THE

SCIENTIFIC PROCEEDINGS

OF THE

ROYAL DUBLIN SOCIETY.

Vol. XIII. (N.S.), No. 36. MARCH, 1913.

ON PURE CULTURES OF *PHYTOPHTHORA INFESTANS* DE BARY, AND THE DEVELOPMENT OF OOSPORES.

BY

GEORGE H. PETHYBRIDGE, PH.D., B.Sc.,

ECONOMIC BOTANIST TO THE DEPARTMENT OF AGRICULTURE AND TECHNICAL INSTRUCTION FOR IRELAND;

AND

PAUL A. MURPHY, A.R.C.Sc.I.

(PLATES XLV, XLVI.)

DUBLIN:

PUBLISHED BY THE ROYAL DUBLIN SOCIETY,

LEINSTER HOUSE, DUBLIN.

WILLIAMS AND NORGATE,

14, HENRIETTA STREET, COVENT GARDEN, LONDON, W.C.

1913.

Royal Dublin Society.

FOUNDED, A.D. 1731. INCORPORATED, 1749.

EVENING SCIENTIFIC MEETINGS.

The Scientific Meetings of the Society are held alternately at 4.30 p.m. and 8 p.m. on the third Tuesday of every month of the Session (November to June).

Authors desiring to read Papers before the Society are requested to forward their Communications to the Registrar of the Royal Dublin Society *at least* ten days prior to each Meeting, as no Paper can be set down for reading until examined and approved by the Science Committee.

The copyright of Papers read becomes the property of the Society, and such as are considered suitable for the purpose will be printed with the least possible delay. Authors are requested to hand in their MS. and necessary Illustrations in a complete form, and ready for transmission of he Editor.

XXXVI.

ON PURE CULTURES OF *PHYTOPHTHORA INFESTANS* DE BARY, AND THE DEVELOPMENT OF OOSPORES.

By GEORGE H. PETHYBRIDGE, Ph. D., B.Sc.,

Economic Botanist to the Department of Agriculture and Technical Instruction for Ireland;

AND

PAUL A. MURPHY, A.R.C.Sc.I.

PLATES XLV, XLVI.

[Read FEBRUARY 25. Published MARCH 26, 1913.]

I. INTRODUCTORY.

IN endeavouring to ascertain the life-history of any parasitic organism, two ways of approaching the subject present themselves.

The first, and perhaps the one hitherto most frequently employed, is to obtain the details from the parasite as it grows upon its host. The second is to grow it in pure culture as a saprophyte on a suitable artificial medium, if such can be found, and study its development under such conditions. It is, of course, both theoretically possible, and it has been found to be the case in some instances, that under artificial conditions the parasite may not develop all the stages in its life-history; but it is also equally possible that under these very conditions the organism may show stages in its cycle of development which are not produced during its career as a parasite. Hence the advantage of approaching the problem by both of the available avenues.

In the case of *Phytophthora infestans*, the parasitic fungus which causes the potato blight, the application of the first method of study has failed so far to reveal with certainty any stage in which sexual organs are produced, although many of its allies amongst the Peronosporaceae have been shown to possess such organs. It is true that certain observers have laid claim to the

discovery of such structures in *P. infestans* when growing on the potato, notably Worthington G. Smith[1] and Smorawski.[2]

It would serve no useful purpose to refer in detail in the present paper to the controversy raised by Smith's publications; suffice it to say that his views, owing largely to the critical observations of de Bary, did not meet with anything like general acceptance. Smorawski's work also can scarcely be said to be convincing; and the bodies described and figured by him as sexual organs do not at all closely resemble the real ones obtained in pure cultures.

One of us spent a considerable amount of time a few years ago in searching for possible oospores of *P. infestans* in the blighted portions of potato-plants. Bodies were found frequently which might have been such structures; but prolonged attempts at causing them to germinate met with absolutely no success; consequently their true nature remained undetermined.

Although only failure has to be recorded as regards the search for oospores along this line up to the present, success has been attained, as will be seen, with pure cultures on certain media.

That *Phytophthora infestans*, exquisite parasite though it is often regarded to be, is capable of being grown on a dead substratum has long been known. Possibly Brefeld[3] was the first to record its growth as a saprophyte when he wrote, in 1883: "Unter den Peronosporeen habe ich mich auf den am meisten wichtigen und characteristischen Pilz der Kartoffelkrankheit beschränkt.—Die *Peronospora infestans* wuchs in künstlicher Ernährung wie Unkraut, fast so üppig, wie sie auf den Kartoffeln wächst." Von Tubeuf states:[4] "*Phytophthora infestans* is more easily reared as a saprophyte [than Exoascus], and occurs in nature as such; hence it approaches somewhat towards the hemi-saprophytes."

Matruchot and Molliard[5] claim to have been the first to grow this fungus in *pure* culture, both on living and non-living substrata, although Hecke[6]

[1] A general account of Worthington Smith's observations is to be found in his book, "Diseases of Field and Garden Crops," London, 1884, particularly in chapter xxxvi. Various earlier articles on the subject were contributed to The Gardeners' Chronicle (and to the Monthly Microscopical Journal, vol. xiv, p. 110, and vol. xvi, p. 120) by this author in 1875 and 1876; while photographs of the supposed oogonia and antheridia are to be found in Quart. Journ. Micros. Science, vol. xv, N.S., 1875, p. 360.

[2] Smorawski, J.—Zur Entwickelungsgeschichte der *Phytophthora infestans* (Montagne) de By. Inaug. Diss. Berlin, 1890.

[3] Brefeld, O.—Untersuch. aus dem Gesammtgebiet der Mykologie. Heft v. Leipzig, 1883, p. 9.

[4] Von Tubeuf, K., and Wm. G. Smith.—"Diseases of Plants induced by Cryptogamic Parasites," London, 1897, p. 7.

[5] Matruchot, L., et Molliard, M.—Sur la culture pure du *Phytophthora infestans* de Bary, agent de la maladie de la pomme de terre. Bull. Soc. Mycol. de France, T. xvi, 1900, p. 209.

[6] Hecke, L.—Untersuchungen über *Phytophthora infestans* de By. als Ursache der Kartoffelkrankheit. Journal für Landwirtschaft, Band 46, Heft ii, 1898, p. 104.

stated a couple of years previously that he had grown the fungus saprophytically, both on gelatine and in liquid media. The two French savants say: "On a déjà observé dans la nature le *Phytophthora infestans* vivant en saprophyte; mais à notre connaissance, personne n'est jamais arrivé à en obtenir de cultures pures, ni sur le vivant, ni sur les milieux artificiels. Tout ce qu'on sait sur le développement de cette Péronosporée résulte d'observations faites sur les plantes attaquées ou d'inoculations pratiquées dans les conditions d'aseptie insuffisante."

It would be interesting to know by whom the fact mentioned by these two sets of authors, and apparently acquiesced in later by Brefeld, that this fungus can grow in nature as a saprophyte, was recorded. We have been unable to find any original statement of this kind; but if it is a fact (which is doubtful), it might have an important bearing on the question of the recrudescence of the disease year after year.

In the paper just cited Matruchot and Molliard reported that they had succeeded in cultivating the fungus free from contamination with any other micro-organisms, on pieces of living potato-tubers; but as to the non-living media on which they obtained pure cultures they are silent. Conidiophores (and presumably conidia) were produced on both kinds of media.

