. All the rest remained undeveloped though retained for twelve weeks. Another set of oospores subjected to alternations of temperature was set up a few weeks later. This time the maximum temperature was raised to 60° C. and the minimum retained at 10° C. After treatment the spores were mounted in hanging drops of Sterile Water and films of Tomato Agar. Of 15 oospores treated, not one germinated.

Alternation of temperature, within the range employed, seems therefore to have negligible effect on encouragement of germination.

#### C. HIGH TEMPERATURE PRETREATMENT OF OOSPORES.

Since infection of healthy tomato plants may take place from soil recently steam sterilised, it would seem that oospores are able to withstand high temperatures for limited periods without damage and it is probable that they may even be stimulated to germinate by exposure for a period to such temperature. In order to test this proposition oospores suspended in water were heated to approximately 210° F. for 20

minutes in order to reproduce to some extent the temperature conditions aimed at in soil sterilisation. A second batch of oospores was treated at a slightly lower temperature, 180° F., for the same period. The heating was done with oospores still attached to roots in a tube of water in a waterbath. After treatment, the oospores were detached and mounted in hanging water drops. Ten were so treated at the higher temperature and no development was obtained. Oospores treated at the lower temperature similarly showed no germination.

#### D. LOW TEMPERATURE PRETREATMENT OF OOSPORES.

The cold treatment was carried out on oospores estimated to be approximately five weeks old and still attached to the parent root. Oospore bearing roots were kept moist, either by placing on filter paper in a tube, one end of the filter paper dipping into water, or by placing directly in a tube of water. Tubes prepared in both these ways were stored in a refrigerator at a temperature of 4-5° above freezing point for periods of 2-4 weeks. On examination of the roots

after two weeks' time, there were no signs of vesicle formation. Some oospores from these roots were then transferred to sterile water hanging drops at 20° C., but after three months these still remained undeveloped.

After four weeks' refrigeration, all the remaining oospores were transferred either direct to hanging drops of glucose solution or tomato extract or to fresh sterile tap water, to soak for two weeks before mounting in hanging drops of Petri Solution. In all cases, the oospores remained ungerminated even after a period of eight weeks. Some slight change in the contents of oospores attached to roots which after four weeks' refrigeration had been soaking in water for a further six weeks, was noted. The oospores appeared turgid and intact but the contents had lost their original granular appearance which had given place to groups of large globules. (Figs. 22 and 23)

This experiment therefore indicates that subjection to low temperature does not encourage germination of oospores of Phytophthora verrucosa.

From the above experiments, it appears that oospore germination is not directly related to any one of the factors considered. Increased aeration did result in a total of 3 germinated oospores of the 28 treated, i.e. 10.7%, and that number includes the majority of the oospores which germinated after subjection to treatment of any kind. In addition, one case of germination was obtained in the group treated by alternation of temperatures.

According to previous observations sporangia, germinating under conditions of ample aeration, develop directly into vegetative mycelium. Uppal (1926) describing two distinct types of germination for conidia of Phytophthora species, mentions that 'direct' germination, or production of mycelium at once, is most frequent in well aerated media, whereas 'indirect' germination or the production of a short hypha terminating in a sporangium which released zoospores, is the common type of germination in poorly aerated media. When vesicles were produced by P. verrucosa, they were on each occasion derived from oospores which had been submerged in stagnant water for several days. It would appear that the vesicles are comparable to sporangia

formed by other species under similar conditions of poor aeration.

The season of year or stage of development of the spore does not appear to have any bearing on whether or not vesicle formation occurs. In the first instance, this observation was made in early February, not long after oospores were first shown to be present in quantity on Tomato roots. Oospore-bearing roots treated similarly towards the end of June behaved in the same fashion, producing vesicles from a number of <sup>oosonia</sup> ~~oospores~~ on detached roots submerged for several days in water (Figs. 16 and 18). In the examples illustrated, the vesicle walls have been ruptured and the zoospores have escaped. The other occasions when vesicle formation was observed were, firstly, on a detached <sup>oosonium</sup> ~~oospore~~ lying for 20 days in liquid Potato extract in February, and again in mid-May when vesicles were found on roots refrigerated for 4 weeks after being soaked at room temperature for 6 weeks. The vesicles found at this stage are shown in Figs. 25 and 26. In Fig. 25 the vesicle wall appears rather thicker than that of previous examined vesicles, but the evacuation of the oospore contents and firm

attachment of the vesicle suggested that it was of the same origin as the previous thin walled structures. Figs. 26 and 27 show other vesicles collapsed at the side of empty <sup>oospores</sup> oospores.

During the following spring, a watch was kept on oospore-bearing roots for the recurrence of vesicle formation. From April to the end of June these structures, either full or after evacuation, were to be found. On three occasions motile spores were observed within the vesicle.

The formation of vesicles, therefore, extended from February, shortly after the formation of oospores, to the middle of July, i.e. during the greater part of the growing period of the host plant. It seems likely then, that there is little correlation between seasonal influence and 'indirect' germination. The evidence that vesicles were formed in most cases on submerged roots, and when supplementary aeration was supplied 'direct' germ tube formation was induced, suggests that aeration is a factor determining the form of germination.

Pretreatment of Oospores in Atmosphere lacking Oxygen.

Since previous experiments have indicated that the greatest proportion of oospores germinated after treatment designed to increase the quantity of Oxygen present, and that in these cases germination tended to be of the 'direct' type, it is suggested that such a theory might be supported by a counter experiment subjecting resting oospores to conditions where oxygen is absent. Should the theory be correct, it might be supposed that the absence of oxygen would impede germination, that few germinating oospores would be found, and that if germination does occur it would be of the 'indirect' type involving production of zoospores. Alkaline pyrogallol was used as the agent for removal of oxygen, 14 grams of this solution being adequate for the removal of oxygen from an 80 cc. capacity jar, though opened on two occasions. The jar carried a ground glass stopper which could be made air tight. Alkaline pyrogallol was placed in the jar and dipping into this were three small glass tubes containing roots bearing verrucose <sup>oosonia</sup> ~~oospores~~ in distilled water.

After a period of 43 hours the first tube was removed and the <sup>oosonia</sup> ~~oospores~~ detached from their roots and

mounted in hanging drops. After a further 23 hours the second tube was removed and treated likewise, and the last tube removed after 6 days. Examination of the oospores two weeks after the last series was mounted revealed no change, and two weeks later all but one were still unchanged. This particular one, subjected to 66 hours' exposure to deoxygenated atmosphere, appeared one month after mounting as an <sup>oosporial</sup> shell. The contents must have been dispersed, but no remnants of a vesicle was observed in this case.

This experiment does, however, bear out the conclusion relating to necessity of oxygen for germination, since of 57 oospores in atmosphere depleted of oxygen, all save one remained ungerminated.

#### Pretreatment of Oospores with $\beta$ indolyl acetic acid.

Attempts were made to induce the germination of oospores of P. verrucosa by pretreatment with  $\beta$  indolyl acetic acid. Two dilute solutions of the chemical were made up to the concentrations of 1:10,000 and 1:20,000. Root fragments known to be bearing verrucose <sup>oosporia</sup> oospores were soaked in water for some days prior

to immersion in the solutions for 12 hours. On removal from the solutions <sup>oospores</sup> ~~oospores~~ were detached and mounted individually in hanging drops as before. In some cases minute fragments of tissue with <sup>oospores</sup> ~~oospores~~ were used.

One month after mounting no germination had been induced in any of the 17 oospores subjected to the weaker concentration nor in oospores given the stronger treatment.

#### GERMINATION OF SPORANGIA.

It was hoped to obtain a culture of P. verrucosa from the germination of a sporangium or zoospores. This suggested a likely line of approach since the question of dormancy does not arise as with oospores. Unfortunately sporangia of this species conforming in all respects with those described by Foister (1940) could not be procured and this project had therefore to be set temporarily aside.

Sporangia of doubtful identity which were found on Toe Rotted roots were found to germinate without

difficulty. Hyphae arose from the apex and branched to form mycelium, or the sporangium ruptured with the release of zoospores which also readily produced mycelium. Cultures were obtained of this species but these showed no inclination to form sexual bodies by which it might be identified.

#### TRANSFERENCE OF DISEASE.

It has so far proved impossible to obtain a pure culture of the fungus P. verrucosa with which to confirm the pathogenicity of the organism. Attempts were made to induce infection by the use of pieces of root tissue (unsterilised or partially sterilised) bearing verrucose <sup>oosporic</sup> oospores, as inoculum. The use of this material was of course unsatisfactory for the reason that several organisms, capable of causing tissue rot, were present in association with P. verrucosa, but in the absence of a pure culture, this appeared to be the only possible means of inducing infection. It was assumed that, if P. verrucosa is the most virulent of the pathogens present, it would

be the first to attack and Toe Rot symptoms would be the first to appear. The inoculum used was in some cases taken direct from the root without sterilisation, which might impede the growth of the fungus. In the series of inoculations of sterile seedlings in Petri plates described below, the inoculum was partially sterilised with 1:2,000 Acriflavine, which has the effect of restricting interference by bacteria.

A. Transference of disease by direct contact.

The most obvious method of producing artificial infection was to imitate as far as possible the events leading to natural infection. A supply of seedlings was raised in sterilised compost and potted up in soil collected from plants known to be infected by the presence of verrucose <sup>oosonia</sup> ~~oospores~~ on their roots. This compost would, no doubt, contain many pieces of diseased root material liable to cause infection of healthy plants with which they came into contact. The compost was mixed with a proportion of sterilised material.

Potting was carried out in May. A later batch was potted up in the last week of June. By the end of July the later batch had been growing in diseased soil

for 5 weeks and during this period infection was negligible. Of 16 plants examined, only 1 displayed the condition of Toe Rot. The root systems of the others remained white and healthy.

Of the earlier batch of plants, 9 were grown in infected soil for 11 weeks, during which very poor growth was made. The plants were all hard and spindly and the leaves blue veined. None of the root systems were healthy and 7 of the 9 plants showed signs of Toe Rot. These 7 and the other 2 plants with tap roots remaining white were all examined microscopically for oospores of P. verrucosa in order to verify its presence as suggested by the symptoms. Though fungal hyphae were present in some quantity and Colletotrichum could be found in most plants, yet oospores of P. verrucosa could not be found. This examination was carried out in late July and it is therefore possible that the majority of ~~oospores~~<sup>oogonia</sup> would have shed their contents, and remained only as oogonial shells attached to the roots. This was a condition found on a batch of the potted plants received at the laboratory at this period. However, oogonial shells could not be seen on the seedlings used for attempted

artificial infection.

After each plant had been partly freed of soil for examination, it was placed in a beaker of water overnight in order that sporangial development might be stimulated, and examined on the following day. Sporangia were found in 2 out of 9 cases, these 2 showing symptoms of Toe Rot. The sporangia formed, however, were nonpapillate but not flattened at the tip, resembling rather the type of sporangium produced by P. cryptogea. The sporangia were found to germinate readily either by zoospore discharge or by extension of their tips with the immediate formation of a germ tube. A culture was obtained by the latter method but was not one of P. verrucosa.

Another technique was employed in order to induce artificial infection by direct contact. By this method the length of time during which healthy material was in contact with the infected fragment used as inoculum was reduced, in order that secondary infection might possibly be decreased.

A number of seeds were sterilised in bleaching powder and allowed to germinate on sterile moist filter paper in a sterile Petri dish. When the roots were

about 15 mms. long, pieces of diseased root, first dipped in Acriflavine solution 1:2,000, were placed in contact with them. After 4 days' contact the seedlings were taken out of the Petri dish and potted in sterilised compost.

During the next fortnight most of the plants damped off; 4 plants alone remained for examination. Of these only 1 showed typical symptoms; another showed the destruction of the base of the stem common in P. cryptogea attack. The other 2 remained healthy.

Once again this examination was carried out in late July when oospores were not found in such quantity, but, if sporangia could be induced to form, the presence of P. verrucosa might be disclosed on the one plant showing Toe Rot symptoms. The entire root system of this plant was therefore transferred for 60 hours to a 0.5% solution of  $\text{KNO}_3$ , but no sporangia could be found after this treatment.

In view of the ease of attack and extent of damage which can result under natural conditions from the presence of the Toe Rot organism, it seems strange that so little evidence of infection could be discovered by these attempts so closely imitating nature.

In the second mode of the infection experiment the exposure to contact, i.e. 4 days, may have been too short, but even in the previous form, involving contact with infected soil throughout growth, the plants, though showing symptoms of Toe Rot, revealed no confirmatory signs in the form of sporangia or oospores.

Only in the course of this study has the seasonal tendency for the formation of reproductive bodies by P. verrucosa come to light. It may be possible, by repeating these attempts at different seasons, that a greater percentage infection will result and the actual presence of the fungus be revealed.

#### B. Transference of disease by Spore Suspension.

Following the disclosure of the means of oospore germination in this species, another method of inducing artificial infection of the Tomato with Toe Rot suggested itself. Since oospores germinate to produce a vesicle which releases motile spores into the surrounding liquid, it was thought that these spores might be the primary cause of infection from the soil, and that a spore suspension might induce infection of

healthy roots immersed in it.

During early February, roots bearing verrucose ~~oospores~~<sup>oogonia</sup> and left for 4 days in sterile tap water showed the development of thin walled vesicles, many of which were empty after release of their contents. Twelve seedlings brought on in commercially sterilised compost, were washed free of adhering particles and supported with their roots submerged in the spore suspension in an open dish. The pH of the suspension was approximately 7.5-8.0.

After 6 days the roots were removed from the suspension and were found to be yellowish in colour in comparison with others submerged over the same period in plain sterile water. Examination revealed no evidence of P. verrucosa, though some algal infection had occurred and small brown dots adhered to root hairs in both the infection experiment and control.

The seedlings from the infected water were potted up in sterilised compost prepared for the commercial Tomato crop. Sixteen days later 2 of the seedlings were washed free of soil for examination. The root system now consisted of new white roots formed from the stem. That part of the original root system

which had been immersed in the spore suspension was reduced to brown shrivelled strands. On both these plants verrucose <sup>oosonia</sup> oospores were found in some quantity, in each case on short rootlets about  $\frac{1}{4}$ " long. These rootlets were slightly brown at their tips and arose from the region exactly at the junction of the shrivelled lower portion and the healthy adventitious roots above them. Two further plants were examined 4 weeks after potting and revealed <sup>oosonia</sup> oospores also but these were not so definitely verrucose as those of the previous pair. It is possible that they were immature <sup>oosonia</sup> oospores of P. verrucosa or those of P. cryptogea. Subsequent examination of the remaining 8 plants at intervals up to 2 months showed no further <sup>oosonia</sup> oospore production. Therefore of 12 plants, 2 demonstrated undoubted infection by P. verrucosa by the actual presence of <sup>oosonia</sup> oospores together with rotting of the terminal portions.

From a seedling thus dipped for 6 days in a spore suspension a culture was obtained on Maltose Agar. The growth was coenocytic and suggestive of a Phytophthora. Apart from short swollen and slightly curved hyphae resembling a young sporangial incept, the mycelium was purely vegetative.

Subsequent subcultures on a variety of other media, including Potato Agar, Oatmeal Agar, Petri solution, Bog soil extract, sterile tap water, 0.5% KNO<sub>3</sub>, potato blocks and a potato agar plate buried in the soil for 7 weeks revealed no indication of sporangial or oogonial production. The culture could not therefore be assumed to be P. verrucosa.

OTHER ORGANISMS FOUND ASSOCIATED

WITH TOE ROT.

P. verrucosa, as recorded by Foister (1940), is the accepted causal agent of the disease known as Toe Rot. In view, however, of the relatively few occasions when the presence of the organism could be determined, i.e. in 55% of the plants showing symptoms, it may be useful to record other fungi whose presence seemed to be equally constant, at least during certain parts of the season. It may be that they contribute to the typical symptoms of the disease, or they may simply be secondary parasites entering the tissue once it has been partly destroyed, and subsequently causing

the extensive rotting of the whole root system met with in advanced cases.

The chief genera encountered during the study of roots showing Toe Rot symptoms were: Asterocystis, Napicladium, Colletotrichum, Rhizoctonia, an occasional Helminthosporium, and frequently Phytophthora cryptogea.

Asterocystis sp.

The presence of Asterocystis was noted almost invariably during the winter months on plants which later showed the presence of P. verrucosa. Indeed one might assume that it was the primary agent of attack, since its resting spores were found in quantity in young roots still white and apparently healthy. The spores were situated in the cortical cells and were found during the period November to January just prior to the appearance of P. verrucosa oospores. During the first half of that time the resting spores were so large as almost to fill the cortical cells, while the later formed spores were considerably smaller, being only 10 $\mu$  in diameter. From February to June smaller Asterocystis spores could be found on

Toe Rot plants, but the genus was not so much in evidence.

Some diseases of Carrot, Oats and Strawberry and Cabbage have been ascribed to Asterocystis spp., but Vanterpool (1930) considers that alone it is of little economic importance to cereals but acting in association with other fungi, it may prove to be slightly pathogenic. Considering the large size and quantity of spores present on Tomato roots during November it seems inevitable that some degree of dislocation of the absorptive system of the root would result, and the early attack of Asterocystis may render the weakened root more susceptible to Toe Rot.

Napicladium sp.

Throughout the year, during the search for oospores of P. verrucosa, the presence of small golden brown dots of approximate diameter  $11\mu$  was frequently noted. On most plants bearing oospores of P. verrucosa, these brown bodies were found to be present in addition.

The colouring matter from these permeated a little way through the wall into the adjacent proto-

plasm, giving the walls an irregular outline (Fig. 33). On various occasions from February to December, brown septate conidiophores were found arising from a swollen base singly or in pairs. It was considered that the swollen base resulted from the development of the brown dot. The conidiophores measured 90-110 x 4  $\mu$ . Towards the tip the conidiophores became slightly zig-zag and bore acrogenously a single elongate ovate 2-3 septate spore 22-26 x 11  $\mu$ . The point of attachment to the conidiophore was a clearly defined tapering structure. Figs. 31 and 32 show the intensity of attack and Fig. 34 shows the spore in detail. This fungus was identified by means of Clements' (1909) key, as Napicladium sp. Some members of the genus are slightly pathogenic, usually on leaves, but none have been reported on the Tomato. The fungus may have gained entry from leaf mould in the compost. It was present in 40% of the Toe Rot plants examined.

Colletotrichum sp.

Attacks of this fungus did not appear to any extent until the growth of the plant was advanced. Older plants examined during June-August bore quantities

of black sclerotia with brown tapering setae. These were frequently found on the root cortex which had become detached from the stele after drying.

C. atramentarium is itself responsible for root rot of Tomatoes (Bewley, 1923). By Dickson (1936) it is regarded as a weak parasite attacking debilitated plants and young plants. In this instance, on account of its late appearance, it is likely to be functioning as a secondary parasite, aggravating and extending the rot begun by a previously attacking organism.

Rhizoctonia sp.

Very frequently rather slender, septate, subfus-cus hyphae were noticed making a straggling growth over the surface of the root. It was recognised as Rhizoctonia sp. The fungus was present on young seedlings from diseased soil as well as on older, partially rotted plants, but the superficial nature of its growth and its sparseness suggested that this fungus was not a primary cause of destruction.

Helminthosporium sp.

No previous record has been found of this genus occurring on Tomato roots. On this occasion, spores were found in quantity on some roots of wilted plants which had been dug up and allowed to dry in a loosely covered glass dish in the laboratory. The lesions could clearly be seen as small sooty patches on the roots and also in greater abundance around the crown. A culture was obtained from a germinating spore and this was grown on several media. Spore measurements were made on each medium with the intention of identifying the species. Growing naturally on the root the spores measured on an average  $43.7 \times 9.25 \mu$ , the range being  $26-70 \mu$  in length by  $7.4-11 \mu$  in width. The septation varied from 5-10, the majority being 3-5 septate. On cultures, septation tended to be greater. The spores were elongate, of an even thickness along their length, dark brown except for both terminal segments, which were hyaline. A short study of Helminthosporium species suggested that this isolate approximated to H. scolicoides.

Phytophthora sp.

Very frequently on roots infected with P. verrucosa and on those where it was not found, evidence of another species was obtained. Immersion of such roots in water led to the production of non-papillate sporangia (Figs. 9, 10, 11) similar to those mentioned by Pethybridge & Lafferty (1919) during their study of Foot Rot of Tomatoes. Less often, smooth walled regular oogonia were found within the tissue. It is probable that this species is P. cryptogea, the Foot Rot organism. This is a common disease of Tomatoes in unsterilised soil and is likely to be present together with Toe Rot in these conditions and would contribute towards the extensive root rotting found on Toe Rot plants.

CONTROL.

Tentative trials for the purpose of suggesting control measures were set up. A proprietary preparation, employing as active agent, organically combined mercury was used with the intention of trying its

effect on diseased plants. A solution of this material was employed as a drench to the soil around the plant. The second treatment involved dipping diseased plants and replanting in sterilised soil. A quantity of plants, raised in sterilised soil, was potted up in compost derived from pots where infected plants had grown, to which was added sterilised compost. After a period of 2 weeks to allow of infection, treatment was commenced. The drench was applied to the soil of one batch of plants, and repeated at 4 weekly intervals. The dipping treatment applied to another batch was carried out only once. As much soil as possible was shaken from the plants and their roots dipped into the prescribed solution of the chemical. They were then repotted in sterilised compost. A third batch was left growing in pots of diseased soil as control.

After 5 weeks had elapsed, the control plants were examined for presence of the disease. Of the 33 control plants, 3 showed Toe Rot symptoms on their roots; the rest retained healthy white roots and intact tap root. Neither the 3 diseased plants nor any of the others revealed P. verrucosa oospores, nor could

sporangia be induced to form by immersing their roots in solutions of Asparagine, Urea, Ammonium sulphate, for 20-70 hours and then transferring back to sterile water for a night before examination.

Since the conditions supplied to the control section had not apparently been conducive to infection, the basis for comparison with the treated lots had broken down. These when examined 10 days later for external signs of Toe Rot had in many cases damped off completely. Of the plants treated with the drench 6 remained; the tap roots of 4 of these had quite disintegrated.

It appears therefore that at this stage of the investigation experiments on control of Toe Rot are premature. It was considered essential that the conditions necessary for infection should be fully understood before attempts at control could be expected to be successful.

DISCUSSION.

In the course of the present study, it has become apparent that of the Toe Rot material examined, only a small proportion gave undeniable evidence of the presence of P. verrucosa by the formation of reproductive bodies on Tomato roots. The occurrence of the disease, as recognised by external symptoms, is widespread, but where the fungus is still in a vegetative condition within the plant, there is no means of identifying the pathogen.

The formation of oospores is observed to be a seasonal feature. None were found until the beginning of February. The fungus may have been present for some time in a vegetative form, since plants had been in contact with diseased soil for 3 months before oospores were first noticed in the spring. Throughout the spring, on the proportion of plants showing the presence of oospores, they occurred in abundance. In July oospores were less frequent, and amongst these were a considerable quantity of <sup>oogonial</sup> ~~oospore~~ shells, suggesting that the season of oospore production was near an end. When oospores are developed their maturation

is completed within a few days. This was demonstrated when on February 9th <sup>oo spores</sup> oögonia, recently formed, were found capable of germinating to form vesicles when they were immersed in water.

Root tissue plated on a variety of media yielded no culture of P. verrucosa and oospores were therefore used to obtain a pure isolate. During this work it became evident that oospores of this species germinated in two ways. The direct means, by production of a branching mycelium, was associated with conditions of good aeration. Where this occurred, germination was apparent in 12-14 days. After that period, though oospores were retained for 2 to 3 months, no further germination resulted. The 'indirect' means of germination, by release of motile spores from a vesicle, occurred in stagnant water where aeration was likely to be inadequate. Prior to the discharge of contents, the vesicle appears granular. Later the contents divide (Fig. 28) and zoospores are formed within the vesicle wall. The zoospores are small, 3.5 $\mu$  in diameter, numerous, hyaline and refractive, they became very active within the vesicle. No rupture of the vesicular wall was apparent but the zoospores

seemed to escape in twos and threes through a lateral perforation. The phenomenon was seen to take place on three occasions.

The production of a vesicle closely applied to the parent ~~oospore~~<sup>oosporium</sup> is not a usual feature of the genus. Commonly, germination of oospores of Phytophthora spp. results in the production of a short hypha at the tip of which a sporangium is formed, corresponding to the type of sporangium dispersing an abundance of asexual spores throughout the season. Germination of oospores of the following species has been observed: P. erythro-septica (Pethybridge, 1914), P. syringae (Lafferty & Pethybridge, 1922), and P. cactorum (Rosenbaum, 1917)<sup>(Blackwell 1943)</sup>. In each case a short hypha bearing a terminal sporangium was produced. In these species the oospores are usually to be found embedded in the host tissue. Since the oospores of P. verrucosa are formed superficially on the Tomato root (Fig. 8) a vesicle directly attached to the ~~oospore~~<sup>oosporium</sup> is in as favourable a position for release of zoospores as if a short intervening tube were present. The occurrence of these closely applied vesicles in P. verrucosa may therefore be correlated with environment. Zoospores thus released directly

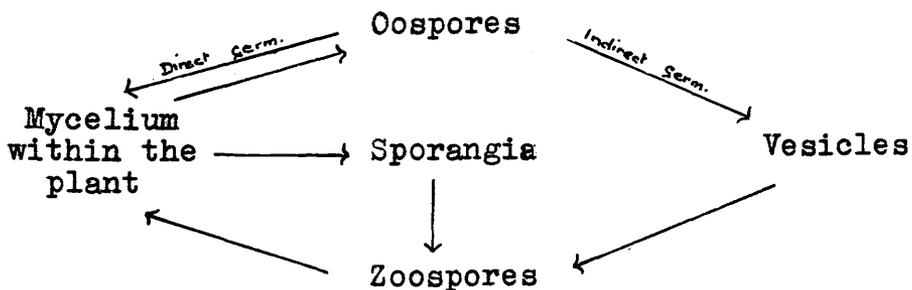
into the soil may cause reinfection during the growing season.

Foister (1940) found sporangia in July near the apices of roots. It appears therefore that infection is spread during the summer by asexual means also.

Around the facts emerging from the present study it is possible to construct a probable life history of the fungus. Oospores formed on young Tomato roots during spring and early summer provide a source of infection after germination, by direct mycelial penetration, or by the release of zoospores from a vesicle. By the latter means, spread of infection to some distance from the original source is possible. Additional infection during the growing season will result from sporangia formed on root tips. The overwintering phase of the fungus is still obscure. The majority of oospores formed during spring and early summer do not appear to function as resting bodies carrying the fungus over to the following year. This conclusion is suggested by the large number of <sup>oocystical</sup> ~~oospore~~ "shells" found from the end of July onwards among a relatively small number of immature oospores. A possible alternative means of overwintering would seem to

be, either by a few remaining oospores adhering to fragments of root tissue or lying free in the soil, or by a mycelial condition in root fragments. The occasion when Toe Rot and oospores of P. verrucosa were successfully induced on Tomato seedlings by immersing the roots in water in which a number of vesicles had discharged their contents, suggests that the spores derived from the vesicle provide the most likely source of primary infection. A sufficient quantity of zoospores to initiate infection could arise from a small number of oospores overwintered in the soil. An alternative suggestion is that the hyphal "knots" recorded in association with this fungus may be concerned in its survival over the winter.

The life history as it is constructed around the evidence set forth may be expressed diagrammatically as follows:-



While some mycological knowledge of the fungus has been obtained, very little information regarding P. verrucosa as a pathogen has accrued. During the course of this work reliance had to be placed on fresh supplies of diseased material and the obstructing feature was the infrequency with which, amongst the quantity of Toe Rotted specimens received, any sure signs of the presence of the fungus could be determined. It has been stated that only 55% of the Toe Rot plants revealed P. verrucosa oospores.

Until a certain means of recognising its presence, even if there only in a vegetative condition, can be devised, experiments involving the artificial production of Toe Rot will remain inconclusive. The promotion of disease by the simple means of planting clean seedlings in compost recently taken from naturally Toe Rotted plants would be expected to be successful. The symptoms were induced in many cases but P. verrucosa could not be identified.

Should a successful cultural technique be devised, isolation would probably result from the vegetative condition within the plant, and recognition of the fungus follow. With this object in view a wide

variety of media was employed and plating carried out at intervals throughout the season. The incidence of other confusing Phycomycetous fungi argued in favour of the alternative method of trying to obtain cultures from the actual fructifications of the species desired. Though this has led to interesting results regarding germination, it was unsuccessful in producing a culture.

Until the matter of proof of the presence of the pathogen is elucidated, experiments on control measures cannot satisfactorily proceed. Since at present there is no means of determining the extent of infection in the controls, no comparison can reasonably be made with treated plants.

#### SUMMARY.

1. The symptoms of Toe Rot of Tomato roots have been detailed and a description of P. verrucosa given.
2. Reproductive structures, on which the identify of the fungus depends, were produced very irregularly.

3. Repeated attempts to derive a pure culture of the fungus from roots plated on a variety of media met with no success. Other Phycomycetous fungi of a vegetative nature were present in sufficient quantity to confuse the issue.
4. An attempt was made to isolate P. verrucosa from oospores which had been subjected to various pretreatments before germination. Initial vegetative hyphae were produced on a few occasions, but subsequent growth could not be sustained.
5. Information was obtained regarding the mode of germination of oospores of this species. Germination may take place in a "direct" method, producing branching hyphae, or "indirectly" with the formation of vesicles and the release of zoospores from these.
6. Toe Rot symptoms have been produced by planting in diseased soil, but ~~oospores~~<sup>oospores</sup> to confirm the presence of P. verrucosa were frequently lacking. P. verrucosa was induced artificially, however, by immersing the roots of seedlings for 6 days in water in which ~~oospores~~<sup>oospores</sup> had borne vesicles and these had become evacuated. ~~Oospores~~<sup>Oospores</sup> were found on these seedlings 16 days after immersion.

7. As <sup>oosonia</sup> oospores of P. verrucosa were not invariably present as evidence of infection by that fungus, a record was made of other fungal flora recurring on Toe Rot plants. None of these appear in themselves to be sufficiently actively parasitic to act as Pathogen. P. verrucosa is therefore still regarded as the primary cause. The Toe Rot symptoms may be produced by the fungus in a vegetative state, unidentifiable, in the absence of <sup>oosonia</sup> oospores, as P. verrucosa.
8. Control experiments were set up. Owing to the sporadic nature of the infection in untreated sections, no true basis for comparison was available. A fuller understanding of the conditions favouring attack is necessary before satisfactory control measures can be planned.
-

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PLATE 1.

Fig.1. Toe Rot plants in a commercial nursery.

Fig.2. Diseased Tomatoes contrasted with healthy plants.

Fig.3. Diseased Tomatoes contrasted with healthy plants.

Fig.4 Severely infected plants with a healthy specimen.





PLATE 2.

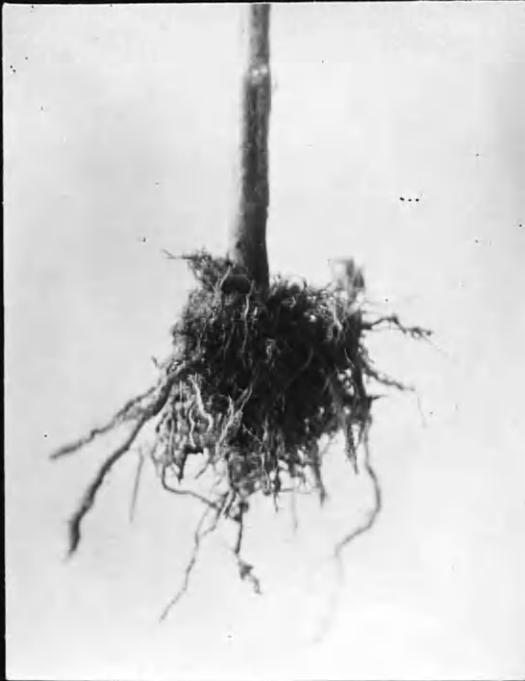
Fig.5. A Tomato seedling with Toe Rot.

Fig.6. The entire extent of root system remaining on a  
badly diseased plant taken from the nursery  
illustrated in Fig.1.

Fig.7. Tomato roots with Toe Rot, cut longitudinally.



5.



6.



7.



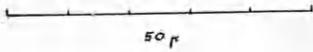
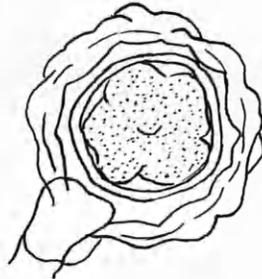
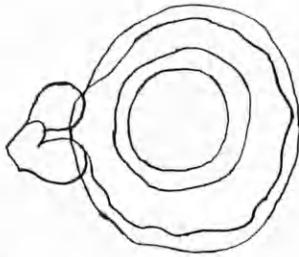
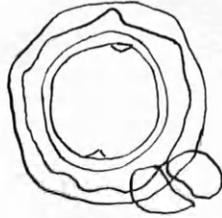
PLATE 3.

Fig. 8. <sup>Oogonia</sup> ~~Oospores~~ of P. verrucosa on Tomato root. x 60.

Fig. 9. <sup>Oogonia</sup> ~~Oospores~~, showing varying degree of verrucosity.



8.



50  $\mu$

9.

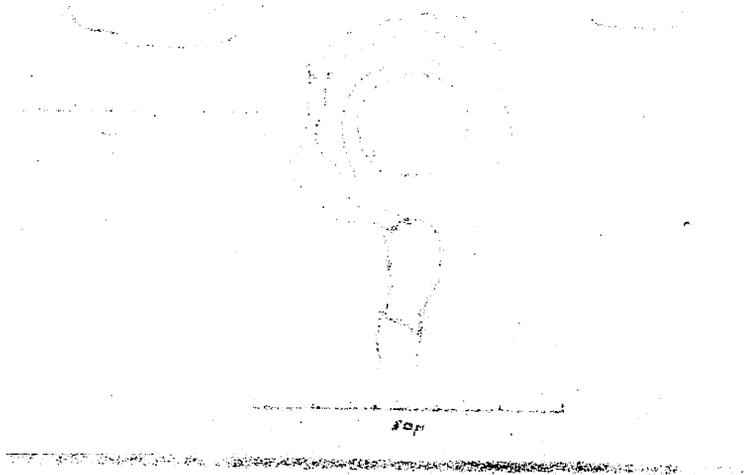
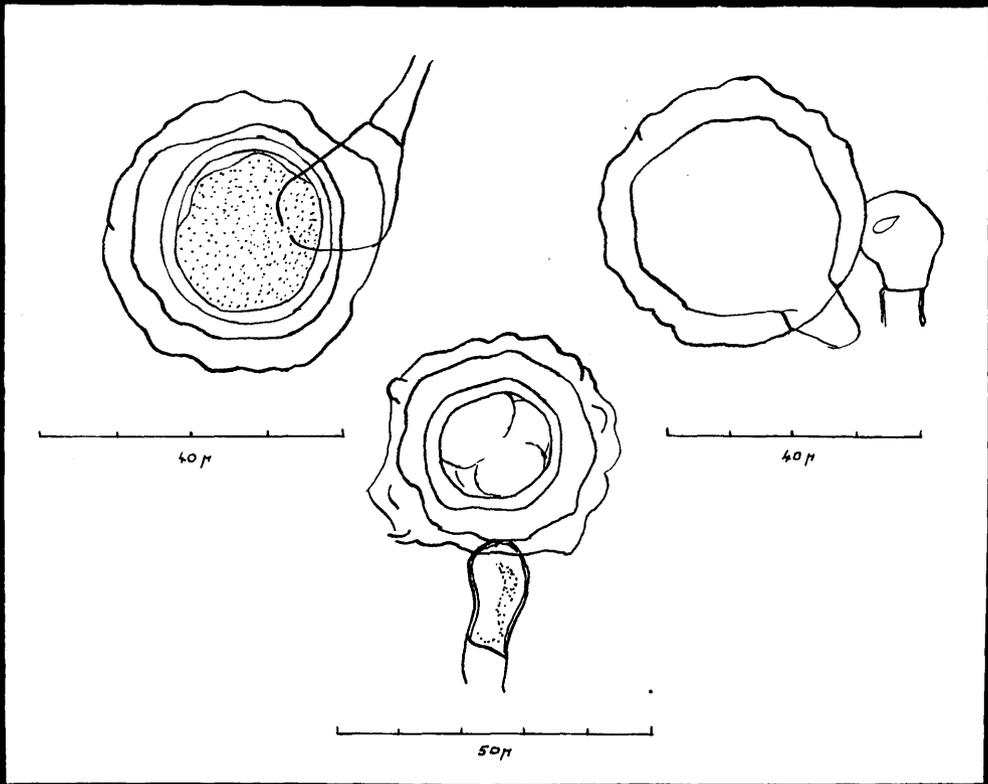