In a further paper,[1] published in 1903, these authors reported that they had succeeded in getting pure, conidia-bearing cultures of the fungus on living pieces of the fruits of the vegetable marrow (*Cucurbita Pepo*) and the Spanish melon (Melon d'Espagne). Pure cultures were also raised on cooked pieces of vegetable marrow, Spanish melon, pear, and turnip, although on the last two the growth was only poor. Conidia were produced on these media or on some of them, but not so abundantly as on the living ones, and their number grew less and less, so that ultimately their production ceased, and the mycelium itself became enfeebled. The fungus also grew on vegetable marrow broth, and produced abundant conidia; but when this was rendered solid by the addition of agar, fewer of these bodies developed. Appreciable growth was also produced in a three per cent. solution of glucose in water. On none of these media was there any development of sexual spores or of chlamydospores. No growth was obtained on cooked potato, or on cooked tomato and various other fruits and roots.

Brefeld,[2] in 1908, gives fuller details of the nutritive solution in which he had found such good growth to take place. It was prepared by cutting young potato-tubers into thin slices, drying them quickly, and then extracting

[1] Matruchot, L. et Molliard, M.—Sur le Phytophthora infestans. Annales Mycologici, vol. i, No. 6, 1903, p. 540.

[2] Brefeld, O., *loc. cit.*, Band 14, 1908, p. 41.

them with cold water. The filtered and sterilized extract, to which a small quantity of beer-wort was added, proved itself an excellent medium, in which copious mycelium was developed as well as conidiophores which were, as regards size, but little behind those found on the potato plant itself. Brefeld found no signs of oospores, and he says[1]:—"In dem Pilz der Kartoffelkrankheit, *Phytophora infestans*, liegt ein sicher erwiesener Fall vor, bei welchem Oosporen nicht zur Ausbildung kommen und nur die Conidienträger auf der Oberfläche der befallenen, hier schnell absterbenden Pflanzenteile beobachtet werden können. . . . Die Oosporen liessen sich bis jetzt in diesen Kulturen mit Nährlösungen auch nicht erzielen, wohl aber können wir nach der leichten Ernährung des Pilzes in Nährlösungen mit allem Grunde annehmen, dass der Pilz von seiner Überwinterung in den Kartoffelknollen saprophytisch in der Erde weiter wächst, über die Oberfläche der Erde kommt und von hier aus in seinen Conidienträgern die Erzeugung der Kartoffelkrankheit in dem oberirdischen Kräutig der Nährpflanze, immer erst in vorgerückter Zeit, etwa im August, bewirkt."

Meanwhile the question of pure cultures of the fungus in artificial media had been taken up in the United States of America, and Clinton,[2] in 1906, reported that he had obtained such cultures in vigorous condition on plugs of living potato and on sterilized corn-meal and water, whilst less satisfactory growths were developed on agar-media containing potato- and pumpkin-juice respectively. In all some twenty-five to thirty media were experimented with by Clinton; but in no case were any sexual organs discovered, although in one instance some peculiar swollen bodies were observed which were suggestive of immature oospores. Further results were published by Clinton[3] in 1909, and, in particular, he found that the fungus grew readily and produced abundant conidia on an agar-medium prepared from the juice of Lima beans (*Phaseolus lunatus*). In no case, however, were any sexual organs observed.

Studies in the cultivation of the fungus had also been carried out by Jones.[4] In an abstract of a paper read before the the Botanical Society of America, in December, 1908, some of the principal results are briefly stated. The media employed were similar in character to those used by Matruchot and Molliard, and by Clinton. In some of these media oogonia-like bodies were obtained frequently; in others, they were developed but sparingly.

---

[1] *Loc. cit.*, p. 116.

[2] Clinton, G. P.—Downy Mildew, or Blight, *Phytophthora infestans* (Mont.) de B., of Potatoes. Rep. Connecticut Agric. Exp. Station for 1905. 1906, p. 304.

[3] Clinton, G. P.—"Artificial Cultures of Phytophthora, with special reference to Oospores." Rep. Conn. Agric. Exp. Sta., for 1907–8. 1909, p. 891.

[4] Jones, L. R., and N. J. Giddings.—Studies of the Potato Fungus, *Phytophthora infestans*. Science, N. S., vol. xxix, 1909, p. 271.

In 1909[1] and 1910[2] Jones gave further particulars of his pure cultures of the fungus which he had grown continuously for four years. Oogonia-like bodies were found in cultures on raw potato, in potato gelatine and on Lima bean agar. In some cases apparently fully developed resting-spores were discovered in his cultures, but no traces of real antheridia were found. The spores are described as having a thick spiny brown outer wall with densely granular contents. They resembled in a general way the oospores of other Peronosporaceae, and bodies similar to them were also seen in potato foliage which had been destroyed by the blight fungus.[3]

The first announcement of the production in pure cultures of undoubted oospores was made by Clinton[4] in 1911, in an article in which the previous literature on the subject is summarized, while full details of the media used and of the results obtained were published later in the same year.[5] Out of about seventy-five media experimented with three are mentioned as having given specially good results, viz., Lima bean-juice agar, a "combination medium" made up from Lima beans, oats, peanuts, potato, sweet corn, wheat, and agar, and lastly oat-juice agar, which is stated to have stood alone so far as the production of oospores is concerned. On it antheridia, oogonia, and oospores were developed; and in the paper quoted these are fully illustrated and described.

It will, we think, at once be conceded that corroboration by other workers of such interesting and important results was highly desirable, and, seeing that we enjoyed particular facilities for work on *P. infestans* owing to the establishment by the Department of Agriculture and Technical Instruction for Ireland of a temporary station in the west of Ireland (where the potato blight is particularly prevalent) for special investigations into the various diseases to which the potato is subject, we commenced early in the summer of 1911 the study of the development of this fungus in pure cultures.

Over twenty media or modifications of media have been experimented with, and many hundreds of cultures have been studied during an uninterrupted period of some eighteen months. True oogonia, antheridia, and undoubted

---

[1] Jones, L. R.—"Resting-Spores of the Potato Fungus *Phytophthora infestans*." Science, N.S., vol. xxx, 1909, p. 813.

[2] Jones, L. R., and A. B. Lutman.—"Further studies of *Phytophthora infestans*." Science, N.S., vol. xxxi, 1910, p. 752.

[3] Since the above was written we have received a fuller description of Jones's work contained in a paper entitled, "Investigations of the Potato Fungus Phytophthora infestans," by L. R. Jones, N. J. Giddings, and B. F. Lutman, and published in 1912 by the Bureau of Plant Industry, U.S. Department of Agriculture, Washington.

[4] Clinton, G. P.—"Oospores of Potato Blight." Science, N. S., vol. xxxiii, 1911, p. 744.

[5] Clinton, G. P.—"Oospores of Potato Blight, *Phytophthora infestans*." Rep. Conn. Agric. Exp. Sta. for 1909–1910. 1911, p. 753.

oospores have been found on one of these media—a modification of Clinton's oat-juice agar; and further than this the study of a new species of Phytophthora,[1] which produces a new and characteristic type of rot in the potato tuber, carried on simultaneously with the cultures of *P. infestans*, has enabled us to throw very considerable light upon the mode of development of these organs in the latter fungus.

Hence Clinton's results are both fully corroborated and substantially amplified.

II.—NOTES ON TECHNIQUE.

The cultures were obtained in the first instance from affected foliage. On keeping this in the laboratory for a day or two under suitable conditions, plenty of aerial mycelium, bearing conidia, is developed. In some cases conidia were allowed to fall on to the surface of a suitable artificial medium in Petri dishes; and not infrequently a pure culture could be obtained by removing a small portion of the mycelium developing from a single conidium to a suitable sterile medium. The important thing is to have the infective material as freshly grown as possible, since under these conditions there is less chance of the presence of the spores of other organisms, such as Fusarium, &c.