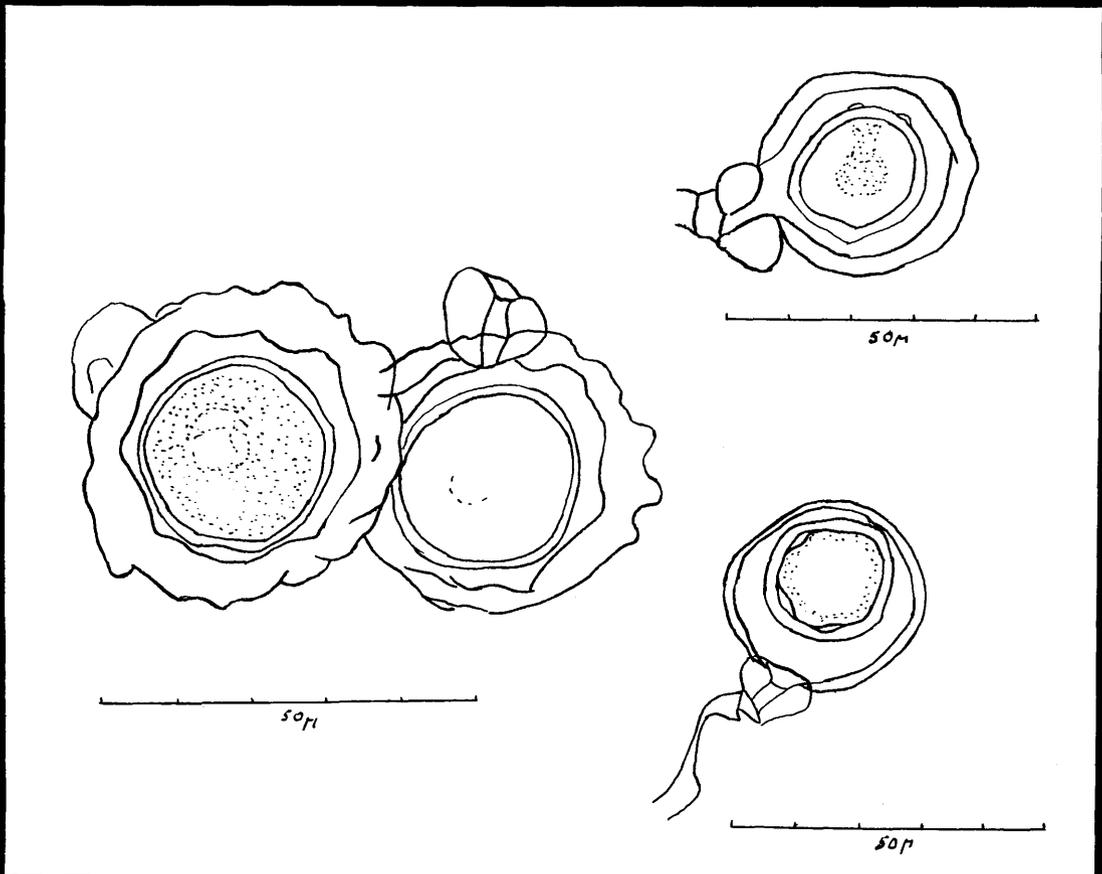
PLATE 4.

Fig.10. Oogonia with paragynous antheridia.

Fig.10a. Oogonia with amphigynous antheridia.



10.



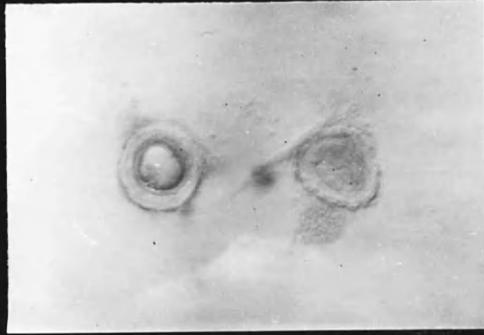
10a.



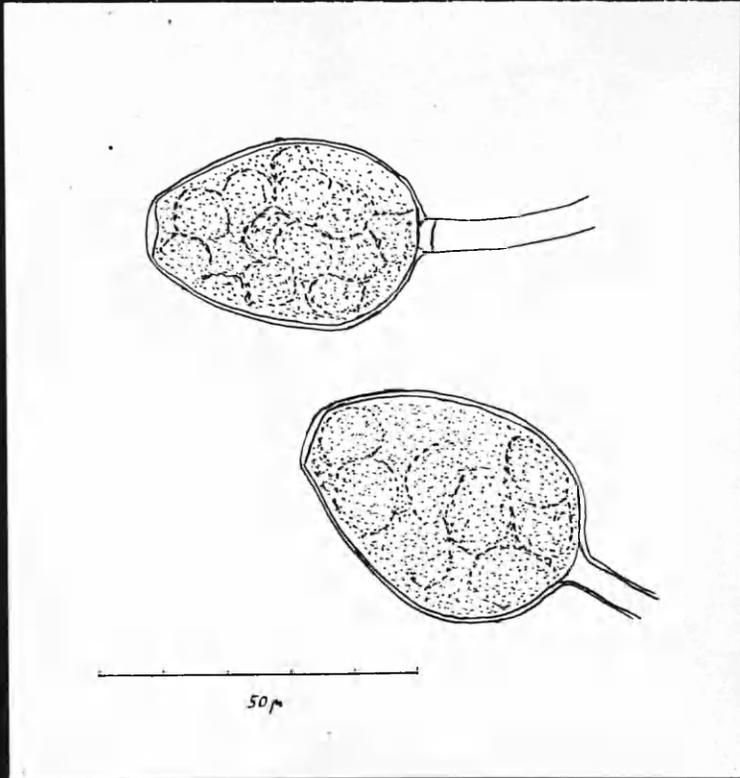
PLATE 5.

Fig.11. Oogonial"shell" with vesicle and mature <sup>oogonium</sup> ~~oospore~~.  
x255.

Fig.12. Sporangia of Phytophthora sp. found on Toe Rot  
root.



11.

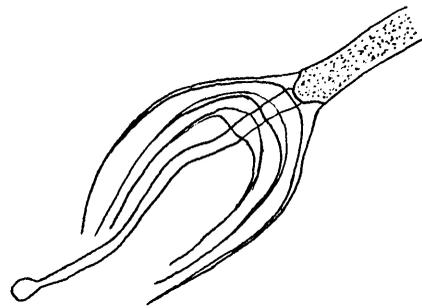
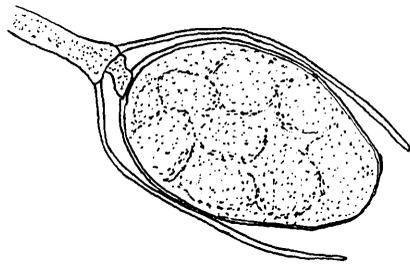


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PLATE 6.

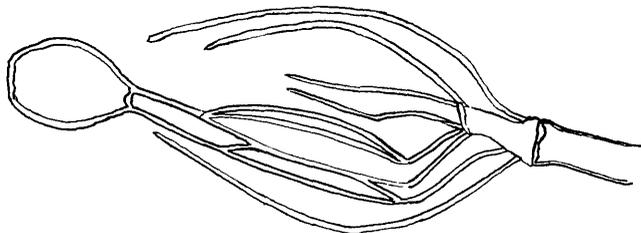
Fig.13. Proliferation of sporangia of Phytophthora sp.

Fig.14. More rare form of proliferation of sporangium  
of Phytophthora sp.



50  $\mu$

13.



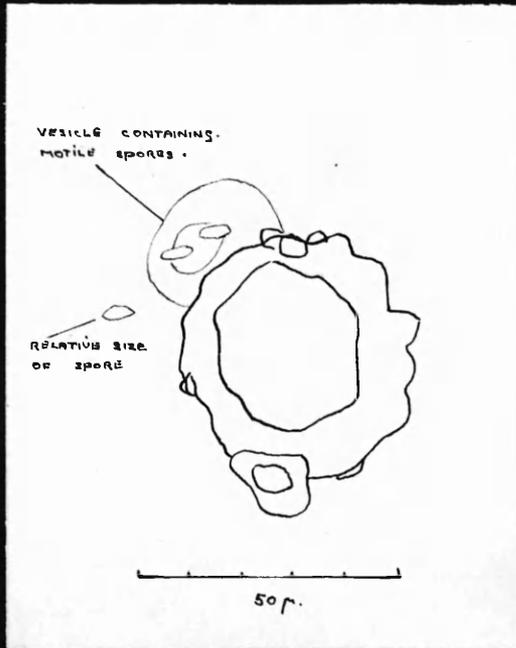
50  $\mu$

14.

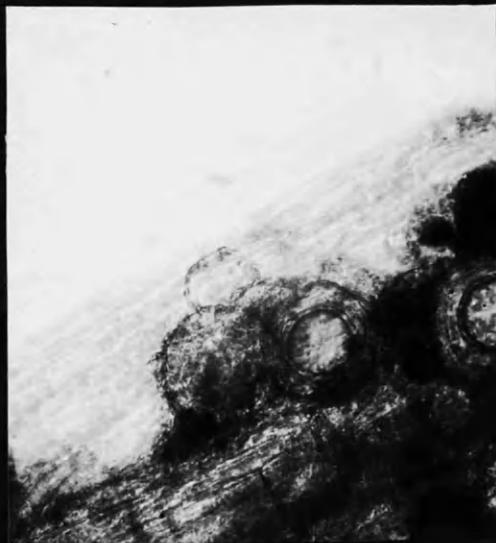
PLATE 7.

Fig.15. P. verrucosa. Oogonial "shell" and vesicle from  
a Tomato root.

Fig.16. P. verrucosa. Oogonial "shell" and empty vesicle  
on a Tomato root.



15.

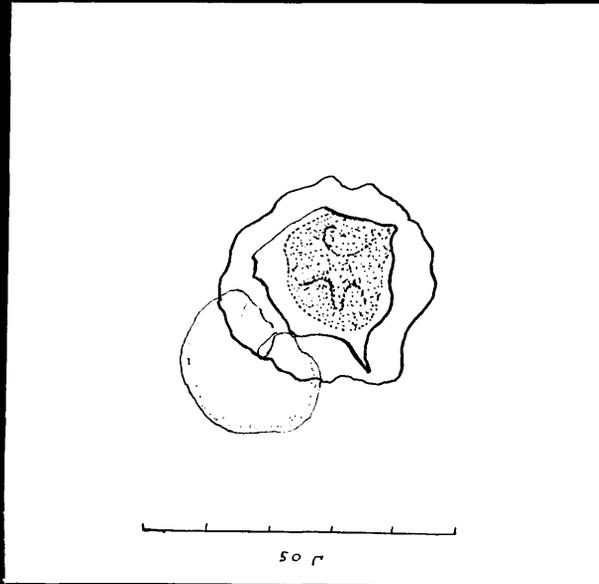


16.

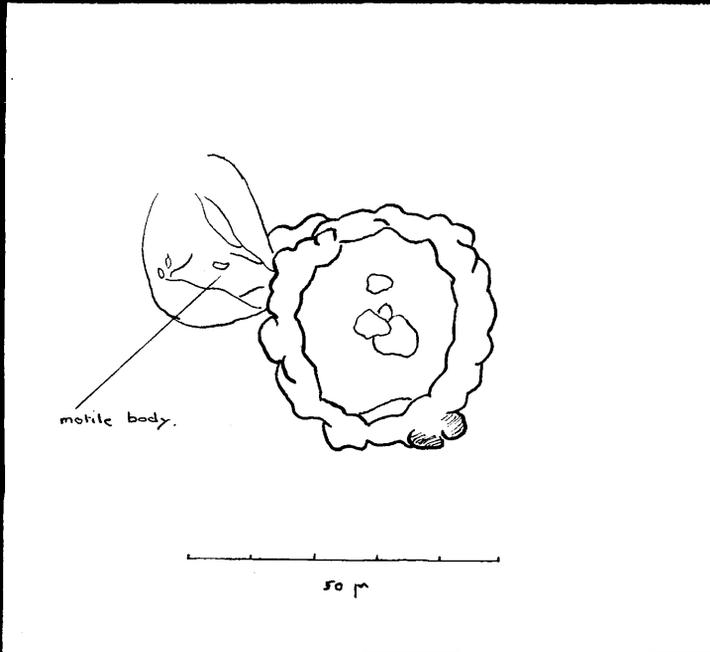
PLATE 8.

Fig.17. P. verrucosa. Oogonial "shell" and vesicle from  
a Tomato root.

Fig.18. P. verrucosa. Oogonial "shell" and evacuated vesicle  
from a Tomato root.



17.

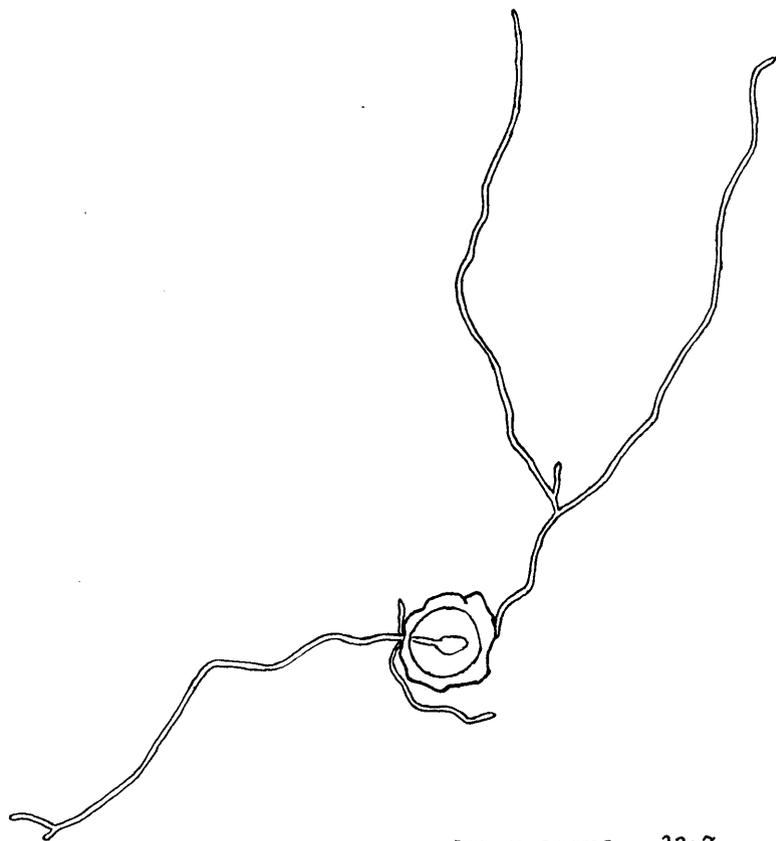


18.

PLATE 9.

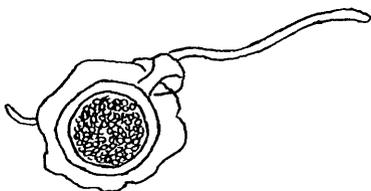
Fig.19. Oospore of P. verrucosa germinating in 14 days after pretreatment by aeration.

Fig.20. Oospore of P. verrucosa germinating in 13 days after pretreatment with H<sub>2</sub>O<sub>2</sub>.



DIAM OF OOSPORE 33.7 $\mu$ .

19.



DIAM. OF OOSPORE 36 $\mu$ .

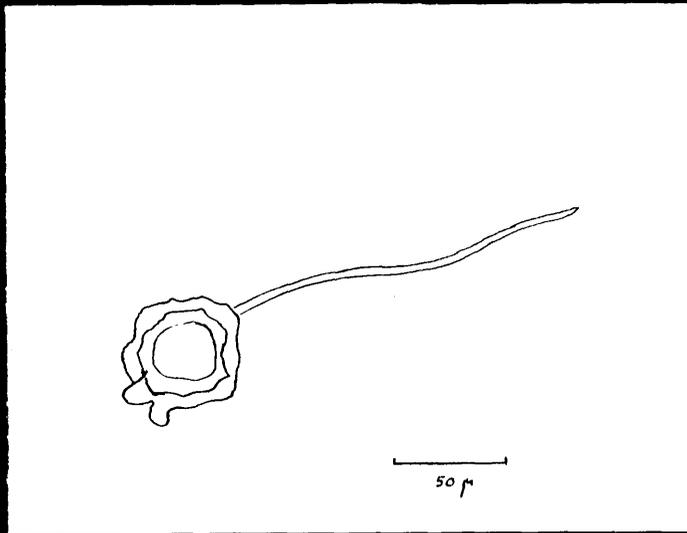
20.

PLATE 10.

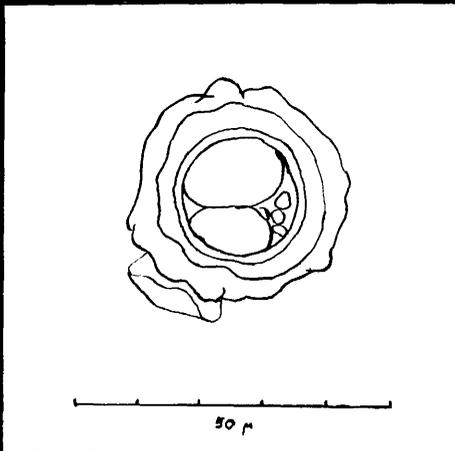
Fig.21. Oospore of P. verrucosa germinating in 12 days  
after pretreatment with alternating temperatures.

Fig.22. Oospore with contents resolved into globules.

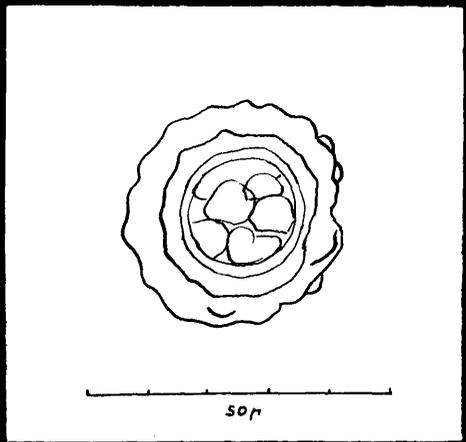
Fig.23. Oospore with contents resolved into globules.



21.



22.



23.

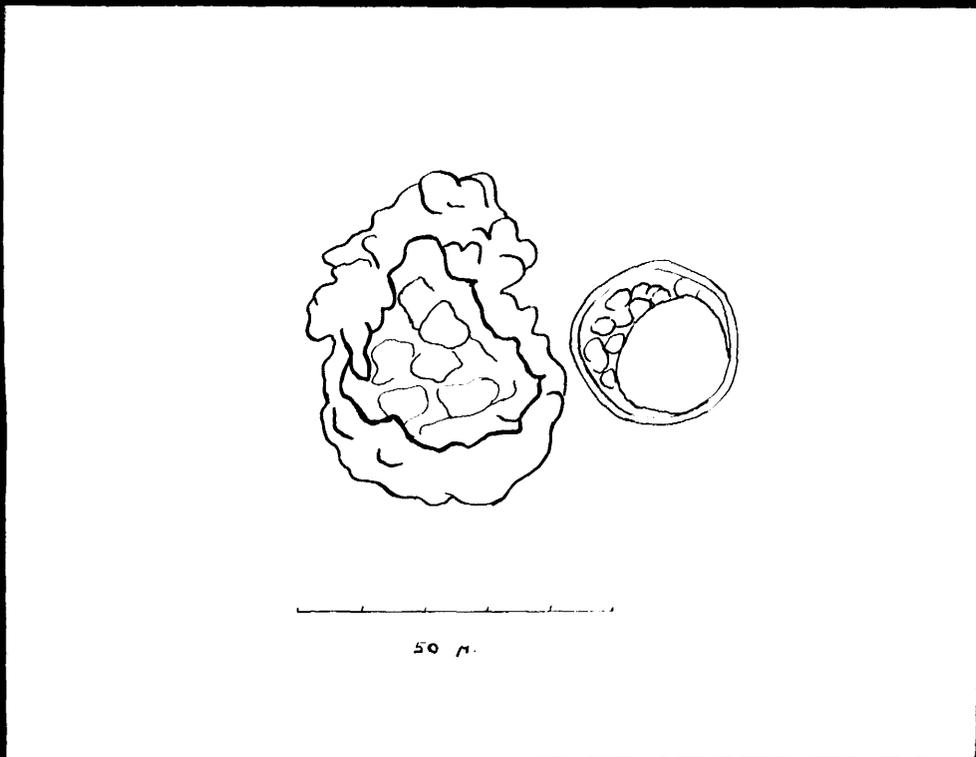


PLATE 11.

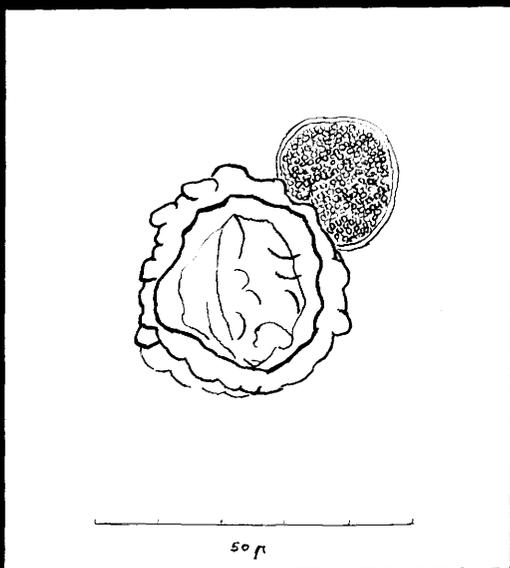
Fig. 24. Oospore shed from an oogonial "shell".

Fig. 25. Oogonial "shell" with vesicle.

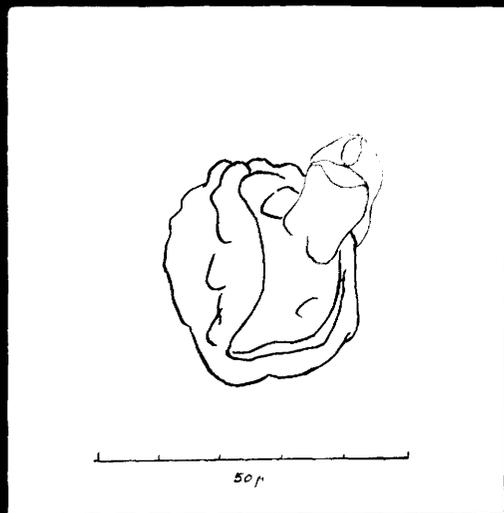
Fig. 26. Oogonial "shell" with vesicle evacuated and collapsed.



24.



25.



26.

PLATE 12.

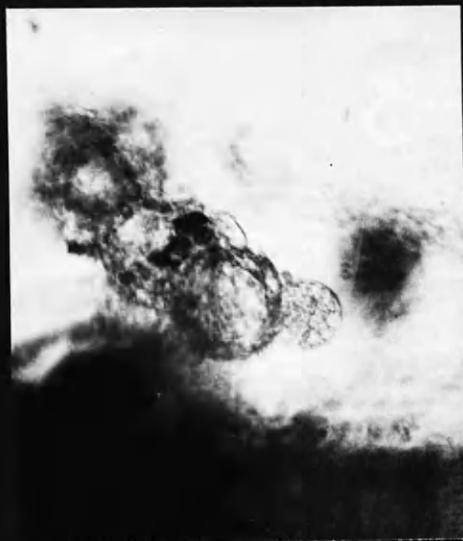
Fig.27. Oogonial "shell" with crushed, empty vesicle,  
on a Tomato root.

Fig.28. Oogonial "shell" with full vesicle, on a Tomato  
root.

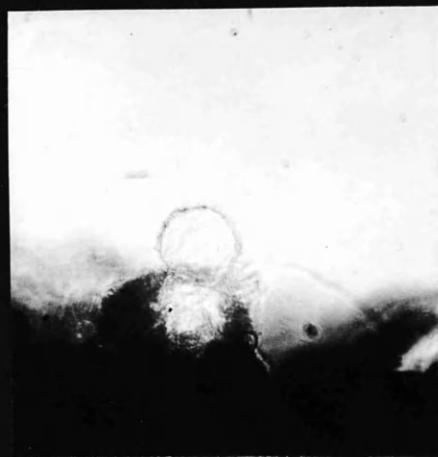
Fig.29. Oogonial "shell" and empty vesicle, immediately after  
the release of zoospores.



27.



28.



29.

PLATE 13.

Fig.30. "Hyphal knots" or haustoria prevalent on roots  
infected with P. verrucosa.

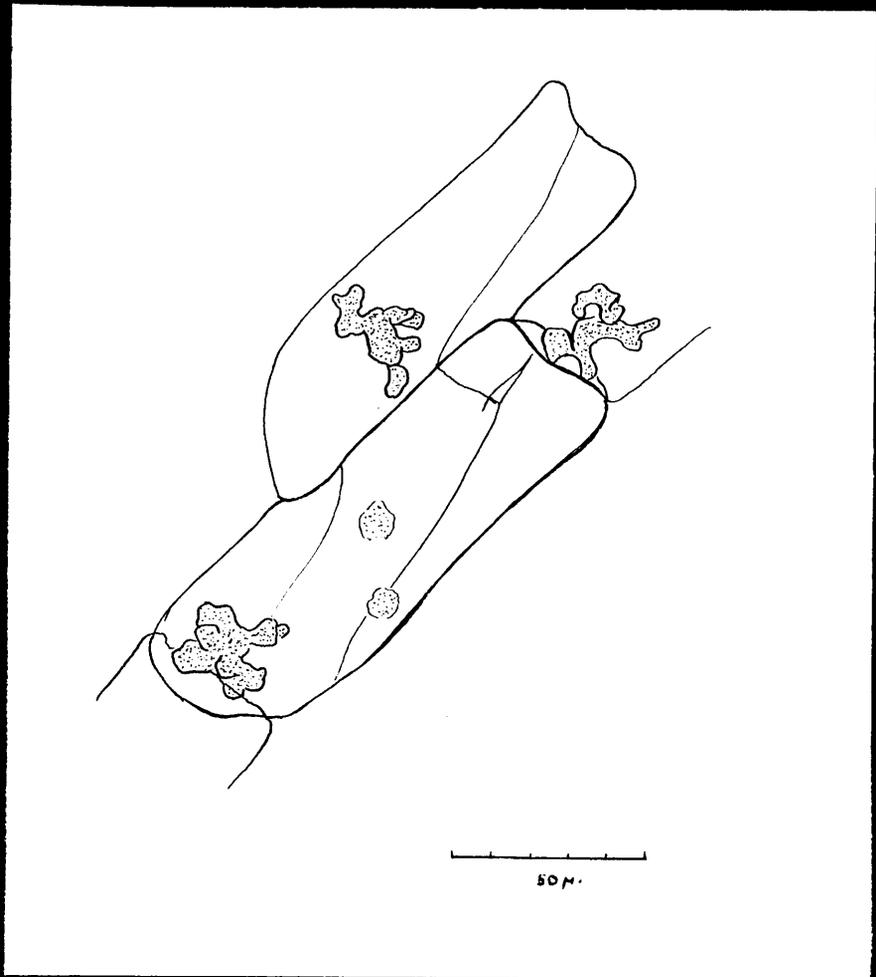


PLATE 14.

Fig.31. Napicladium sp. Conidiophores on root. x66.

Fig.32. Napicladium sp. Conidiophores on root. x66.

Fig.33. "Dots" on Tomato root associated with  
Napicladium sp. x330.

Fig.34. Spores of Napicladium sp. x312.



31.



32.



33.



34.

Additional Paper presented for  
the degree of Doctor of Philosophy.

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A ROOT ROT OF CINERARIA,  
AND THE PHYTOPHTHORA SPP. INVOLVED

by

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B.Sc. Hort. (London)

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Glasgow. 1944.

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A ROOT ROT OF CINERARIA,  
AND A STUDY OF THE PHYTOPHTHORA SPP. INVOLVED.

INTRODUCTION.

Cinerarias were grown to a considerable extent in Ayrshire nurseries for the Pot Plant trade before the war. Many nurserymen annually lost an appreciable proportion of their stock as the result of a root rot disease associated with wilting of the leaves. This disease made its appearance at all stages in the growth of the Cineraria. The periods when plants were most susceptible, however, were during the seedling stage and just prior to flowering.

Isolations made from fragments of diseased root tissue in almost every case resulted in a culture of a Phycomycetous fungus resembling a Phytophthora sp.

REVIEW OF PREVIOUS WORK.

Few records exist of research into the cause of a Cineraria Root Rot. The only direct references to a naturally occurring Root Rot ascribed to Phytophthora

spp. are those of Pethybridge & Lafferty (1919) in Britain, and in Victoria (Australia) of Brittlebank & Fish (1927) who name as the causal organism P. cryptogea. Tomkins, Tucker and Gardner (1936), working on a Cauliflower root rot caused by P. megasperma, reported the same fungus present on Cinerarias affected with Root Rot. Symptoms of wilting were artificially induced in Cineraria by Tomkins & Tucker (1937) using an isolate of P. cryptogea from China Aster. In 1935 Drechsler recorded Pythium spp. on Cineraria roots, though the fungus was not isolated.

MATERIAL AND METHODS.

Cineraria plants with symptoms of Foot Rot and Wilt were obtained from three sources, namely, The Gardens Auchincruive, a Prestwick Nursery and the Plant Husbandry Department Auchincruive. The 6 distinct isolates of Phytophthora sp. used throughout the following experiments were selected from plants representing all 3 sources. Two seedlings from the Plant Husbandry Department provided isolates F<sub>2</sub>, G<sub>1</sub>. Three plants from the Gardens yielded isolates J<sub>2</sub>, R<sub>1</sub>, H<sub>1</sub>, and one isolate R<sub>4</sub> was taken from a diseased plant received from the Prestwick Nursery. Of these plants some were Cineraria grandiflora and some Cineraria stellata; it appears that the Wilt or Foot Rot may occur on either species.

Isolation of the pathogen was carried out on Potato Agar medium composed of the extract from 200 grams of potato pulp and 15 grams of Agar per Litre of medium. Subcultures were made on an Oatmeal Agar consisting of 60 grams of oatmeal and 17 grams of Agar per litre, and in Petri solution.

The inoculum used for inducing infection in healthy

plants was ordinary Potato Agar culture or inoculum prepared from crushed oats or cracked wheat in the following manner. Test tubes were one third filled with crushed oats, as supplied for stock feeding, or cracked wheat. The tubes were then sterilised in an autoclave for 15 minutes. Separate tubes of water were sterilised simultaneously and afterwards added to the dry grain so as to saturate it and leave very little free liquid in the tube. Media so prepared were incubated for a few days, to test for sterility, and then inoculated with fragments of agar cultures of the various isolates. Growth on moist, cracked wheat and oats proceeded rapidly, and when the tubes were well filled with white flocculent mycelium, the material was ready for use as inoculum on healthy plants. One tube was sufficient to infect a plant in a  $4\frac{1}{2}$ " pot. The method of introduction of the inoculum is described in the appropriate section.

The dilute solutions of Malachite Green used in identification of the isolates were prepared according to Leonian's recommendation (1934). The Dye was made up in nutrient solution to the concentrations of 1:2,000,000; 1:3,000,000; 1:4,000,000; 1:8,000,000; and 1:12,000,000. Dissolving the dye in water first,

results in precipitation, in the higher concentrations.

To avoid this, Leonian added the dye directly to a nutrient solution of the following composition:-

Witte Peptone	2.0 g.
Dihydrogen potassium phosphate	0.5 g.
Magnesium sulphate	0.5 g.
Succinic acid	0.2 g.
Dextrose	5.0 g.
Distilled water	1,000 ml.

The method adopted for the preparation of the dilute solutions of Malachite green for the present work was as follows. Three litres of the above nutrient solution were prepared. 0.01 g. of the crystalline dye were weighed accurately and dissolved in 1 litre of the nutrient medium. Thus the solution was of the concentration of 1 part per 100,000 or 0.00001 g. of Malachite green in 1 ml. of solution. This medium served as a stock solution from which to derive further dilutions.

3 ml. of stock soln. (0.00003 g. dye) were added to  
357 ml. nutrient soln.

The resultant solution contained 3 parts dye  
per 360,000,000  
or 1 part per 12,000,000

5 ml. of stock soln. (0.00005 g. dye) were added to  
395 ml. nutrient soln.

The resultant solution contained 5 parts dye  
per 400,000,000  
or 1 part per 8,000,000

6.

10 ml. of stock soln. (0.0001 g. dye) were added to  
390 ml. nutrient soln.

The resultant solution contained  
1 part dye per 4,000,000.

20 ml. of stock soln. (0.0002 g. dye) were added to  
380 ml. nutrient soln.

The resultant solution contained 2 parts dye  
per 4,000,000  
or 1 part per 2,000,000.

10 ml. of stock soln. (0.0001 g. dye) were added to  
290 ml. nutrient soln.

The resultant solution contained  
1 part dye per 3,000,000.

The diluted solutions were tubed, 5 ml. per tube,  
and sterilised by autoclave. Inoculation was carried  
out a few days later.

The same basic Leonian's medium was used in the  
liquid cultures to determine the effect of a pH range on  
the growth of the isolates. A litre of solution was  
prepared and divided into 5 portions of 180 ml. each.  
These were to be adjusted by the addition of varying  
quantities of N/20 NaOH to form a series of 5 pH values  
ranging from 4.5 - 6.5 approx. To determine the amount  
of N/20 NaOH to be added in each case, preliminary titra-  
tions were made using 5 ml. volumes from the remaining  
100 ml. of the nutrient solution. The alkali was intro-  
duced from a burette and the end points determined

colorimetrically. From this reading the greater quantity of NaOH required similarly to adjust the pH of the larger 180 cc volumes could be estimated. Afterwards a more accurate reading was made of the pH of each solution by electrometric means. The series arrived at was pH 4.55, 5.1, 5.8, 6.0 and 6.4.

The effect of varying pH on the growth of Phytophthora isolates was tried also on an agar medium. In this case, the pH values were adjusted by means of buffer solutions, for greater accuracy.  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  and pure Citric acid were used and the solutions made up in distilled water.

For each pH value, 120 ml. of buffered solution were prepared and placed in a 500 ml. flask.  $1\frac{1}{2}$  litres of Potato extract were made up and to it 1.5% of agar added gradually, shaking at intervals so that the agar might be evenly distributed through the Potato extract. 240 ml. of this Potato agar were added to each flask of buffer solution, agitating the suspension in order to ensure uniform distribution throughout the series.

The flasks of agar medium were then sterilised by autoclave and 15 plates poured from each. A small quantity from each flask was set aside at the same time

in order that the exact pH might be determined by means of the quinhydrone electrode. The range of pH arrived at was -

3.67, 4.10, 4.59, 5.96, 6.39, 6.68, 7.17.

The media of the 3 lower pH values did not set well. Growth took place on these plates, however, and the results are recorded.

The pea broth used to encourage the formation of reproductive organs was prepared according to Leonian (1934). 3 ounces of young canned peas were mashed and added to 500 ml. of distilled water. This material was brought to the boil, the heavy matter allowed to settle out, and the liquid then poured through absorbent cotton until clear. Flasks of this clear medium were sterilised and then it was poured into Petri plates.

#### SYMPTOMS.

The disease is first apparent when the aerial portions of the Cineraria show a tendency to wilt. The lower leaves are affected first, but still retain their green colour; later the young leaves droop. First the

laminae become flaccid, then the petioles lose their turgidity. The central axis of the plant usually remains upright. The condition appears suddenly, in 1-2 days.

In cases of severe attack, shading and watering failed to produce any recovery. When sun heat became more intense and pots dried out, some plants showed a tendency to wilt more readily than others. These were probably plants in which fungal invasion was still not far advanced, and they were capable of recovery when shade and water were provided. The infected plants, however, still remained less tolerant of extremes than plants free from disease.

When wilted plants were shaken from their pots, a pinkish coloration of the roots, in contrast with the white roots of healthy specimens, was a constant feature, though in some cases it appeared only in patches. In advanced stages of the disease the roots were brown and a soft odourless rot was evident. The cortex sloughed easily by pulling the root from the soil. In the crown of the plant, rotting of the xylem and pith was frequently involved.

These symptoms appeared on both Cineraria stellata

and Cineraria grandiflora. (Fig. 1)

ISOLATION OF THE CAUSAL ORGANISM.

Roots which showed the pinkish discoloration of the stele were selected from diseased plants at varying stages of development. These were cut into short lengths, surface sterilised in 0.1% Mercuric chloride and plated on Potato Agar. Fungal growth was observed after 2-3 days.

The fungi isolated included Fusarium spp. and on three occasions Ascochyta sp., but the majority of the platings produced, usually as the only growth, a phycomycetous fungus resembling either Pythium or Phytophthora. It is difficult to distinguish between these genera. The presence in some of these Phycomycetes of amphigynous oogonia, and the tendency to release zoospores directly from the sporangium with no external vesicle, suggests that the genus is Phytophthora. From March and throughout the summer, all but one of the diseased plants whose roots were plated produced Phytophthora cultures.

In view of the ease and frequency of *Phytophthora* isolations, sometimes emerging as pure cultures, it seems most likely that isolates of this genus are the cause of Root Rot of the *Cineraria*.