In other cases pure cultures have been obtained by lightly touching fresh aerial conidia-bearing mycelium with a sterile, moistened platinum loop and transference to a suitable slant in a test-tube. Of course, some of the cultures obtained in this way are impure, but with care a considerable proportion of them can be obtained pure from the start.

Another method employed was to prepare, under as strictly aseptic conditions as possible, blocks of living potato-tuber tissue and infect them from the original material. *Phytophthora infestans* develops fairly rapidly on the living potato, and by this means the presence of common saprophytic fungi, such as Penicillium, Mucor, &c., can be avoided.

Since the fungus does not develop very rapidly on the artificial media used, it was soon found that Petri dishes were entirely unsuited for the prolonged cultures necessary; consequently nearly all our cultures were carried out on slants in test-tubes, or, if liquids were being used, in shallow layers in small flasks.

In removing portions of cultures from tubes, steel lancet-pointed needles were used which were kept standing in strong alcohol. Immediately before being used they were removed and the spirit on them ignited. By this means the

[1] A full account of this new species (*P. erythroseptica*) will be found in Scient. Proc. Roy. Dublin Soc., N.S., vol. xiii, No. xxxv, 1913, p. 529.

needles were sterilized without becoming unduly heated, and without suffering from the corrosion which follows from the frequent strong heating of a steel needle in a flame. Platinum needles are, of course, much too flexible for this work.

The oogonia being of a distinct brown tinge were easily discernible in the media under a low-power dissecting microscope, although they are just beyond the limit of vision by the unaided eye. In examining them, small portions of the media containing them were removed; and, as a general rule, each individual oogonium or oogonium and its adhering antheridium was dissected out of the medium under the dissecting microscope, mounted in a drop of water and covered with a cover-slip. Excess of water, if any, was removed by means of blotting-paper, and the preparation was then irrigated with a drop of a $2\frac{1}{2}$ per cent. solution of caustic soda. This tended not only to clear up somewhat any small opaque portions of still adhering medium, but to some extent cleared away the rather deep-brown colouring-matter in and around the oogonium. When the clearing process had gone far enough, the soda was neutralized by irrigating the preparation with a drop of weak acetic acid solution. If the clearing was allowed to go too far, and particularly if no spore was present in the oogonium, the latter was frequently apt to swell up and burst.

More or less successful attempts were made in our earlier preparations to obtain the oogonia free from the semi-opaque starchy medium (oat-agar), in which alone they developed, by a process of digestion of the latter with malt-extract. This plan did not, however, offer any special advantages; and the method described of first mechanically dissecting away under the microscope the greater part of the medium from around the oogonium proved itself, with a little practice, to be in the end the simplest and most satisfactory.

It is perhaps unnecessary to add that the strictest control was exercised over the cultures, both by microscopic examination and by control cultures, to obtain and to keep them pure; and there is no room for doubt but that the sexual organs described do belong to *Phytophthora infestans*, and to no other fungus.

III. Pure Cultures on Media in which no Sexual Organs were found.

(1) *Growth on sterile, raw Potato.*—It is commonly but erroneously supposed that *P. infestans* produces a more or less soft wet rot in potato tubers. As a matter of fact, however, tubers when infected with this fungus, whether naturally or artificially, remain hard and firm, showing the well-known and characteristic dark and sunken areas on the skin, unless the

attack is followed by that of another, or of other organisms, when the subsequent fate of the tuber will depend upon the nature of these secondary organisms, and upon the conditions under which the tuber is kept. In reality, therefore, the rot produced in a tuber by this fungus is essentially a form of *dry-rot.*

In practice it is not an easy matter to grow the fungus in a whole tuber in such a way that one can be *absolutely* sure that the growth will remain pure all the time. Our methods of procedure with whole tubers, and the results, have been as follows:—Clean, healthy tubers, with unblemished skins, were selected and carefully washed in running water, a soft brush being used. They were steeped for a period in a dilute solution of either formalin or mercuric chloride, and then dried. Inoculation was made with a small portion of mycelium, generally bearing conidia, from a pure culture of the fungus on an artificial medium, through a shallow stab into the skin of the tubers. The tubers were then allowed to stand in a covered glass dish, sometimes on a piece of moistened filter-paper, sometimes without this, at room-temperature.

As a rule, in from five to seven days, a somewhat dark, sunken area is formed around the original point of inoculation; aerial mycelium, bearing conidia, may or may not develop at the inoculation-wound. This dark, sunken area gradually extends; and in the course of four weeks or so the greater part of the skin of the whole tuber may have become similarly affected; on the other hand, in some cases, after the lapse of a similar period, the diseased area may be much smaller.

Inoculated tubers lose some of their water more quickly than control tubers, treated similarly (i.e. stabbed, but with a sterile needle), do. This water tends to condense and to collect on the lower surface of the tuber between it and the bottom of the glass dish. When this is the case or when the inoculated tuber has been placed on moistened filter-paper at the outset, the lower part of the tuber becomes affected more rapidly than the upper. Experiences of this kind and others which have been encountered lead us to believe that the skin of a potato must not be looked upon as a mere physical membrane impervious to water, for its properties, with regard to the passage of water both inwards and outwards, appear to be radically altered when the living cells adjacent to it become killed. This idea is supported by the experiments of Stoward,[1] who found that certain chemical substances in solution pass much more readily into a tuber through the skin over dead portions of tissue

[1] Stoward, F.—"The Effect of certain Chemical Substances on the Vitality of the Buds of Potato Tubers, and their Disinfective Action on Potato Blight (*Phytophthora infestans*)." Proc. Roy. Soc. Victoria, vol. xxiv (New Series), Pt. 2, 1912.

than over still living areas. The diseased areas on the surface of an inoculated tuber are distinctly harder to the touch than the still healthy areas, and they are also tougher when cut with a knife. As a rule, the darkening of the skin precedes its sinking in.

On cutting open such inoculated tubers the tissues show the mottled brown or rusty markings so characteristic of the attacks of the fungus, as seen in naturally affected tubers. The extent of the browning depends largely on the time which has elapsed since inoculation took place, but perhaps also to some extent upon the individuality of the tuber.[1]

It is commonly supposed that this brown discolouration of the dead tissue is the result of the action of the fungus in question; but Matruchot and Molliard maintain that this is not the case. They state that aseptically obtained cylinders cut from the tissues of tubers and artificially inoculated with *P. infestans* remain for an indefinite period white and firm, retaining the same aspect as non-inoculated controls, and simply drying up like the latter do. We have paid considerable attention to this question of the browning of affected tissue, and find that it *does occur* in pure cultures of the fungus on cylinders of raw potato-tissue prepared aseptically; and we cannot but conclude that the browning is due to the action of the fungus, as is generally believed.

One of our critical experiments on this point deserves to be described in detail. Twelve cylinders of living tissue were prepared under as strictly aseptic conditions as possible, and were transferred with the greatest possible precautions to sterile test-tubes. These were then kept for a period of ten days at room (summer) temperature, when close scrutiny showed that nine of them were sterile, whilst the other three had become contaminated. Each of these nine cylinders was then inoculated from a pure culture of *P. infestans*. At the time of using this culture it was subjected to microscopic and also cultural control,[2] to make sure that it was what it purported to be—namely, a pure culture.

The nine inoculated cylinders produced a good growth of the fungus and they became typically browned, as we have observed in other pure cultures.