PROOF OF PATHOGENICITY.

A number of isolations were made and pure cultures of these obtained. Artificial inoculations of healthy plants were carried out using pure cultures of each of these isolates grown on crushed oats, cracked wheat, or potato agar. The inoculum was introduced into the soil near the roots of healthy plants. Injury to plant roots was avoided as much as possible by knocking the plants out of their pots and pushing pieces of culture into the side of the soil ball. As a rule 3-5 points of infection were introduced into the root ball of each plant in a  $4\frac{1}{2}$ " pot. The *Cinerarias* were then replaced in their pots without further root disturbance.

The plants were kept in a slightly heated greenhouse and given ample root moisture. Controls were also set up.

As a result of these inoculations 64% of the plants produced wilt symptoms and root rot. Some of the isolates attacked more readily than others, but all were capable of producing symptoms of Root Rot. The disease usually became apparent in 4 weeks and sometimes the symptoms could be distinguished in 8 or 9 days.

Roots from artificially infected plants were surface sterilised and plated on potato agar; these again yielded Phytophthora cultures.

DESCRIPTION OF THE ISOLATED  
PHYTOPHTHORA SPECIES.

A series of 6 isolates of Phytophthora was derived from 6 wilted plants grown at Auchincruive and at a Prestwick nursery. These were labelled F<sub>2</sub>. G<sub>1</sub>. H<sub>1</sub>. J<sub>2</sub>. R<sub>1</sub>. R<sub>4</sub>.

Growth on Potato Agar.

When these isolates were cultured on Potato agar medium, their growth and behaviour were found to be dissimilar. R<sub>4</sub> in Potato agar culture produced a hyaline, prostrate, mycelium, more dense in some parts than others, suggesting the appearance of a snowflake. The individual

hyphae were of even thickness along their length, branching sparsely, and of average diameter  $5.0\mu$ . Some thin walled spherical or oval bodies with granular contents were present, which were intercalary or terminal. Zoospores were observed within these structures, thus suggesting the possibility that they were zoosporangia. They measured  $14-16 \times 26-29\mu$ . Thick walled intercalary structures of diameter  $16\mu$  were also found; these were probably chlamydospores.

The mycelium of  $H_1$  was also prostrate, spreading and hyaline, but of an even density and close growth. It formed a superficial tough, clear skin over the agar, and was barely distinguishable to the eye unless the Petri plate was held against the light, when the margin of growth could be discerned. Growth was very rapid. The hyphae of  $H_1$  were coarse, infrequently branching and occasionally septate. The diameter was  $4-7\mu$ .

The mycelium of isolates  $J_2$ .  $R_1$ .  $F_2$ .  $G_1$ . bore more resemblance one to another; all produced a small amount of thin, aerial, white mycelium over the prostrate, spreading, hyaline growth. At  $20^\circ$  C the increase in diameter was much slower than that of the above mentioned isolates.

Microscopically the mycelia of  $J_2$ .  $R_1$ .  $F_2$ .  $G_1$ . showed

a close resemblance. Hyphae branched almost at right angles and the young branches were of a similar diameter to the hyphae from which they arose. For the most part the mycelia were coenocytic, but septa were found in older hyphae of all 4 isolates. The average diameter of the hyphae was 5.5  $\mu$  but this was not constant throughout the length. In some parts the hyphae appeared to be distended and in others restricted. A restriction occurred particularly at the point of origin of a lateral branch from its parent hypha.

Growth and Reproductive Organs formed in Potato Agar.

	Type of Growth	4 weeks	6-8 weeks
F <sub>2</sub>	Low, sparse, aerial mycelium, much branched	Vegetative	Vegetative
G <sub>1</sub>	Some sparse, aerial growth	"	"
H <sub>1</sub>	No aerial growth. Mycelium spreading rapidly	"	"
J <sub>2</sub>	Some aerial growth. Mycelium much branched	"	"
R <sub>1</sub>	Low, sparse, aerial mycelium	"	"
R <sub>4</sub>	Mycelium prostrate, hyaline, "snowflake" appearance	Sporangia round Intercalary or Terminal Chlamydo spores.	

In order further to study the variability in behaviour, the isolates were inoculated on to a number of media designed to promote the formation of sporangia and oogonia. It was thought that these inoculations might serve as a basis for comparison between the isolates. Such media were, Oatmeal agar, Petri solution, and plugs of Cineraria tissue. In addition, actively growing mycelium was transferred to solutions poor in nutritive value, e.g. sterile distilled water, or sterile Bog soil extract. It was hoped that such treatment would induce the production of sporangia in the liquid.

#### Oatmeal Agar.

Petri plate cultures on this medium were maintained at 24° C. This appeared to be a favourable temperature for the growth of all isolates. The cultures were examined at intervals of from 2 to 8 weeks.

A profuse growth developed on oatmeal agar. The aerial mycelium of isolates R<sub>1</sub>. H<sub>1</sub>. G<sub>1</sub>. and J<sub>2</sub>. was abundant, white and flocculent. That of F<sub>2</sub> was less plentiful, and the aerial growth of R<sub>4</sub> was no more than a superficial "frosting" on the agar plate. The average hyphal diameter of each isolate was approximately 4.7  $\mu$ .

On this medium the incidence of reproductive organs was again variable.  $R_4$  produced thick walled, spherical chlamydospores, and, on one occasion, distinct pyriform sporangia measuring  $44 \times 18 \mu$ . (Fig. 2) in which motile zoospores were present. The mycelia of  $F_2$ .  $R_1$ .  $J_2$  were purely vegetative. That of  $H_1$  bore irregularly shaped granular bodies, which might have been sporangia. The mycelium of  $J_2$  showed the presence of thin walled intercalary swellings.

Growth and Reproductive Organs formed on Oatmeal Agar

	Type of Growth	4 weeks	6-8 weeks
$F_2$	Abundant white, flocculent, aerial mycelium	Vegetative	Vegetative
$G_1$	"	"	"
$H_1$	"	A few irregularly shaped, granular bodies	Irregularly shaped bodies
$J_2$	"	Vegetative	Intercalary swellings
$R_1$	"	"	"
$R_4$	Surface "frosted"	Round Intercalary sporangia and few pyriform sporangia	Round Intercalary sporangia

### Petri Solution.

Tucker (1931) found that some species which rarely produce sporangia on solid media could be induced to form them in Petri solution. Tubes containing this solution were inoculated with pieces of agar cultures of F<sub>2</sub>. G<sub>1</sub>. H<sub>1</sub>. and R<sub>4</sub>. After incubation at 24° C. for 5 weeks the mycelia of F<sub>2</sub> and G<sub>1</sub> remained purely vegetative. In the culture of H<sub>1</sub> some very small bodies, 11 μ in diameter, resembling sporangia, were found. R<sub>4</sub> developed the round sporangia recorded on Potato agar but differing from those more typical of Phytophthora, which were found associated with the same isolate on Oatmeal agar.

The foregoing cultures on 3 different artificial media revealed a remarkable variation in behaviour of strains obtained from one type of host plant, and further cultural studies emphasised this variability.

### Growth on plugs of Cineraria Tissue.

In the taxonomic keys relating to Phytophthora spp. (Tucker 1931) (Leonian 1934), the form of antheridium (i.e. whether paragynous or amphigynous) is a major feature in classification. If sexual organs could be induced to form, they would prove a valuable means of

differentiation between the strains and an aid to identification of the species. So far the isolates have shown no tendency to form reproductive organs on artificial media. It is possible that they may require conditions of substrate more closely resembling the host plant for their development.

Stems of Cineraria stellata were cut into convenient lengths to fit into test tubes and split lengthwise to expose the soft inner tissue on which the fungus may grow more readily. The plugs were supported and kept moist by pads of damp cotton wool at the base of the tubes. Sterilisation was carried out at one atmosphere for 20 minutes. Two days later inoculations were made of all 6 isolates, using as inoculum small cubes of agar cultures. The tubes were incubated at 24° C.

No growth was produced by J<sub>2</sub> nor R<sub>4</sub> on Cineraria plugs. Abundant flocculent mycelium formed in 4 days on the plug inoculated with H<sub>1</sub>, in 6 days from R<sub>1</sub>, in 10 days from F<sub>2</sub>, and in 14 days a slight weft developed from G<sub>1</sub> with very small beaked or papillate sporangia.

The occurrence of sexual organs was infrequent. The first to be observed were found on F<sub>2</sub> two weeks after inoculation of the plug. The oogonia were golden brown,

smooth walled and spherical, drawn out slightly within the amphigynous antheridia. The oogonia measured 25-29.7  $\mu$  in diameter, or 37  $\mu$  to the base of the antheridium. Oospores measured 18.6-22.3  $\mu$  diam. A later inoculation failed to produce oogonia until after a 13 week interval when the plug had almost dried out. Oogonia were then found in abundance. (Fig. 3)

The only other isolate to form oogonia was R<sub>1</sub>. They developed on this culture after a period of 5 weeks. Many golden brown oogonia were found, measuring 25.5-30  $\mu$  in diameter, and with amphigynous antheridia. (Fig. 4) The measurement from the tip of the oogonium to the base of the antheridium was 37  $\mu$ .

The other isolates showed no sexual stage.

#### Attempts to Induce Sporangial Formation.

The transference of the fungus from a highly nutritive substratum to a liquid medium in which there is little nutritive value has been found to have the effect of inducing members of this genus to form sporangia (Pethybridge & Lafferty 1919). In the application of this method to the isolates from Cineraria, three techniques were employed.

A. Well nourished aerial hyphae were taken from agar cultures, avoiding where possible any fragments of the agar substratum.  $F_2$ .  $G_1$ .  $H_1$  were taken from Oatmeal agar cultures,  $J_2$  from Potato agar culture, and  $R_4$  from Dox agar. Tubes of sterile distilled water were inoculated with this vigorously growing material.

In 2 weeks' time,  $H_1$  and  $R_4$  had been induced to form spherical thin walled sporangia in abundance. The sporangia of  $R_4$  measured 20-35  $\mu$  in diameter and were apparently terminal on slender hyphae as in Fig. 8.  $F_2$  was the only strain which produced sporangia typical of the genus Phytophthora and these were formed after 7 weeks' growth. These sporangia were elongate-ovate, somewhat irregular in outline, and measured 22.2-15 x 11-7.5  $\mu$ . They possessed granular contents (Fig. 6). The other isolates,  $G_1$  and  $J_2$  remained vegetative. Oval intercalary chlamydospores were found on the mycelia.

B. Six isolates were grown on a highly nitrogenous medium consisting of Potato agar to which 1%  $KNO_3$  had been added. The pH of this medium was 5.5. After a few weeks' growth, subcultures of these well nourished mycelia were made in liquid Bog soil extract of pH 4.6.

The tubes were incubated at 20° C. for 3 weeks before examination.

Isolate F<sub>2</sub>, under this treatment, produced no sporangia comparable with those which were induced by transference of the isolate to distilled water. A few intercalary swellings were present.

G<sub>1</sub> bore a few small ovate sporangia on very strong hyphae. Intercalary swellings of this isolate were seen to be germinating in situ; one or more hyphal tips emerging from all sides of a single swelling.

J<sub>2</sub> formed many large ovate sporangia, quite distinct from the intercalary swellings which were also present and which germinated similarly to those of G<sub>1</sub>.

The formation of large ovate sporangia in R<sub>1</sub> culture (Fig. 7) resembled the behaviour of J<sub>2</sub>. Those of R<sub>1</sub> measured 44.4 x 25.8 μ. Intercalary swellings germinating in all directions by hyphal protuberances, were present in groups.

H<sub>1</sub> produced no true sporangia, but some short lateral hyphae were abnormally swollen, making "thumb-like" projections from the main hyphae. R<sub>4</sub> formed spherical thin walled sporangia.

C. The technique adopted by Leonian (1934) was to grow the isolate for 3-4 days in a clear Pea Broth, prepared from canned peas. After that period he washed the mycelium repeatedly and transferred it to a small quantity of distilled water. Four days later he obtained a clear segregation between those species producing sporangia only, those producing oogonia, and those remaining sterile.

This technique was followed in order to encourage sporangial development in the present isolates. The results obtained were as follows:-

- F<sub>2</sub>. Occasional sporangia, oval, nonpapillate  
av. 30.6 x 21.6  $\mu$  .
- J<sub>2</sub>. Occasional sporangia, oval, nonpapillate  
av. 31.5 x 21.5  $\mu$  . (Fig. 5)
- H<sub>1</sub>. Round intercalary sporangia.
- R<sub>4</sub>. Round intercalary sporangia.
- R<sub>1</sub>. Vegetative.

From the foregoing cultural work it was apparent that there existed a wide variability among the isolates in their reaction to different media. Although a Phytophthora species could be isolated without difficulty from Cineraria plants with wilt symptoms, in each case an apparently different strain was obtained. Each

possessed distinctive reactions to cultural treatments distinguishing it from other isolates.

Similarly when reisolates from artificial inoculations were grown in culture, it was found that some of these differed slightly in behaviour one from another and also from the strains which were introduced into the plant, yet all were apparently Phytophthora species.

The sporadic occurrence of reproductive organs and the difference in response of the isolates to cultural treatments can be summarised in the following table omitting reference to chlamydospores.

	Potato Agar	Oatmeal Agar	Petri Soln.	Plugs	Agar → Water	KNO <sub>3</sub> → Bog Ext.	Pea broth → Water
F <sub>2</sub>	Veg.	Veg.	Veg.	Oogonia Antheridia	Sporangia	Veg.	Sporangia
G <sub>1</sub>	Veg.	Veg.	Veg.	A few small beaked sporangia	Veg.	Few small ovate sporangia	
H <sub>1</sub>	Veg.	Veg.	Small sporan- gia	Veg.	Round Sporangia	Veg.	Round sporangia
J <sub>2</sub>	Veg.	Veg.		No growth	Veg.	Large ovate sporangia	Sporangia
R <sub>1</sub>	Veg.	Veg.		Oogonia Antheridia			Few round swellings
R <sub>4</sub>	Round sporan- gia	Round sporangia and few true pyri- form sporangia	Round sporan- gia	No growth	Round Inter- calary Sporangia	Round sporangia	Round sporangia

Thus the presence of ovate nonpapillate sporangia of the type associated with the genus Phytophthora has been recorded for F<sub>2</sub>, G<sub>1</sub> (very small), H<sub>1</sub> (very small) J<sub>2</sub>, R<sub>1</sub>, R<sub>4</sub>, though each produced the organs under different cultural conditions. R<sub>4</sub> showed on most occasions round sporangia suggestive of Pythium spp., but, on oatmeal agar, produced on one occasion the Phytophthora type of sporangium (Fig. 2). Oogonia together with antheridia were found only in isolates of F<sub>2</sub> and R<sub>1</sub>. In both cases the antheridia were amphigynous. (Figs. 3, 4)

The marked dissimilarity in behaviour suggests that more than one species may be present on Cinerarias affected with Root Rot. Reproductive structures, however, were so few and infrequent in their occurrence that it was difficult to identify them, by morphological features alone.

So far the isolates seem to fall into 4 groups:

- F<sub>2</sub>. R<sub>1</sub> - Each formed oogonia with amphigynous antheridia and had large sporangia.
- J<sub>2</sub> - Similar sporangia were present but no growth could be induced on Cineraria plugs. The sexual stage was not observed.

- G<sub>1</sub>. H<sub>1</sub> - These resembled each other in the inclusion of very small sporangia in their life histories.
- R<sub>4</sub> - Formed round sporangia, and on one occasion pyriform sporangia were found on oatmeal agar.

Tucker (1931) and Leonian (1934) recognised that the size of oospores varied according to the conditions under which they were formed. Measurements of reproductive organs were useful guides in identifying species, but owing to their inconsistency one must also take into account the response of the species to stated cultural conditions. Such conditions are the temperature range (Tucker 1931 and Leonian 1934) or concentration of Malachite green (Leonian 1934) which the species would tolerate. These characteristics, together with the pathogenicity of the species on apple and potato tubers (Tucker 1931), were found to be more specific and, when considered in conjunction with morphological details, of greater taxonomic value.

ATTEMPTS TO CLASSIFY THE CINERARIA ISOLATES.

The taxonomy of Phytophthora species is not yet clearly defined. Many named species exist, differing sufficiently in behaviour to merit classification by Tucker (1931) as distinct species. More recently, Leonian (1934) advocated that the grouping be simplified by retaining a few of the main species and collecting under these the forms showing a close relationship to them. Thus fewer true species would exist and within each there would be some degree of variation.

The difficulty in identifying members of the genus Phytophthora lies in the fact that the conditions under which the genus has been cultivated artificially are not universally regulated, and the approximately similar environment supplied allows scope for considerable variation within the species. Leonian (1934) showed how successive subcultures of the same individual may differ widely, and therefore no list of invariable characteristics can be relied upon to identify each species.

Such keys to identification as are available were compiled by Tucker (1931) and Leonian (1934). These are based on (a) the response of the organism to culture

on certain media; notably by the production or absence of sexual organs or sporangia: (b) the tolerance of the species to extremes of temperature or concentrations of Malachite green: and (c) differential pathogenicity on Apple and Potato tubers.

Along the lines suggested by these studies, the forms derived from the present investigation were subjected to a number of treatments. From the results of these a few of the strains may roughly be classified.

Cultures on various media have been made, and the resulting types of growth compared in a previous section. Subsequently, experiments were carried out to determine the pathogenicity on Potato tubers and the tolerance of the isolates to varying temperatures and to different concentrations of Malachite green, according to recommendations made by Tucker (1931) and Leonian (1934). In addition, trials were made to indicate the range of pH which would support the growth of the various species.

#### Pathogenicity on Potato Tubers of Phytophthora Isolates from Cineraria.

Cultures of the fungus on potato agar were used for inoculation of the tubers. Potatoes were washed and

after surface sterilisation of the tuber with alcohol, 3 V-shaped incisions were made equidistantly spaced on three sides of the tuber. The flap of tissue so made was raised up and a piece of agar inoculum bearing the Phytophthora was introduced underneath. Each potato was thus inoculated 3 times with the same isolate. Controls were provided by tubers similarly cut but not inoculated. The tubers were kept moist under inverted sterilised bell jars and incubated at 24°C.

Results:

- J<sub>2</sub> - After 6 days the tissue surrounding all inoculated lesions had turned brown and rotted, while all control cuts remained healthy. Reisolation of the fungus from the margin of the lesion was successful. The isolate was pathogenic to potato tubers.
- R<sub>1</sub> - Two inoculations proved pathogenic in 7 days. All controls remained healthy.
- F<sub>2</sub> - All inoculations proved pathogenic after 7 days. Later *Penicillium* contamination set in.
- R<sub>4</sub> · G<sub>1</sub> }  
 H<sub>1</sub> } After 20 days, inoculated cuts still remained healthy. A thin callus formed on the potato lining the cut. The introduction of these 3 isolates was repeated on a second series of tubers, and again they were nonpathogenic.