---

[1] Experiments carried out by one of us since this paper was written show that different varieties of potatoes differ considerably in their reaction towards the fungus. In a variety like "Shamrock," which in the field is practically immune to the blight both as regards foliage and tubers, the tubers rot much more slowly when inoculated with *P. infestans* than do those of a variety such as "British Queen," which possesses no marked resistance to the disease.

[2] Practically all of the common moulds, as well as species of Fusarium, &c., will develop on wort-gelatine, as will also the majority of ordinary bacteria. *P. infestans* makes absolutely no growth on this medium. Portions from the pure culture when placed upon wort-gelatine slants produced absolutely no growth; the slants remained absolutely sterile. The microscope revealed no foreign organisms in the culture. Hence we concluded that it was pure.

After the lapse of periods of time varying from nine to twenty days small portions of the browned tissue were removed from eight of the tubes and transferred as carefully as possible to slants of wort-gelatine. This was no easy matter, since, as mentioned above, the diseased tissue becomes very tough, and to cut off small portions from the affected cylinders situated in the bottoms of test-tubes without at the same time running considerable risk of contamination presents some difficulties. In two cases Penicillium, in another an unidentified mould, and in a fourth bacteria developed on the wort-gelatine slants. We have every reason for believing that these contaminations arose during the process of transference, for in the remaining four cases the wort-gelatine slants remained absolutely sterile. Finally, the remaining portions of the eight affected cylinders were removed and subjected to microscopical examination, when absolutely no bacteria and no fungus other than *P. infestans* could be discovered.

A somewhat similar experiment was carried out on whole tubers. These were washed, disinfected, dried, and inoculated with the pure culture. After about three weeks the tubers were cut open under strictly aseptic conditions, when the browned, diseased areas were found as usual. Small portions of this browned tissue were removed and planted on one oat-agar and three wort-gelatine slants. The three latter remained absolutely sterile, while on the oat agar *P. infestans* developed in characteristic fashion, but no other organism of any kind was present.

If therefore there is an organism which is associated with *P. infestans* in causing the brown discolouration, it does not grow either on wort-gelatine or on oat agar; and since the pure culture originally used had been obtained from a long series of periodical transferences from oat agar to oat agar, it is practically impossible to believe that any such organism could have been present; and we are forced to the conclusion that *P. infestans* is alone responsible for the well-known browning.

Jones found, as stated above, that oogonia-like bodies were formed in his pure cultures on raw potato. We have never seen them on this medium; but it must be admitted that our search for them here has up to the present not been so prolonged or so thorough as has been the case with other media. A pure culture on Lima bean agar, which Professor Jones was good enough to send us early in 1911, contained the fungus in active growth and in a normal condition of virulence; but microscopic examination of this particular culture failed to reveal the presence of any bodies suggestive of sexual organs in it.

(2) *Growth in raw Potato-juice.*—Six medium-sized tubers were well washed, peeled, chipped into small portions, and then well squeezed through

a potato-masher. About 80 c.c. of juice were thus obtained, to which was added an equivalent quantitity of water. This was allowed to stand over night in a tall cylinder, when the starch-grains and other suspended solid matter became sedimented. The supernatant liquid was siphoned off and filtered through a Chamberland filter into a sterile flask, the sterile juice being then distributed into sterile flasks. A portion of the unfiltered (non-sterile) juice was also transferred to a sterile flask. Ten flasks were inoculated from a pure culture of *P. infestans*, and were allowed to stand at room-temperature for a considerable period, while side by side with them stood similar uninoculated flasks containing the juice as controls. During this time, somewhat to our surprise, the fungus made little or no apparent growth in any of the flasks.

The experiment was repeated at a later date, flasks containing the filtered (sterile) and unfiltered juice being inoculated with both conidia and small portions of mycelium from a pure culture. No growth whatever occurred in the unfiltered juice, possibly because it rapidly underwent putrefactive decomposition at the hands of bacteria. The filtered juice remained sterile, but where inoculated with conidia no growth occurred. When mycelium from a pure culture, however, was placed in the filtered juice, it not only remained alive for about three weeks, but increased in amount to a small extent. This freshly developed mycelium frequently presented curious deformities in structure some of which are illustrated in figs. 1, 2, and 3, Plate XLVI. We do not consider these structures as being attempts at the formation of sexual organs; but in many instances they certainly would seem to be malformed or abortive conidia,[1] their condition being due in all probability to the fact of their being submerged in a liquid, and not produced, as is normally the case with *P. infestans*, in the air. Our experience as well as that of other workers, such as Himmelbaur, seems to show that the production of hyphae, with abnormal growth, is more or less common in several species of Phytophthora when cultivated on artificial substrata.

(3) *Growth in Potato-juice Agar and Potato-juice Gelatine.*—The potato-juice is prepared in the cold as above, and then boiled and filtered. It is stiffened by the addition of 10 per cent. to 12 per cent. of gelatine or 1·25 per cent. of agar. The juice is naturally slightly acid, and the addition of gelatine renders the medium still more acid. In some cases the acidity of the gelatine was neutralized before use—in others not.

On these media growth certainly takes place, and conidia are to some

[1] Some of the structures figured by Jones as occurring in potato-gelatine cultures would also seem to be susceptible of a similar interpretation.

extent produced; in some cases they were observed submerged in the medium. But, on the whole, growth is decidedly poor, and no signs of oogonia or antheridia were observed.

(4) *Growth on Potato-juice-wort Agar and Gelatine.*—These media were prepared similarly to the foregoing except that an amount of beer-wort equivalent to the amount of potato-juice was added.

Growth is very similar in these two media. For the first week or so the mycelium develops and remains submerged; then aerial mycelium arises and a few conidia are borne. No sexual organs were observed, but in the agar medium swollen hyphae were quite abundant, which appeared as if they might be the early stages of oogonia, but they were never observed to develop into these bodies. The fungus remains alive in the agar medium for many months.

(5) *Growth on "Salep" Agar.*—Salep is a preparation made from the dried roots of certain orchids. It was used by Bernard[1] in his work on the fungi living in the roots of orchids, and subsequently by Klebahn[2] and Himmelbaur,[3] for the culture of certain species of Phytophthora. We first endeavoured to make up an agar medium containing this substance in accordance with the recipe given by the last-named worker, but found that it would not set solid when cold. On leaving out the tartaric acid and the inorganic salts, which seemed superfluous when ordinary tap-water was used, a medium was obtained which set satisfactorily.

On this medium *P. infestans* makes slow and somewhat scanty growth. A fair amount of aerial mycelium is developed, on which a considerable abundance of conidia occurs, whilst there is also a fairly good development of submerged mycelium, which ultimately permeates the whole of the medium. No signs of sexual organs were observed in the cultures in this medium.

(6) *Growth on Lima Bean Agar, filtered and unfiltered.*—This medium was prepared in accordance with the directions given by Clinton, but instead of straining finally through cheese-cloth as recommended,[4] we filtered through Chardin's Agar filter-paper. We also used this medium without any final filtering or straining. Considerable care is necessary in making the unfiltered medium in order to avoid frothing up during subsequent sterilization. On the unfiltered medium *P. infestans* grows quite luxuriantly, little less so indeed than on the Quaker-Oat agar medium presently to be described.

---

[1] Bernard, N.—Rev. Gén. de Bot., xvi, 1904, p. 408.

[2] Klebahn, H.—"Krankheiten des Flieders," Berlin, 1909. p. 37.

[3] Himmelbaur, W.—Zur Kenntnis der Phytophthoreen. Jahrb. d. Hamburg. Wiss. Anstalten, 28. Beiheft 3, 1910, p. 43.