Pathogenicity of Isolate R<sub>4</sub> on Apple.

An apple was inoculated with isolate R<sub>4</sub> in the same way as potato tubers had been treated. As control, a second apple was cut but not inoculated. After 6 days under moist conditions at 25° C. all the inoculated lesions had produced a brown rot.

The Relation between Temperature and Growth of Cineraria Isolates.

A large bulk of Potato agar was prepared in order that the medium might be constant in composition throughout the experiment. The pH was 5.8. Plates were inoculated with the six strains of Phytophthora derived from Cineraria plants, the inoculum being approximately of uniform size and planted exactly in the centre of the plate to allow of equal growth on all sides. These plates were incubated at temperatures ranging from 12-35° C. Owing to limited incubator space, the whole range of temperatures could not be tried at once, but the complete series of isolates to be treated at one particular temperature were incubated together. Once set, the incubators maintained a fairly steady temperature. The lower range 12-14° C. was achieved by incubating the

plates under sterile inverted bell jars placed in a cold greenhouse during late autumn, and there considerable variation in temperature was experienced. The recordings, therefore, for these lower temperatures are approximations.

The experiment was carried out in replicate.

Recordings of the mean diameter of growth were made after 96 hours. The growth made by each strain at a given temperature showed only a slight variation, but the average reading was taken as the final figure. The table of Growth-diameter readings for one experiment is reproduced and the results of the experiment are indicated graphically. (1)

Diameter of Growth in mms. recorded after 96 hours' exposure  
to a range of temperatures.

Centi- grade.	16.5-18° Av. 17.2°	20°	24°	27°	29°	30°	32°	35°
F <sub>2</sub>	35 Aver. 35 <u>35</u> 35 35	36 Aver. 40 <u>40</u> 41 41	47 Aver. 49 <u>49</u> 49 50	41 Aver. 42 <u>42</u> 43	9 Aver. 10 <u>14</u> 22	- -	- -	
G <sub>1</sub>	37 39 <u>40</u> 40 44	41 42 <u>42</u> 43 43	43 43 <u>43.5</u> 44 44	38 41 <u>40.2</u> 41 41	6 11 <u>13</u> 15 20	trace trace	trace -	
H <sub>1</sub>	68 <u>68</u> 68	Plate full					76 <u>78</u> 80	- -
J <sub>2</sub>	37 39 <u>38.5</u> 39		48 49 <u>49</u> 50	39 39 <u>39</u> 40	10 13 <u>18</u> 30	trace trace	- -	
R <sub>1</sub>	37 37 <u>38</u> 38 39	40 41 <u>41</u> 41	42 48 <u>47.5</u> 51	42 43 <u>44</u> 44 47	20 20 <u>22</u> 27	8 <u>8.5</u> 9	- -	
R <sub>4</sub>	49 50 <u>51</u> 52	58 60 <u>60</u> 61	68 69 <u>69</u> 69	60 61 <u>61.5</u> 62 63	30 36 <u>35</u> 39	- -	- -	

Examination of the graph shows that, for each strain, there is an upper temperature limiting to growth. Some strains thrive at a higher temperature than others; 35° C. inhibits the growth of all. In each case the limiting effect of increased temperature acts rather suddenly. Vigorous growth may be brought abruptly to an end by an increase of 1 or 2 degrees.

#### Low temperatures.

The effect of low temperature on the growth of the isolates was also tried. Potato agar plates were inoculated with isolates F<sub>2</sub>, J<sub>2</sub>, H<sub>1</sub>, R<sub>1</sub>, and R<sub>4</sub>. They were incubated at 24° C. for one day in order that initial growth might develop in the fresh agar. The circumference of growth was outlined on the under glass of the Petri dishes and the series was transferred to a cold store room which maintained a temperature throughout the experiment of 8-9° C. Every 24 hours the temperature was recorded and the circumference of growth marked.

All isolates continued to grow, though slowly, at the low temperature. The growth of H<sub>1</sub> was most rapid, increasing in radius by 7 to 8 mms. each 24 hours. R<sub>4</sub> increased in radius by 3 to 4 mms. per 24 hours, F<sub>2</sub> by

2 to 3 mms.  $R_1$  and  $J_2$  grew only very slightly at that temperature; 2 mm. was the greatest increase observed in 24 hours.

Differential Growth in Malachite Green Solutions.

Leonian (1934) found that the response of different species to the presence of small amounts of Malachite Green in the growth medium was a fairly constant specific character on which classification within the genus might be based. His cultures were confined to a liquid medium, the formula for which was closely followed in this work, except for the substitution of Witte peptone for the proteose peptone used by Leonian. The formula for the nutrient medium and the method of dissolving in it the minute quantities of Malachite Green have already been stated in the section "Material and Methods". Dilutions containing 1 part per 2,000,000, 1 part per 3,000,000, 1 part per 4,000,000, 1 part per 8,000,000 and 1 part per 12,000,000, were prepared. Tubes were selected for uniformity in diameter and shape. The media were tubed (5 ccs. per tube) and sterilised at one atmosphere for

15 minutes. A few days passed before inoculation. Inoculum was cut from potato agar plates of the various *Cineraria* isolates using a sterilised platinum wire bent to form three sides of a 1 mm. square. In this way the size of the inoculum used in each tube could be made nearly identical. One piece of inoculum was introduced into each tube, and each treatment repeated 4 times. The tubes were kept at a temperature of 20° C., and after 2 weeks they were examined and the amount of growth measured and recorded.

1. = A trace of growth around the inoculum fragment.
2. = Growth up to 2 mms. in length.
3. = Growth approx. 3 mms. in length.
4. = Considerable growth throughout the liquid.

	1:2 millions	1:3 mill.	1:4 mill.	1:8 mill.	1:12 mill.
F <sub>2</sub>	0 0 0 0	0 0 0	1 2 2	2 2 2 2	2 3 3
G <sub>1</sub>	0 0 0 0	0 0 0 0	0 1 1 1	2 2 2 2	2 2 3 3
H <sub>1</sub>	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
J <sub>2</sub>	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 1	0 1 2 3
R <sub>4</sub>	0 0 0 0	0 0 0 0	0 1 1 1	3 3 3 3	3 3 3 3

Some differences between the isolates became apparent in their reaction to the presence of small quantities of the dye.  $H_1$  would not tolerate even the most dilute solution of 1 part of Malachite Green in 12 million; 1:8 million was the greatest concentration in which the growth of  $J_2$  developed.  $F_2$ ,  $G_1$  and  $R_4$  still produced slight growth in 1:4 million, and  $F_2$  was slightly less intolerant of that concentration than the others. One tube of  $F_2$  showed a trace of growth in a solution of 1:3 million, a concentration too strong to support the growth of any of the other isolates.

A repetition of this experiment was carried out a year later, using the same isolates with  $R_1$  in addition, and also 6 re-isolates obtained from artificially inoculated plants in order to compare the results and probable identity of these with the original isolates. The designation of these re-isolates was as follows:-

$F_2$ a.	from a plant inoculated with $F_2$ .
$R_1$ y1	" " " " " $R_1$ .
$R_1$ y2	" same " " " $R_1$ .
$J_2$ y	" a " " " $J_2$ .
$H_1$ x	" " " " " $H_1$ .

The concentration 1:2,000,000 was omitted, since in the previous experiment no growth was obtained in that concentration. In these experiments a more accurate means of estimating the amount of growth was devised. After the fungus had grown for 2 weeks the tubes were centrifuged for 5 minutes at 2,000 revolutions per minute, which lightly compacted the mycelium at the base of the tube and, as these were of uniform size and shape, the amount of fungal growth for comparative purposes was taken as the depth in mms. of fungal material in the tubes. As before

1. = Trace of growth.
2. = Up to 2 mms. growth

and higher figures relate to the depth of compacted fungal hyphae.

	1:3 millions	1:4 mill.	1:8 mill.	1:12 mill.
F <sub>2</sub>	0 0 0 0	0 0 0 0	0 0 0 0	0 1 1 1
J <sub>2</sub>	0 0 0 0	0 0 0 0	0 1 1 1	1 1 2 2
R <sub>1</sub>	0 0 0 0	0 0 0 0	1 1 1 1	1 2 2 2
G <sub>1</sub>	0 0 0 0	0 0 0 0	1 1 1 1	1 2 2 2
H <sub>1</sub>	0 0	0 0 0	0 0 0	0 0 0 0
R <sub>4</sub>	0 0 0 0	0 0 0 0	2 2 2	2 2 2

## Re-isolates

F <sub>2</sub> a.	8 8 8	8 8 8	8 8 8	8 10 10
R <sub>1</sub> y1	0 1	1	1 1	2 2 2 2
R <sub>1</sub> y2	2 2	1 1	1 1	2 2
J <sub>2</sub> y	0 0 0 0	0 0 0 0	1 1 1	1 1 1
H <sub>1</sub> a		0 0	0 0	0 0 0

H<sub>1</sub>a and J<sub>2</sub>y were the only 2 re-isolates whose reaction to Malachite Green corresponded to that of the original isolates J<sub>2</sub> and H<sub>1</sub>.

It appeared that the original isolates F<sub>2</sub>, J<sub>2</sub>, G<sub>1</sub> and R<sub>4</sub> were, in this experiment, more sensitive to Malachite Green than in the previous one, and were on the whole more sensitive than the re-isolates which had not been so long in artificial culture. This sensitivity may have been due to decrease in vigour as a result of repeated subculturing.

A repetition of the experiment was planned, after allowing each strain a period of growth on plugs of potato tissue, in order to find if "rejuvenation" and increased sensitivity of the strains resulted. A comparison was made with the strains in their present state.

	1:3 millions	1:4 mill.	1:8 mill.	1:12 mill.
F <sub>2</sub>	0 0 0 0	0 0 0 0	1 1 1 1	1 2 2 2
J <sub>2</sub>	0 0 0 0	0 0 0 0	0 0 1 1	2 2 2 2
R <sub>1</sub>	0 0 0 0	0 0 0 0	1 2 2 2	2 2 2 2
G <sub>1</sub>	0 0 0 0	0 0 0 0	0 0 1 1	2 2 2 2
H <sub>1</sub>		0 0 0	0 0 0	0 0 0
R <sub>4</sub>	0 0 0 0	0 0 0 0	2.5, 2.5, 2	3 3 3
H <sub>1a</sub>			0 0	0 0 0
R <sub>1y1</sub>	0 0 0	0 0	0 0	0 0 1
J <sub>2y</sub>	1 1	0 0 1	2 2 2	2 2

Repeat inoculations from strains not passed  
through potato.

F <sub>2</sub>	0 0	0 0 0	1 1	2 2
J <sub>2</sub>	0 0 0	0 0 0	1 1	2 2 2
R <sub>1</sub>	0 0 0	0 0 0	2 2	2 2 2
G <sub>1</sub>	0 0 0	0 0	1 1	2 2
R <sub>4</sub>	0 0	0 0 0		4 4
J <sub>2y</sub>	0 0	0 0		2

The period of growth on sterilized potato plugs seemed to have very little effect in increasing the tolerance of the isolates to concentrations of Malachite Green.  $F_2$  was again capable of growth in a concentration of 1:8,000,000 and  $R_1$  and  $R_4$  produced slightly increased growth at the concentrations they were able to tolerate before "rejuvenation".  $J_{2y}$  displayed the greatest effect of the interval of growth on potato, yet one would expect that this isolate would not show such staling symptoms as the original isolate, since it had been brought more recently into culture.

A graph based on the experiment following "rejuvenation" is appended, showing the relative tolerance of concentrations of Malachite Green among the isolates.(11)

### Review of Results.

A study of the foregoing experiments dealing with the effects of Temperature and different concentrations of Malachite green on growth, and with pathogenicity for Potato tissue, indicates that there is a tendency, among the isolates of Phytophthora to group themselves in a manner which is repeated in the results of each experiment.

The graphs show this grouping very well. Isolates  $J_2$ ,  $F_2$  and  $G_1$  repeatedly showed a very similar growth response to a range of temperatures. The variance of maximum temperature for each was within 1-2 degrees.  $R_4$  lies outside the main group and differs from  $H_1$  which produces wider growth and tolerates higher temperatures than other isolates.

On Malachite green media, again the responses of isolates  $F_2$ ,  $G_1$  and  $J_2$  approximate more closely than the others.  $R_1$  resembles this group in the series of experiments carried out before transference of the isolates to Potato for "rejuvenation". In the graph made from a later experiment  $R_1$  is slightly more tolerant than other isolates in that group.  $R_4$  again behaves independently and  $H_1$ , and  $H_1$  x its re-isolate, resemble one another closely but are distinct from all the others.

In pathogenicity tests on Potato tubers, once more the resemblance held between  $F_2$  and  $J_2$ . Isolate  $R_1$  joins this group and these produce disease within 7 days.  $R_4$  and  $H_1$  segregate themselves as non pathogenic.  $G_1$ , usually with the former group, this time falls into the latter as it has been found to be non-pathogenic.

A further series of experiments dealing with the response of the isolates to varying degrees of acidity was carried out to find if a similar grouping exists under these conditions.

The effect of Varying pH on Phytophthora isolates from Cineraria Plants.

In Potato Agar Culture.

Culture media of a series of pH values were prepared as described under the heading "Material and Methods". The range arrived at was pH 3.67, 4.19, 4.59, 5.96, 6.39, 6.68 and 7.17.

Three plates of each pH value were inoculated with each of the following strains of Phytophthora F<sub>2</sub>, J<sub>2</sub>, H<sub>1</sub>, R<sub>1</sub>, R<sub>4</sub> and incubated for 4 days at 23° C. At the end of that time the diameter of growth was measured in cms. and recorded in the following table from which a graph is drawn (III).

	pH						
	3.67	4.19	4.59	5.96	6.39	6.68	7.17
F <sub>2</sub>	3.3	4.3	4.3	4.1	3.8	3.6	3.9
	3.3	4.4	4.3	4.1	3.9	3.6	4.0
H <sub>1</sub>	4.3	9.4	Full	Full	Full	Full	Full
	5.0	9.5	Full	Full	Full	Full	Full
	5.5	9.6	Full	Full	Full	Full	Full
J <sub>1</sub>	2.9	3.3	3.3	3.2	2.8	2.6	3.0
	2.9	3.4	3.3	3.2	2.9	2.6	3.2
		3.5	3.5	3.4	3.0	3.1	
R <sub>1</sub>	2.8	3.3	3.5	3.2	3.1	2.9	3.3
	2.9	3.6	3.6	3.3	3.1	3.0	3.3
		3.7	3.7	3.4	3.2		
R <sub>4</sub>	-	-	2.5	4.6	5.0	3.4	5.9
	-	-	3.3	5.0	5.1	4.2	6.0
		2.4	3.7	5.1	5.2	4.3	

From a study of these results it appeared that over a wide range of pH, the response of strains F<sub>2</sub>, R<sub>1</sub>, J<sub>2</sub> was little affected; with a 2.3 increase in pH the decrease in diameter growth was no more than 4-5 mms. As the acidity increased still further there was a sharp decrease in growth. At pH 6.68 there was a decrease in the growth of all isolates; this, surprisingly, increased again as the pH exceeded 7.0. R<sub>4</sub> behaved

differently. It increased in growth over the range 4.1 - 6.4 to a considerable extent in contrast to the decline in growth of the above mentioned isolates. The growth of  $H_1$  filled the plate at pH values from 4.5 to neutrality.

Once more it appeared that the grouping of the isolates follows the previous tendency.  $F_2$ ,  $J_2$ ,  $R_1$  showed similarity in behaviour.  $R_4$  and  $H_1$  each behaved independently. In a previous experiment, in which isolate  $G_1$  was included, the graph for the development of  $G_1$  closely followed that of isolates  $F_2$ ,  $J_2$ , and  $R_1$ .

#### REINOCULATION OF CINERARIA PLANTS.

Consideration of the cultural work carried out with isolates of Phytophthora from Cineraria suggests that these include more than one species. The behaviour of isolates  $F_2$ ,  $J_2$  and  $R_1$  show so close a similarity under all the treatments given as to be considered a single species.  $G_1$  reacted in the same way to temperature, acidity and the presence of Malachite green, but was non-pathogenic on Potato tubers. Except for this one

response the strain resembled the previous ones.  $H_1$  differs consistently from other isolates.  $R_4$  is usually distinct, and in culture produced round sporangia in abundance.

In order to determine whether one or all of these groups is responsible for the disease under consideration, artificial inoculations of healthy plants were made at intervals during all seasons of the year. Each of the 6 isolates was used separately.

The order of their infective power was found to be as follows:  $R_1$ ,  $J_2$ ,  $F_2$ ,  $G_1$ ,  $H_1$ ,  $R_4$ .

The actual results of the inoculations were tabulated.

Strain	Successful Inoculations		Delayed Symptoms		Unsuccessful inoculations	Total	% Successful inoculations
		Incub. days.		Incub. weeks.			
$R_1$	10	7-32	1	15	-	11	100%
$J_2$	5	6-25	3	6-17	1	9	89.0%
$F_2$	8	8-28	-		4	12	66.7%
$G_1$	5	8-40	2	6-14	4	11	63.6%
$H_1$	3	17-35	1	10	7	11	36.3%
$R_4$	1	17	1	7	6	8	25.0%
Total	32		8		Successful Infection		64.5%

Positive indication of infection was shown much more rapidly by the first 4 strains. Those of H<sub>1</sub> and R<sub>4</sub>, though they occasionally produced disease, were of a less virulent character.

Thus the same grouping of isolates is met with again in Inoculation experiments. Isolates F<sub>2</sub>, J<sub>2</sub>, G<sub>2</sub>, R<sub>1</sub> may be regarded as the major parasites with the remaining 2 isolates as secondary forms.

#### DESCRIPTION OF THE ISOLATES.

##### F<sub>2</sub>.

Mycelium on agar media spreading, prostrate, hyaline, with sparse, low, aerial development. Sterile on agar media. Sporangia in cultures transferred from Oatmeal agar to sterile tap water (Fig. 6) elongate-ovate, irregular in outline, 11-22.2 x 7.5-15  $\mu$ . Larger sporangia formed in a culture transferred from Pea broth to sterile water, 30.6 x 21.6  $\mu$ . Oogonia after 4 weeks' growth on plugs of Cineraria tissue, Golden brown, 25-29.7  $\mu$  diam. Oogonial walls thick

and smooth. Oospores 18.6-22.3  $\mu$  diam. All antheridia observed amphigynous.