[4] Conn. Ag. Ex. Sta. Rep., 1907-8, p. 898.

The aerial mycelium, bearing a prolific crop of conidia, clothes the whole surface of the slant with a dense growth, whilst submerged mycelium permeates the whole of the substratum. The fungus does not remain alive on this medium for so long a period, however, as on Quaker-Oat agar. On the filtered medium the growth is still good, but considerably less luxuriant than on the same medium unfiltered. Some thirty or forty cultures were made on these two media, but they did not extend over a period of many months. Both Jones and Clinton found immature sexual organs on Lima Bean agar, but they were not to be found in any of our cultures.

(7) *Growth in Oat-extract Agar.*—This medium was prepared from 200 grams of very finely ground Quaker Oats, extracted with 1,000 c.c. of cold tap-water. The powdered oats were allowed to stand covered with the water in a corked flask for about five days, a few drops of chloroform having been added to prevent decomposition. Subsequently the whole was well shaken up for some hours on a shaking machine, and then allowed to stand. After sedimentation, the supernatant liquid (about 650 c.c.) was siphoned off and heated in the inner part of a double saucepan until the whole of the chloroform was driven off, and 2 per cent. agar was added. The medium was somewhat turbid, but was not filtered. On subsequent sterilization in the autoclave a curdy precipitate was formed, which settled down as a more or less flocculent mass at the bottom of the tubes.

P. infestans grows very well on this medium, and soon covers the surface of the slant with a thick felt of aerial mycelium, on which conidia are developed in abundance. The mycelium also makes extensive submerged development. On the whole the growth here is but little less than on Quaker-Oat agar, but no sexual organs were ever seen.

This medium, although slightly turbid, is in thin layers, sufficiently transparent to admit of use with advantage for growing certain species of Phytophthora, either in Petri dishes or in films on the lower surface of a cover-glass in a moist chamber.

(8) *Other Media.*—No growth whatever took place on the following media:—Cooked potato; sterilized bread, carrot and potato-stalks; potato-stalk extract-agar; wort-gelatine; wort-agar; beef-broth-peptone-gelatine and -agar.

IV. Pure Cultures on Media in which Sexual Organs are formed.

(1) *Clinton's Oat-juice Agar.*—The following is the method given by Clinton for preparing this medium:—"Fifty grams of ground oats, such as are ordinarily fed to horses, are stirred into about 300 to 350 c.c. of water, and steam from an autoclave, by means of glass- and rubber-tubing connected

with the stopcock, is run into this in a covered dish for half an hour. This cooks the material without burning, and at a uniform temperature. The coarse sediment of the oats is then strained off through an ordinary fine wire strainer, and 10 grams agar is added to the liquid, which is again treated to the steam for half an hour to melt the agar thoroughly. Some water passes over with the steam during these cookings, so that what little, if any, is needed to bring it up to the required 500 c.c., is added after the whole is drained into a graduated cylinder. After the added water is uniformly distributed by repouring, the medium is placed in the test-tubes, and these are sterilized in the autoclave for fifteen minutes under 7 to 10 lbs. pressure."

We followed these directions closely, except that steam was not generated from an autoclave, but from a glass flask, and it was passed into the mixture in the first instance for rather less than the half hour, since with the oats we used there was a tendency for the medium to become too stiff to be poured easily if steamed for this length of time. We also found that the half hour's steaming after addition of the agar was not sufficient to dissolve the whole of it thoroughly; but by keeping the mixture well stirred during pouring into tubes it could be distributed fairly uniformly through them, and subsequent sterilization completed its solution.

When this medium is inoculated with conidia, the early growth of mycelium is confined to its surface, but in due time aerial mycelium becomes visible to the unaided eye. The period which elapses before this occurs varies from three to nine days, the usual interval being from five to seven days. If Petri dishes are used, the whole surface becomes covered with a thick growth of mycelium in about a week after it has become visible to the naked eye, at room-temperature. On slants in test-tubes during a similar period a fairly dense felt of mycelium is also formed, which completely fills the space available in the tube for some considerable distance up the slant. Conidia are produced in large numbers; and the fungus remains alive in a tube of this medium for several months. Oogonia were also observed, but only sparingly; out of about 150 cultures carried out on it during a period of seven months these bodies were only abundantly present in one case. No antheridia were developed in any of our cultures on this medium; but, in spite of their absence, apparently normal thick-walled oospores were developed in some cases. (See figs. 5 and 6, Plate XLV.)

There are certain disadvantages connected with this medium which caused us to give up its use in favour of the one next to be described. It was troublesome to prepare and difficult to pour in a clean fashion into tubes. Its most serious drawback, however, was that certain starch-containing cells in which the grains had become swollen during cooking and sterilization, but not

sufficiently so as to cause the cells themselves to burst, remained distributed here and there throughout the medium. These cells were about the same size as the oogonia; and since their walls were also brown, it was impossible to distinguish them from the oogonia without the use of the compound microscope. Hence when looking over culture-tubes for the presence of oogonia with a pocket-lens, the presence of these bodies was the cause of considerable inconvenience.

Ground Quaker-Oats Agar.—This is the medium which has given us the best results; it is prepared as follows:—

Thirty grams of Quaker Oats[1] are ground in a small hand-mill, with adjustable frictional surfaces, to as fine a powder as possible. This is then stirred into 500 c.c. of cold water (rain-water or soft water), and placed in the inner vessel of a double saucepan, cold water being placed in the outer. The saucepan is then warmed up; and in from ten to fifteen minutes the oatmeal and water form a rather thin gruel. At this stage 10 grams of strip agar, cut into small pieces, are added, and the heating is continued until the latter has become completely dissolved, the latter process being facilitated by constant stirring. The medium thus prepared is free from lumps, and can easily be poured into test-tubes which are then sterilized in the autoclave, slanted, and allowed to cool. No water is added to make up for the small quantity lost during the cooking process. If the Quaker Oats are not ground fine before using, the medium will be lumpy and stiff. The annoying brown cells, present in our preparations of oats, according to Clinton's method, are entirely absent in this medium.

The growth of *P. infestans* on this medium is similar to that on Clinton's oat-juice agar, but somewhat more luxuriant. The aerial mycelium is more copious, and conidia are formed in very great abundance. Conidiophores which have produced ten conidia on the same hypha are not uncommon, and one was observed which had borne thirteen without becoming branched.

Oogonia were borne by the fungus on this medium in far greater abundance than on Clinton's oat-juice agar. Out of seventy-seven of our earlier cultures on it only eleven failed to produce any, and thirty-four of the remainder produced them very abundantly.

Antheridia have been found on this medium as well as oogonia, but up to the present in only three cultures of one and the same series, although, in all probability, they will continue to develop more abundantly as time progresses.

[1] Quaker Oats is a proprietary article of food made in Canada by the Quaker Oats Company for Quaker Oats, Limited, London. It appears to consist of partially cooked, crushed oat caryopses, without the pales.

The oogonia are found embedded in the medium mainly along a U-shaped course following the outline of the surface of the slant, and distant a few millimetres from its edge. The apex of the slant is usually free from oogonia. Since they are closely pressed against the glass, as a rule, they can be seen readily with a pocket-lens. They have also been found elsewhere, such as on the surface of the slant, and even on the aerial mycelium, but in smaller numbers.

Again and again they have been found in contact with a solid body in the medium, most frequently a small portion of the aleurone layer of the grain which has escaped grinding. This fact, and their abundance in contact with the walls of the tube, seem to suggest that possibly some mechanical obstacle to the forward progress of a hypha may act as a stimulus to oogonia formation ; but this may, perhaps, be only a coincidence.