H<sub>1</sub>a.

Mycelium spreading very rapidly on all media; prostrate and hyaline on Potato agar, abundant aerial mycelium on Oatmeal agar. Neither Sexual reproductive structures nor true sporangia observed. Irregularly shaped, granular vesicles on Oatmeal agar. In Petri solution small round, thin-walled structures formed, 11.2  $\mu$  diam. Isolate nonpathogenic on Potato tubers.

J<sub>2</sub>.

Mycelial growth on Potato agar resembled that of isolates F<sub>2</sub>, R<sub>1</sub>, G<sub>1</sub> in prostrate, hyaline appearance and rate of growth. On Oatmeal agar, profuse aerial growth developed. Sterile on Potato and Oatmeal agars. In 0.1% KNO<sub>3</sub> pyriform sporangia found, average size 20 x 40.5  $\mu$  germinating by apical hyphae. These resembled the sporangia formed by isolate R<sub>1</sub> under similar conditions. Oogonia not observed. Clusters of chlamydospores, germinating at several points, occurred in cultures transferred from 0.1% KNO<sub>3</sub> to Bog soil extract. Reaction of growth to temperature conformed to those of F<sub>2</sub>, G<sub>1</sub>, and R<sub>1</sub>. Growth ceased

at 31° C. Pathogenic on Potato tubers.

G<sub>1</sub>.

Mycelial growth resembled those of R<sub>1</sub> and J<sub>2</sub>. Vegetative on all solid media and in Petri solution. A culture transferred to Bog soil extract developed very small ovate, microsporangia, also clusters of chlamydospores, germinating like those of J<sub>2</sub>. On Cineraria plugs a few small beaked structures formed, possibly sporangia. No other reproductive organs could be induced. Reaction to temperature resembled that of R<sub>1</sub>, F<sub>2</sub> and J<sub>2</sub>. Non-pathogenic on Potato tubers.

R<sub>1</sub>.

Mycelium on Potato agar prostrate, hyaline, and spreading; aerial growth short and sparse. On hard Oatmeal agar (12% agar), growth abundant, superficial, white and flocculent. On softer Oatmeal agar this was present in reduced form. Sterile on Agar media. A few ovate, non-papillate sporangia (18.5-26 x 33-44.4 $\mu$ ) formed when actively growing mycelium was transferred to Bog soil extract and to 0.1% KNO<sub>3</sub>. Sporangia germinated by apical hyphae. Oogonia, after 5 weeks' growth on plugs of Cineraria tissue, golden brown, smooth walled,

26  $\mu$  diam. or 37  $\mu$  to base of antheridium. Antheridia amphigynous (Fig. 4); Chlamydozoospores formed in 0.1%  $\text{KNO}_3$  liquid culture. Maximum temperature for growth, 31° C. Pathogenic on Potato tubers.

R<sub>4</sub>.

Prostrate, hyaline, spreading mycelium on Oatmeal and Potato agars. Aerial hyphae absent. "Snowflake" appearance of mycelium on Potato agar. Sporangia developed on Potato agar within 2 weeks, spherical, intercalary, 20-35  $\mu$  diam., containing motile zoospores. This type of sporangium most frequently associated with R<sub>4</sub>, but a culture on Oatmeal agar produced a few sporangia more typical of Phytophthora. These were pyriform, borne terminally, 18.5 x 44.4  $\mu$  (Fig. 2). Oogonia not observed. The temperature range tolerated by this isolate was similar to that of J<sub>2</sub>, F<sub>2</sub>, G<sub>1</sub> and R<sub>1</sub>, but at 25° C. the growth of R<sub>4</sub> was more vigorous than that of any of the other isolates. R<sub>4</sub> was non-pathogenic on Potato tubers.

IDENTIFICATION OF THE STRAINS.

An attempt was made to determine the species of six isolates of the genus Phytophthora, by means of the keys drawn up by Tucker (1931) and Leonian (1934). To these were related the results of cultural work already described in this paper.

R<sub>1</sub>.

Consideration of the isolate R<sub>1</sub>, of which the life history is most complete, revealed a spreading growth on agar media at 20° C. and the maximum temperature for growth was 30-31° C. Oogonia failed to develop on Oat-meal agar or on any other agar medium tested. But they were present, with amphigynous antheridia, on tissue plugs. Sporangia, formed in a soil extract, were non-papillate.

From these features the fungus might be identified according to Tucker (1931) as one of the following species:

P. erythroseptica

P. richardiae

P. cinnamomi

P. cambivora

P. cryptogea

By a process of elimination the isolate can be distinguished from P. erythroseptica by the smaller oospores observed, i.e. less than  $30\mu$  in diameter. The pathogenicity of  $R_1$  on potato tubers, together with the smaller sporangia and the fact that the optimum growth of the isolate is below  $25^{\circ}$  C., distinguishes it from P. cambivora.

The pathogenicity on potato tubers and absence of oogonia on oatmeal agar distinguish the isolate  $R_1$  from P. richardiae. There remain, therefore, P. cryptogea and P. cinnamomi.

Inter calary swellings were recorded in a culture transferred to Bog soil extract. The presence of these vesicles rare in P. cryptogea, together with the lack of sexual organs on oatmeal agar, suggest that the isolate is P. cinnamomi.

Transference of a Pea broth culture to distilled water failed to produce any sporangia or sexual organs, though some hyphal swellings developed. Restricted growth continued in the presence of 1 in 8 million Malachite green. From these additional features Leonian (1934) would classify the isolate as P. cinnamomi. Thus the previous conclusion, based on Tucker's classification, is confirmed.

J<sub>2</sub>.

The behaviour of J<sub>2</sub> closely resembled that of R<sub>1</sub>. Temperature relations were the same. Aerial mycelium formed on oatmeal agar and no oogonia were observed. Sporangia were of similar dimensions to those of R<sub>1</sub>. Intercalary vesicles, germinating by hyphal tips, formed in Bog soil extract cultures of J<sub>2</sub> also. J<sub>2</sub> was pathogenic on potato tubers. These 2 isolates might be regarded as the same species, but oospores of J<sub>2</sub> were not observed. Provisionally, therefore, J<sub>2</sub> will be classed with R<sub>1</sub> as P. cryptogea or P. cinnamomi.

The nature of growth in Distilled water after 3 days in Pea broth, on which Leonian based his key, does, however, reveal a difference between J<sub>2</sub> and R<sub>1</sub>. J<sub>2</sub> produced a very few scattered sporangia. This feature points to the isolate being P. citrophthora (Leonian 1934). This is unlikely since the sporangia of the isolate are non-papillate and the temperature relations recorded by Tucker (1931) do not agree. On account of the rare occurrence of these sporangia J<sub>2</sub> might well be classed with the group in which they are absent, thus resembling R<sub>1</sub>. It would then be classified as P. cinnamomi.

F<sub>2</sub>.

The temperature relations and the behaviour of this isolate on potato agar were similar to those of R<sub>1</sub>. F<sub>2</sub> was also pathogenic on potato tubers. Non-papillate sporangia and oogonia with amphigynous antheridia were formed. The oogonia measured 25-30 $\mu$  in diameter. Hence, according to Tucker (1931) the identity of the isolate might be -

P. cryptogea                      P. richardiae  
P. cambivora      or      P. cinnamomi.

From P. richardiae, the isolate can be distinguished by temperature relations (P. richardiae failed to grow at 10° C. (Tucker 1931)), by pathogenicity on potato tubers, and by the size of oogonia (those of P. richardiae are greater than 35 $\mu$  in average diam.).

The pathogenicity of F<sub>2</sub> on potato tubers, together with the smaller sporangia and the optimum growth of the isolate F<sub>2</sub> occurring below 25° C., distinguish it from P. cambivora.

The dimensions of sporangia and oogonia found in F<sub>2</sub> cultures conform more closely to P. cryptogea. The absence of oogonia on oatmeal agar cultures, however,

and the presence of groups of vesicles on cultures of F<sub>2</sub> suggest that this isolate is again P. cinnamomi.

According to Leonian's key, the production of sporangia in sterile water after transference from Pea broth, together with the toleration of 1 in 8 million Malachite green would classify this isolate as P. citrophthora. Again, as with isolate J<sub>2</sub>, it is probable that since the production of sporangia is occasional, the fungus might be identified as P. cinnamomi.

### G<sub>1</sub>.

The behaviour of the vegetative mycelium of G<sub>1</sub> was very similar to that of R<sub>1</sub> and J<sub>2</sub>. No aerial growth was formed. The temperature range was the same as those of R<sub>1</sub> and J<sub>2</sub>. Intercalary vesicles, germinating by hyphal tips, were again present. In the reproductive structures the similarity could not be traced. No oospores were observed and the only sporangia which could be induced were very small. The isolate was not pathogenic on potato tubers. It is suggested that the rarity of sporangia and oogonia, the occurrence of vesicles on a few occasions only, and the non-pathogenicity of the isolate on potato tubers, place it near to P. cambivora.

The optimum temperature for growth of the isolate is, however, much lower than that of P. cambivora. The identity of G<sub>1</sub> is therefore doubtful. Apart from pathogenicity on Potato tubers, G<sub>1</sub> most closely resembles the isolates described above and identified as P. cinnamomi.

#### R<sub>4</sub>.

The identify of R<sub>4</sub> is difficult to determine. The beautifully radial hyaline growth and the reactions to temperature and Malachite green suggest that it might be P. citrophthora. Microscopically the appearance of the fungus does not bear out this conclusion. The pyriform sporangia, when found, were not markedly papillate. The abundance of spherical, intercalary sporangia are not a feature of P. citrophthora.

#### H<sub>1</sub>.

The temperature range of isolate H<sub>1</sub> alone distinguishes it from all the other isolates from the Cineraria. H<sub>1</sub> produced abundant and rapid growth from 20° C. to 32° C. No growth was recorded at 35° C. Oogonia, sporangia and chlamydospores were not observed. The isolate was non-pathogenic on potato tubers.

Relating these features to the key drawn up by Tucker (1931), the identity of this isolate might be P. cambivora. This species is described briefly (Tucker 1931) as follows:- "Hyphae on agar media usually sterile. Vesicles occasionally developing, and oogonia rarely. Non-pathogenic on potato tubers. Optimum growth temperature is between 25-30° C. No growth occurred at 35° C."

This description bears out the probable identity of the isolate.

H<sub>1</sub> formed no sexual bodies on transference from Pea broth to sterile water. It was markedly intolerant of the most dilute solution of Malachite green. Hence Leonian (1934) would group it as one of the following species

P. cambivora      P. colocasiae      P. porri.

*last* The latter species ceases growth at a temperature below 31° C. H<sub>1</sub> continued growth when the temperature was reduced to 8° C. P. colocasiae does not grow at so low a temperature. The papillate and pedicellate sporangia characteristic of P. colocasiae were not observed. The identify of the isolate P. cambivora is therefore confirmed.

Thus there appears to be at least two well defined species involved in the Root Rot of Cineraria.

P. cinnamomi ----- isolates R<sub>1</sub>. J<sub>2</sub>. F<sub>2</sub>.

P. cambivora ----- " H<sub>1</sub>.

In addition G<sub>1</sub> and R<sub>4</sub> have not satisfactorily been classified under existing schemes.

#### THE CAUSAL ORGANISM.

Inoculation experiments have shown that symptoms of Root Rot of Cineraria were induced, within a few weeks, by the introduction into the surrounding soil of Isolates R<sub>1</sub>, J<sub>2</sub>, F<sub>2</sub> and G<sub>1</sub>. R<sub>1</sub>, J<sub>2</sub> and F<sub>2</sub> have since all been identified as P. cinnamomi. G<sub>1</sub> very closely resembles this species except for pathogenicity on Potato tubers. The % successful infection induced by isolates H<sub>1</sub> and R<sub>4</sub> was considerably lower. H<sub>1</sub> was identified as P. cambivora and R<sub>4</sub> was unclassified.

From the present study it would appear therefore that P. cinnamomi is responsible in greatest measure for the development of symptoms of Root Rot in Cineraria. P. cambivora, though parasitic, is less virulent than P. cinnamomi.

SUMMARY.

1. The symptoms of a Root Rot of Cineraria have been described and a number of fungal isolations made from diseased plants.
2. Considerable differences and variations were observed between these isolates, yet all apparently belonged the genus Phytophthora.
3. Six isolates, from a variety of sources, were selected for further, more detailed experiments.
4. The isolates were cultured on several solid and liquid media, and their growth characters compared.
5. The influence of temperature, pH, and a range of concentrations of Malachite green on the isolates has been studied.
6. Each of the Cineraria isolates was separately identified on the basis of their growth and response to various treatments, and finally, in spite of apparent considerable variation, the majority of these isolates were identified as P. cinnamomi.
7. Descriptions are given of the characteristics of each isolate.
8. Reinoculations of Cineraria plants revealed that the three isolates identified as P. cinnamomi

were those which most rapidly produced symptoms of Root Rot. P. cambivora and isolate R<sub>4</sub> were less virulent parasites.

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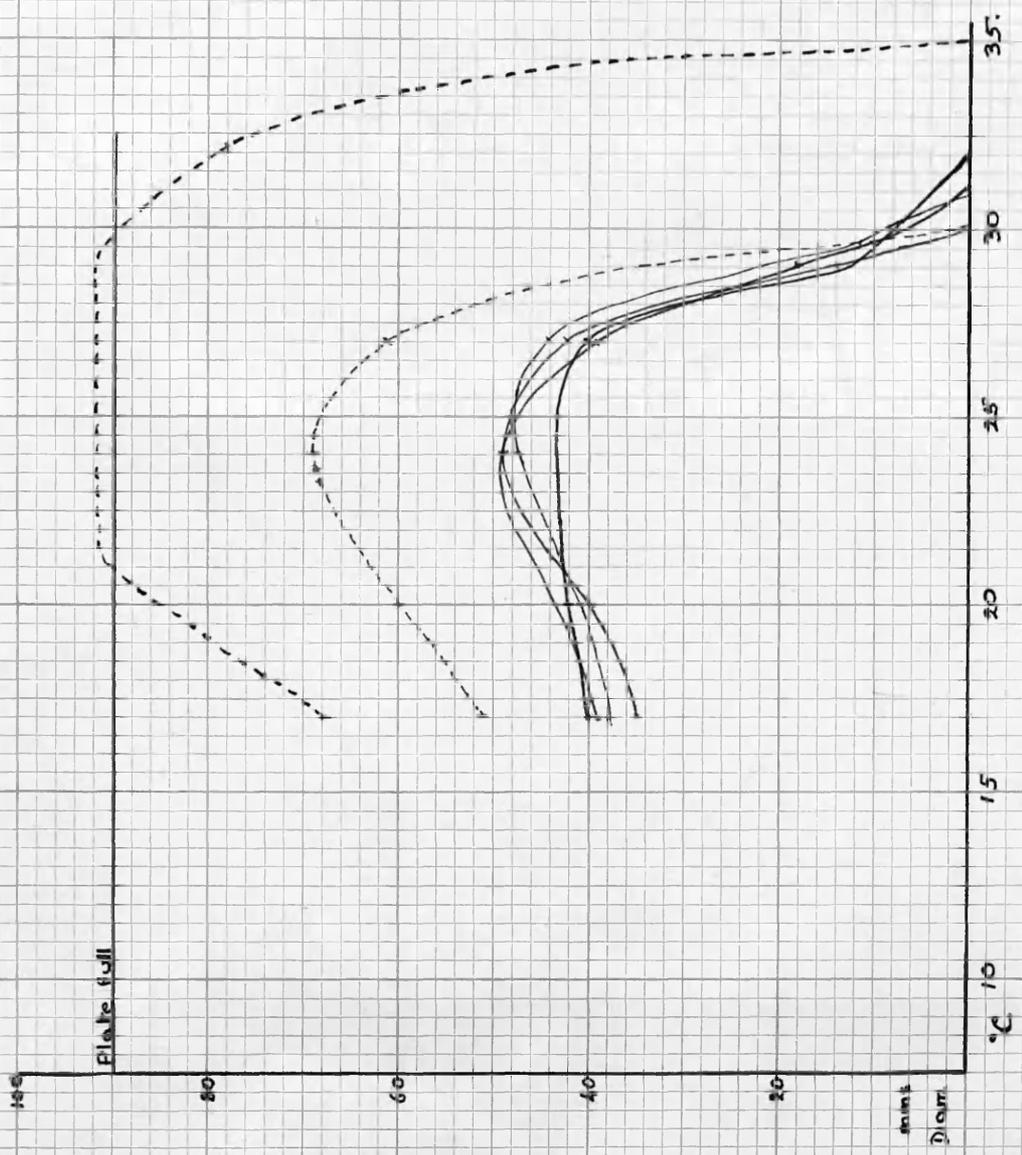
REFERENCES.

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TEMPERATURE — GROWTH CURVES.