Submerged mycelium does not appear to penetrate far into that portion of the medium at the bottom of the tube, which does not constitute part of the actual slant.

A photograph showing some of the oogonia, as seen under the low power of the microscope, embedded in the medium is reproduced in fig. 3, Plate XLV.

V. General Account of the Development and Structure of the Sexual Organs.

(1) *Influence of Medium, Time, and Illumination on Development of Oogonia.*— From a considerably greater number of preliminary isolations, and after much useful experience had been gained in the successful handling of them, nine pure cultures were selected as starting-points, and from them, by continual transfers at suitable intervals, nine series of cultures were kept going and were submitted to extremely close macro- and microscopical observation for many months. The isolations were all made from Clifden material; and we have no reason to suppose that the nine series of cultures represent so many different "strains" of the fungus. In all of the nine, oogonia were produced; and in one series antheridia were associated with many of the oogonia. It seems probable that the formation of antheridia is merely a matter of time ; and that on prolonged culture these bodies will also appear in cultures of the remaining eight series.

Speaking broadly, we have found that when a culture has once commenced to form sexual organs, it continues to do so in the subsequent transfers without intermission; and although the relative abundance of these bodies may vary somewhat in the successive cultures, as a rule, the subsequent

transfers from cultures rich in oogonia become themselves, in due time, also well provided with them.

It was found that oogonia developed sooner, more abundantly, and with greater regularity on Quaker-Oat agar than on Clinton's oat-juice agar. Thus six out of the nine series produced oogonia on Clinton's medium, only one of them abundantly, and the other five somewhat spasmodically; but when the nine were transferred to the Quaker-Oat medium, oogonia soon began to be produced in all of them, and in most of them quite plentifully.

As regards the time taken for oogonia to make their first appearance, it would appear that continued culture on the oat-medium, with several transfers to fresh tubes, is desirable before these bodies develop, at least in any quantity.

In the case of the series M. 7, oogonia were first found, and in abundance, about one month from the start of the pure culture, and in the second transfer. In the case of series C. 1, about ten days later. In the case of the other members of the nine series, a few showed some oogonia, and the others none over a period of about six months, during which they were being grown, with transfers at suitable intervals, on Clinton's Oat agar. Oogonia first began to appear in relative abundance and with constancy when the transfers were made on to our own Quaker-Oat medium at the end of February, 1912.

When a small piece of a culture in which oogonia have already developed is transferred to a fresh tube, the time taken for oogonia to develop in the new culture varies considerably. The shortest time observed by us has been six days, but it is frequently very much longer than this.

On two occasions sets of parallel cultures were made with a view of ascertaining whether the oogonia would develop better in total darkness than under the alternating conditions of diffused daylight and the darkness of night. The results showed in both cases that the oogonia developed more rapidly and more abundantly in total darkness.

(2) *Development of Oogonia and Oospores in the absence of Antheridia, i.e., parthenogenetically.*—The oogonia arise, as a rule, as terminal swellings on fairly stout lateral hyphae; but they may also arise laterally on a hypha. (See fig. 7, Plate XLVI.) The contents consist at first of finely granular protoplasm, without oil-drops, similar to that found in the general mycelium.

As the terminal portion of the hypha proceeds to swell, its contents become more dense (see fig. 4, Plate XLVI); eventually oil-drops appear; and its wall, which becomes the wall of the oogonium, becomes brown in colour, thus hiding from view to a great extent the subsequent changes undergone by the contents.

This reddish-brown colouring matter is a very characteristic feature of the oogonium; and in addition to staining the wall of the oogonium itself, it diffuses out into and stains the surrounding medium. In this way the thickness of the wall becomes considerably exaggerated in appearance. When the colouring matter is removed, or at any rate is rendered less dense by treatment with alkali, it is seen that the wall is in reality not very thick, although it is distinctly thicker than the walls of the ordinary hyphae, and its double line of contour is readily made out. (See fig. 6, Plate XLVI.) Clinton describes the oogonium wall as becoming thickened by the deposition on the outside of the original coat of a more or less irregular, thick, reddish-brown coat. We regard this deposit as being part of the medium which has become stained by diffusion. As a result of branching two oogonia, and in some cases even three, are borne on one lateral hypha. (See fig. 5, Plate XLVI.)

The oogonia are distinctly brittle, and slight pressure on the cover-glass suffices to break them open and liberate the contents or the spore if present. Figs. 5 and 6, Plate XLV, are from photographs showing such crushed oogonia. As regards shape the majority of oogonia are pyriform, but a few were observed which were almost spherical (see fig. 4, Plate XLV); and all stages were observed between forms which were practically spherical and those which were distinctly pear-shaped or even so much elongated as to be almost club-shaped. The oosphere and oospore occupy, as a rule, only the terminal swollen portion of the oogonium, although one or two cases were observed where the spores themselves were somewhat pyriform. Fig. 7, Plate XLVI, illustrates one of these.

A septum is, in most cases, formed at the base of the oogonium, shutting off its contents from the hypha which bears it. Sometimes, however, this septum is absent, as is shown in fig. 6, Plate XLVI.

Even in the absence of antheridia, apparently normal, thick-walled oospores are produced in the oogonia, which can be discerned after treatment with alkali, or by applying judicious pressure to the cover-glass. Out of 258 oogonia specially examined one by one for the purpose, 87, or roughly one-third, contained oospores with more or less thick walls.

The average transverse diameter of the oogonia was found to be 38μ, and it varied between the limits of 31μ and 46μ. These measurements agree fairly closely with those given by Clinton for the oogonia which developed in his cultures.

The oogonium wall is smooth, or at the most shows some occasional irregularities, and cannot be described as sculptured. Its outline is of course

rather obscured by the brown stain which, as previously stated, diffuses from it into the surrounding medium.

The oospores are spherical bodies (with the exception of a few more or less pear-shaped ones which have occasionally been seen) with a thick, colourless, smooth, hyaline wall.[1] Apparently the whole of the protoplasm in the oogonium does not go to form the oosphere and subsequent oospore, for what appear to be remaining portions of it occur between the wall of the spore and that of the oogonium. The diameter of the oospores varies from 28μ to 34μ, the average diameter being found to be about 30μ. The thickness of the walls of the oospores measures from 2–3μ, but is slightly greater (4μ) when antheridia are present.

The spores are filled with colourless, densely granular protoplasm, especially towards the periphery; the central portion is much clearer and more transparent, and the contents thus resemble those described and figured by de Bary for the oospores of other members of the Peronosporaceae.

A few attempts have been made to germinate the oospores in hanging drops; but so far they have met with no success.

(3) *Production of Oogonia and Oospores in the presence of Antheridia.*—Although the oogonia and oospores described above were produced in abundance and with great regularity for many months, the most careful and prolonged search failed for a long time to disclose the presence of antheridia.

It was not until a period of some fifteen or sixteen months had elapsed that the presence of antheridia was observed, and even then only in the cultures of one series (M. 7), which had been all along one of the most robust and prolific as regards the production of oogonia and oospores parthenogenetically. Meanwhile, one of us had been studying for a couple of years the development of a new species of Phytophthora (*P. erythroseptica*), which causes a serious rot of potato tubers, temporarily designated as "doubtful" rot.[2] Owing to the knowledge gained by the study of this organism, a detailed description of which will be published simultaneously with this paper,[3] we were in a position to understand at once by analogy the course of events in the development of the antheridia, oogonia, and oospores of *P. infestans*. Our attempts to get *P. infestans* to form its sexual organs on Quaker-Oat agar in cover-glass film cultures in a moist chamber met with no success, and consequently the stages

[1] No spores were observed resembling in any degree the resting spores with protuberances on their walls figured by Jones, and recalling *Artotrogus hydnosporus*.