Diam. growth measurements in mms. after 96 hours on Potato Agar  
 [pH 5.8]

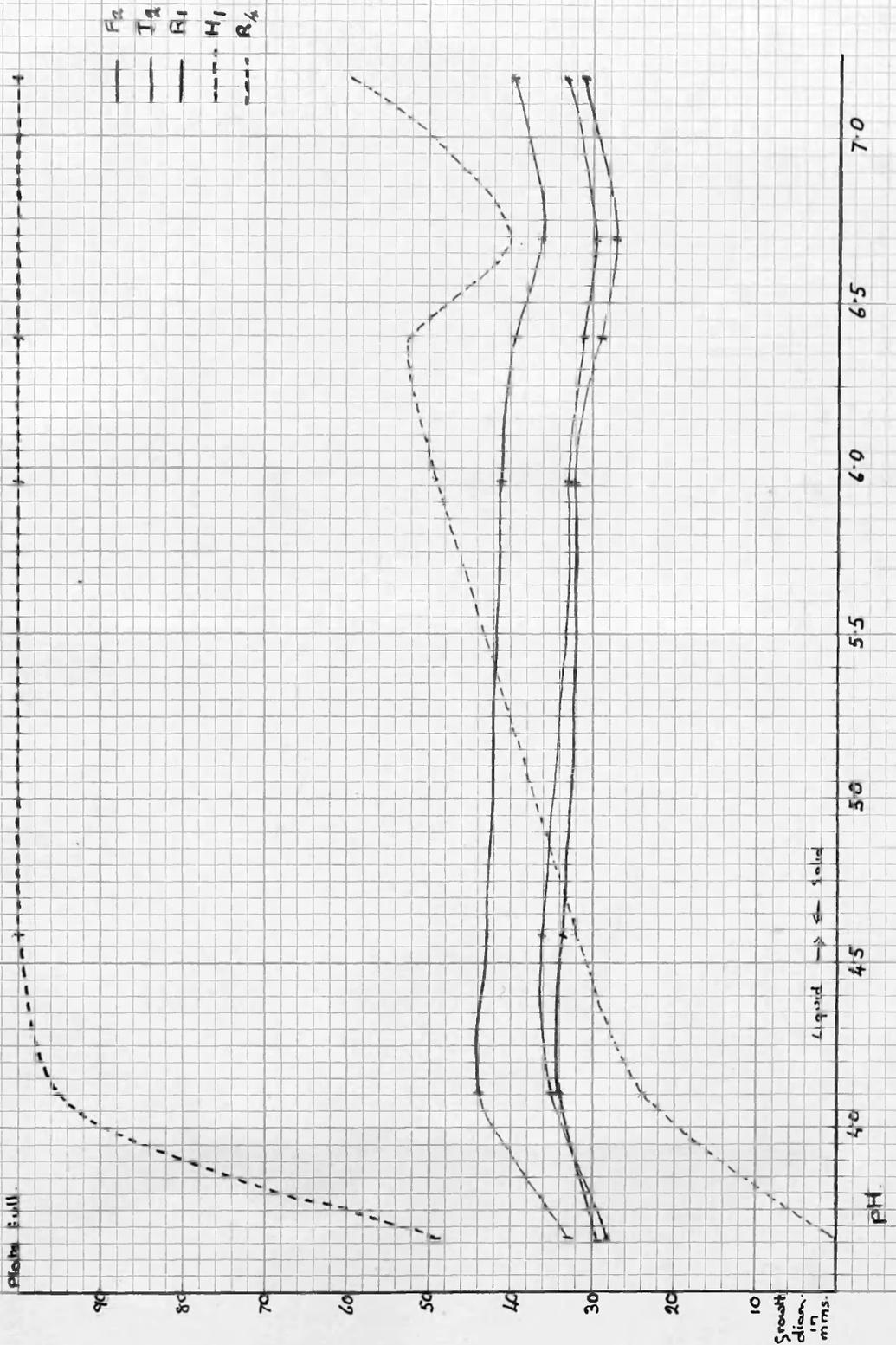
P<sub>1</sub> ———  
 T<sub>2</sub> ———  
 R<sub>1</sub> ———  
 G<sub>1</sub> ———  
 H<sub>1</sub> ———  
 A<sub>1</sub> - - - -





ACIDITY — GROWTH CURVES.

Diam. growth measurements in mms. after 96 hours growth at 23°C.



ACIDITY — GROWTH CURVES.

Diam growth measurements in mms. after 96 hours growth at 23°C.

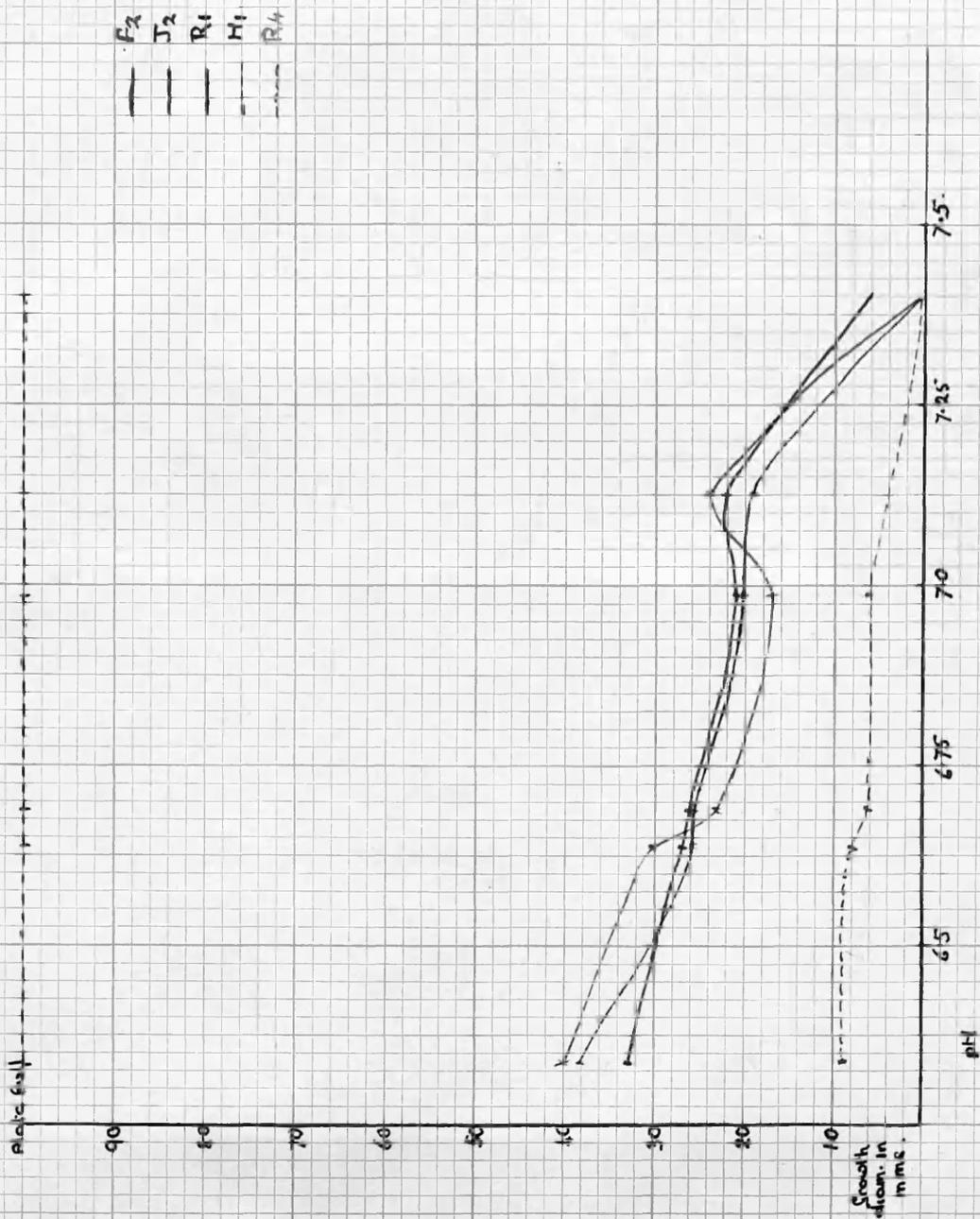


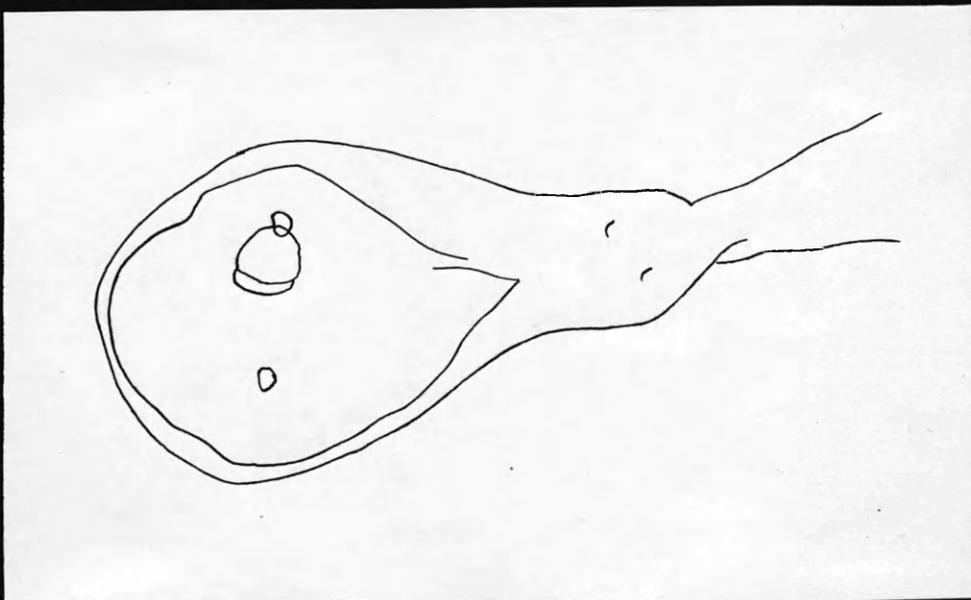
PLATE 1.

Fig.1. Cineraria with Root Rot.

Fig.2. Sporangium of isolate R<sub>4</sub> on oatmeal agar.  
44 x 18 μ.



1.



2.

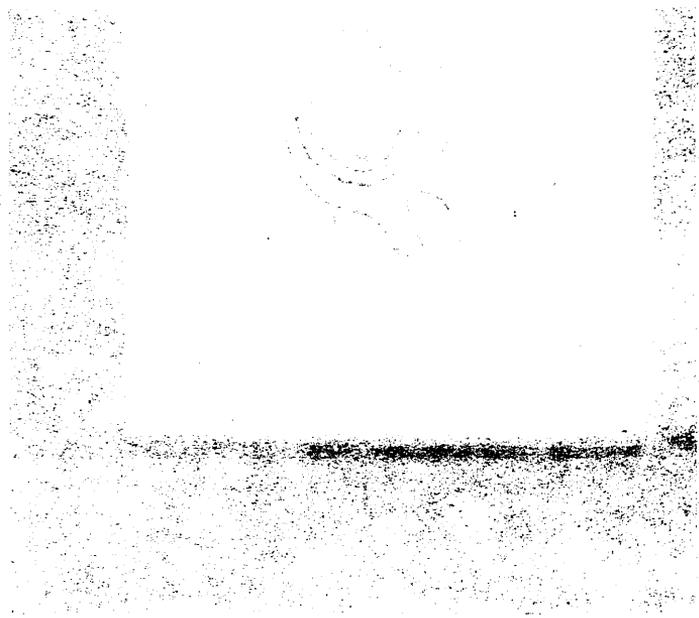


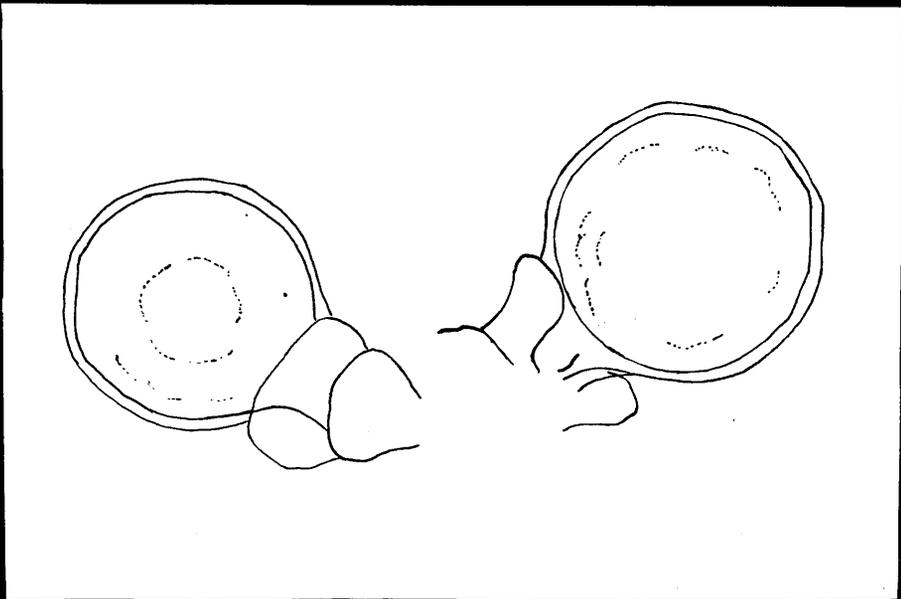
PLATE 2.

Fig.3. Oogonium of  $F_2$ , from a plug of Cineraria tissue.  
diam.28.5  $\mu$ .

Fig.4. Oogonia of  $R_1$ , from a plug of Cineraria tissue.  
average measurement 37 x 26  $\mu$ .



3.



4.

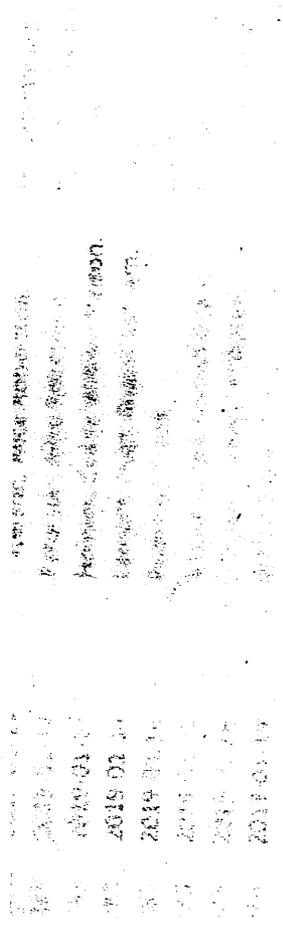
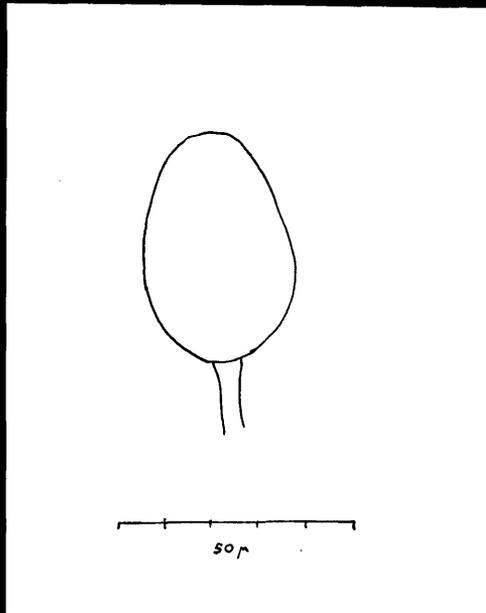


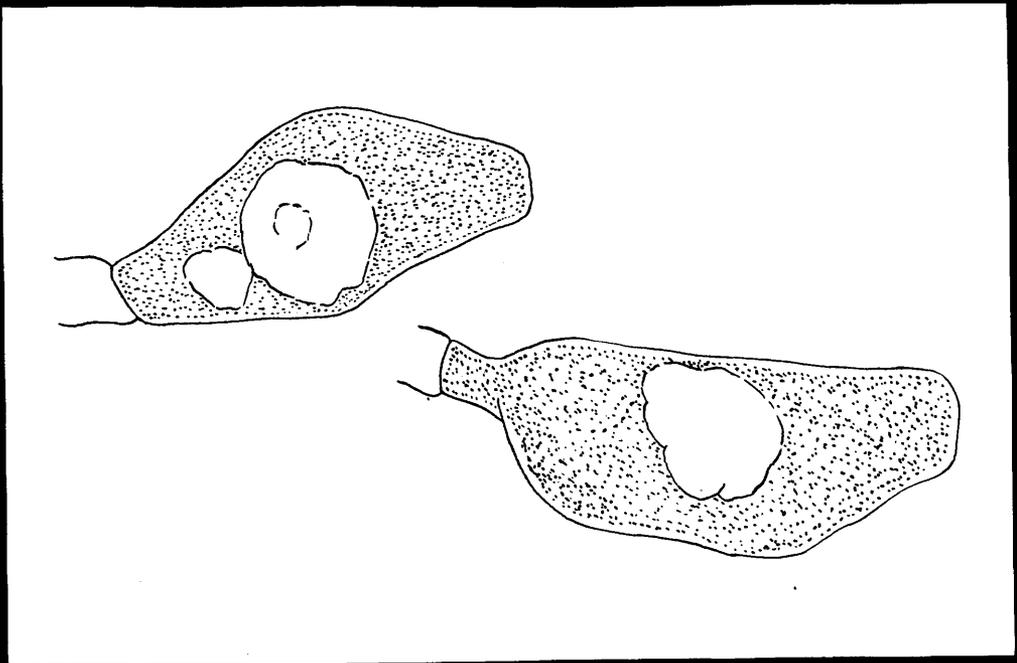
PLATE 3.

Fig.5. Sporangium of  $J_2$ , from a pea broth culture transferred to sterile water.

Fig.6. Sporangium of  $F_2$ , from an oatmeal agar culture transferred to sterile water. Measurements 22.2-15 x 11-7.5  $\mu$ .



5.

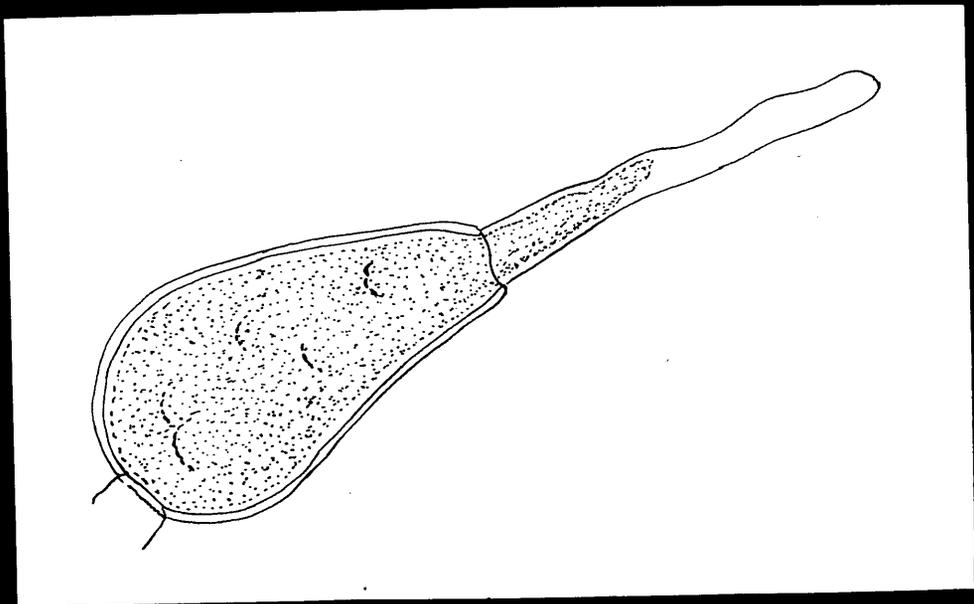


6.

PLATE 4.

Fig.7. Sporangium of  $R_1$ , from a 0.1%  $KNO_3$  culture transferred to bog soil extract. Measurement 40.7 x 20.4  $\mu$ . Germ tube 30 $\mu$  long.

Fig.8. Sporangia of  $R_4$  formed when actively growing mycelium was transferred to sterile water. Diam. 28 $\mu$ .



7.