[2] See Journal Department Agric. and Tech. Instruction for Ireland, vol. xii, 1912, p. 357.

[3] Pethybridge, G. H.—"On a form of Rot in the Potato tuber caused by a new species of *Phytophthora*, having a method of sexual reproduction hitherto undescribed." Scient. Proc. Roy. Dublin Soc., N.S., vol. xiii, No. 35. 1913.

of development were not actually observed in the case of this fungus; but the final state of affairs which results is as follows:—The *antheridia* are subspherical or oval structures borne on the tips of hyphae, or apparently in some cases as sessile lateral outgrowths on them. The *oogonia* are pear-shaped structures, and are borne on different hyphae from those carrying the antheridia. The oospore is contained within the spherical portion of the oogonium, the lower, tapering, or funnel-shaped portion of which is *actually within, and is surrounded by the antheridium.* Hence the oogonium appears at first sight to be a spherical structure, sessile on the summit of the antheridium; but in reality it is pyriform; and its lower portion, which is within the antheridium, is continued out through the base or side of the latter, at which point it is continuous with the mycelium. As a rule, when an antheridium is present, there appears to be no transverse septum at the base of the oogonium where it joins the hypha which bears it.

An oogonium, containing an oospore, with its lower portion within the antheridium, is shown in the photograph reproduced in fig. 7, Plate XLV. In this case the hyphae bearing the antheridium and the oogonium respectively were removed during the dissection of the structure from the medium; but figs. 11 and 12, Plate XLVI, will illustrate more fully the connexion of the oogonia and antheridia, with their respective hyphae.

The course of events in *P. infestans* is in all probability similar to that in *P. erythroseptica*, in which the antheridium is formed first, as a lateral or terminal structure on the younger portions of the mycelium. The incept of the oogonium develops on a separate hypha, and enters the antheridium either at or near its base. This "oogonial incept," the top of which is sometimes slightly swollen, remains within the antheridium possibly for some little time—and perhaps fertilization may occur at this period—but gradually grows up through it, and finally breaks out through the top of the antheridium, when it swells out and produces the oogonium proper (i.e. the portion in which the oosphere is ultimately rounded off) in a comparatively short space of time. An oosphere becomes rounded off from a portion of the protoplasmic contents of the oogonium; and from it a thick-walled oospore, similar to that formed in other Peronosporaceae, is developed.

This method of development of the sexual organs is very unusual, and necessitates a revision of the genus *Phytophthora*. A discussion on this point will be found in the paper on *P. erythroseptica* just alluded to.

VI. General Conclusions.

The results obtained by us confirm the work of Clinton, and show that in pure cultures in certain artificial media, *Phytophthora infestans* does form

sexually produced spores or oospores. Whether, however, these spores are, *strictly speaking*, formed sexually or not—that is, whether an actual process of fertilization occurs or not—cannot be decided at present.

In the absence of antheridia, Clinton found that the oogonia did not do more than develop oospheres; but we have found in at least one-third of the cases examined that under such circumstances, both in Clinton's medium and in our own Quaker-Oat agar, oospores were produced; and we look upon such spores as having been formed parthenogenetically. These spores resemble those formed when antheridia are present, except that in many cases their walls appear to be slightly less thickened.

Even when antheridia are present it is difficult to see how the oosphere can be fertilized, for it is completely shut off from the antheridium by the funnel-shaped base of the oogonium, and no signs of a fertilization track have been observed. It is of course possible that a union of the male and female elements may occur soon after the entrance of the oogonial incept into the interior of the antheridium; but if fertilization occurs at this stage, it occurs *before* the formation of the oosphere, which would represent an unusual state of affairs.

Clinton was not able to trace the points of origin of the oogonia and antheridia, but states that they seem to arise on separate hyphae. Our observations show that this is actually the case, and moreover they explain Clinton's difficulty in finding antheridia, except such as were in contact with oogonia which were already well on in their development. Clinton states that the antheridia observed by him often show the *superimposed* "oogonial thread"; but we find that this structure, which is in reality the lower part of the oogonium itself, is actually within the antheridium, and not superimposed upon it.

Whether the fungus produces oospores in the potato-plant or not is a question which will have to be settled by further research. As stated before, we (as well as other workers) have found thick-walled spores in the tissues of various parts of the potato-plant, which have been destroyed by *P. infestans* which may possibly have been such bodies, although as a rule they appear to be smaller than the spores obtained in pure cultures. Many of them, too, have been seen to be surrounded by a kind of halo of brownish material which may possibly be the remains of the oogonium wall. If such bodies are produced in the potato-plant, they would doubtless find their way ultimately to the soil, and probably play an important part in keeping the fungus alive over the winter, and in causing infection of the potato-crop during the following season.

EXPLANATION OF PLATES.

All drawings were made with the help of a camera lucida under a Leitz microscope with objective 9 and ocular 2, giving a magnification of approximately 730 diameters, and are reproduced reduced to about two-thirds of the original size, viz. 486 diameters. Our thanks are due to Mr. H. A. Lafferty for the drawings (made under our supervision) of figs. 1, 2, 3, 11, and 12. The contents of the spores in figs. 7, 8, 9, and 10 are necessarily somewhat diagramatically represented. The reproductions of photographs in Plate XLV are from the original, untouched negatives.

PLATE XLV.

Fig. 1. Pure culture of *P. infestans* on Quaker-Oat agar (to which some lamp-black was added previous to sterilization in order to increase the contrast between the fungus and the medium), showing eight days' growth at room-temperature. Conidia were abundant. (Reduced.)

Fig. 2. Pure cultures of *P. infestans* on Oat agar slants in test-tubes. (Reduced.)

Fig. 3. Oogonia as seen embedded in the Quaker-Oat agar medium under low power of microscope (Leitz Objective 3, Ocular 2).

Fig. 4. A young spherical oogonium (with oosphere faintly visible) grown on Clinton's Oat-juice agar. × 625.

Fig. 5. A spherical oogonium (touching a hair from the Oat), after gentle pressure on the cover-glass has caused it to burst, showing the parthenogenetically formed oospore within. Grown on Clinton's Oat-juice agar. × 375.

Fig. 6. A pyriform oogonium burst open, with the liberated oospore. Grown on Clinton's Oat-juice agar. × 375.

Fig. 7. Oogonium and antheridium of *P. infestans* grown on Quaker Oat Agar. The oogonium contains a ripe oospore which nearly fills it; and the less dense central portion of its contents is faintly discernible. The funnel-shaped base of the oogonium within the antheridium is clearly visible; but the hyphae bearing the antheridium and oogonium are not present in this instance, having been broken away, in all probability, during the preparation of the specimen. × 730.

PLATE XLVI.

Figs. 1, 2, 3. Abnormalities or deformities seen in mycelium growing submerged in potato-juice sterilized by filtration through a Berkefeld candle. They are probably to be regarded as abortive conidial growths.

Fig. 4. An early stage in the development of an oogonium; no antheridium is present. (Quaker-Oat agar.)

Fig. 5. A "twin"- oogonium in which two oospores would probably have been formed. A distinct wall separating the oogonium from the hypha which bears it is present in this case. (Quaker-Oat agar.)

Fig. 6. An oogonium containing a young, parthenogenetically formed oospore. The lower limit of the wall of the oogonium is clearly seen, but there is no septum closing off the oogonium from the hypha which bears it. (Quaker-Oat agar.)

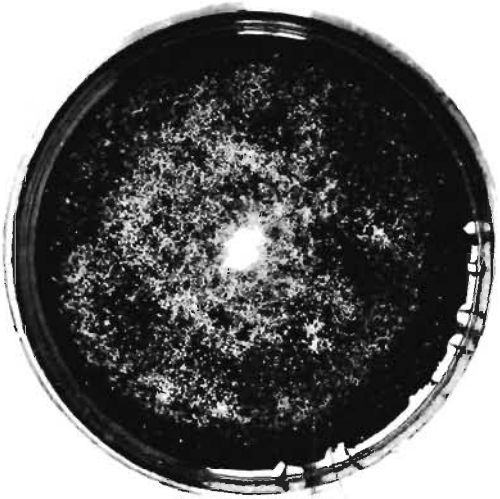
Fig. 7. An oogonium borne laterally on a hypha and containing a young pear-shaped oospore, formed parthenogenetically. (Quaker-Oat agar.)

Fig. 8. An oogonium (containing a practically ripe oospore) with its lower portion within an antheridium. The hyphae at the base of the antheridium are probably the oogonial and antheridial hyphae; but it was impossible in the preparation to determine this with absolute certainty. *og* = oogonium, *os* = oospore, *an* = antheridium. (Quaker-Oat agar.)

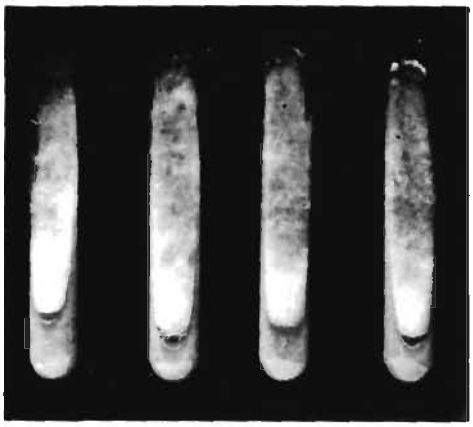
Fig. 9. An oogonium (with a practically ripe oospore) with its antheridium. The antheridium is probably a terminal structure borne on the hypha *a*; the funnel-shaped lower portion of the oogonium, within the antheridium, is probably continuous with the hypha *o* (at the back); but the connection could not be made out with absolute certainty. (Quaker-Oat agar.)

Fig. 10. An oogonium (with a practically ripe oospore) with its lower portion within the antheridium, which is a sessile structure on the hypha *aa*. The hypha bearing the oogonium was broken off during the removal of the adhering medium. (Quaker-Oat agar.)

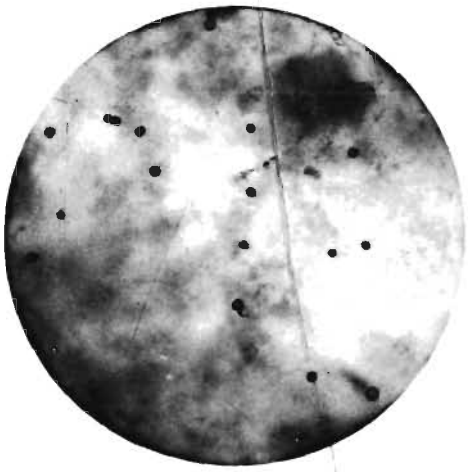
Figs. 11 and 12. Oogonium, oospore, and antheridium. The irregularities in the oogonium wall are indicated by shading (except over the oospore). In fig. 11 the antheridium is probably a lateral outgrowth of a hypha, the end of which is seen at *a*, the other portion of it being absent. *o* is the hypha which bears the oogonium, and it was definitely traced into the antheridium and seen to be continuous with the funnel-shaped base of the oogonium. In fig. 12, the antheridium is a terminal structure, borne on the hypha *a*; and its contents are represented somewhat contracted away from its walls. *o* is the hypha bearing the oogonium; its passage into the antheridium and continuation as the funnel-shaped base of the oogonium were clearly discernible. *m* is a small portion of adhering medium. (Quaker-Oat agar.)



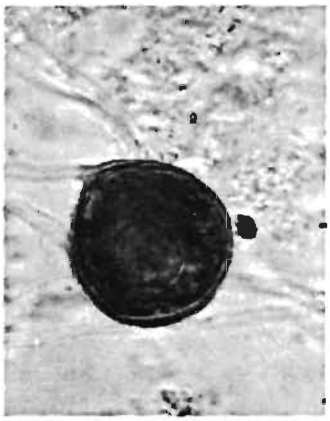
1



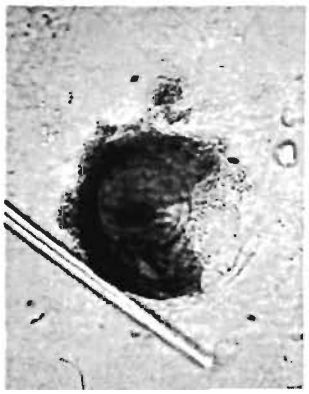
2

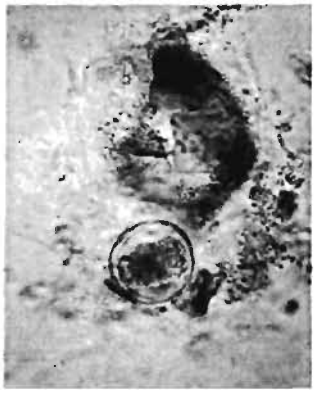


3



4

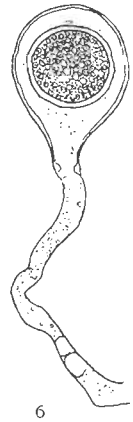


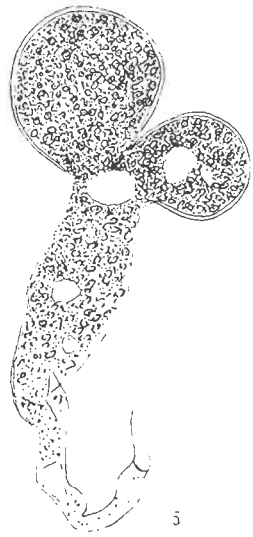


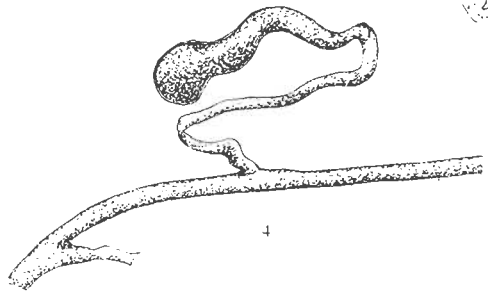

1

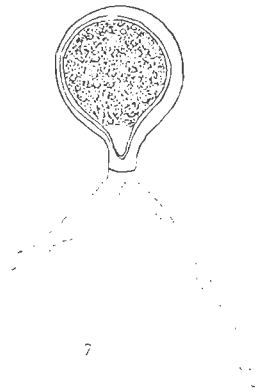

2

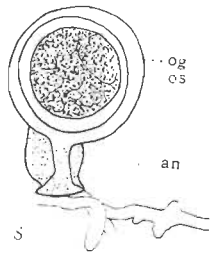

3


6

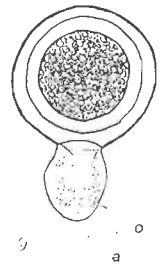

5


4


7



8



9