Illustrations of the Structure and Life-History of Phytophthora infestans, the Fungus causing the Potato Disease.

By

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With Plates XXXI and XXXII.

Some time ago I was commissioned to prepare for the Science and Art Department, South Kensington, a series of drawings illustrating the structure and life-histories of certain parasitic fungi; the intention being that these drawings should eventually be enlarged to form wall-diagrams suitable for museum and teaching purposes. I soon found that if the drawings were to be what I should wish them to be—i. e. to form a properly connected series, and to be taken from original preparations—the task promised to develope into one of greater magnitude than had been anticipated. In fact it became necessary to go over the entire field.

On the completion of the drawings from which the diagrams illustrating the phenomena in the life of the potato disease fungus (Phytophthora infestans) were to be selected, it was suggested to me that they form in themselves a series which would probably be welcomed as bringing together facts about this fungus in a connected form, whereas what other original figures exist are scattered through various foreign periodicals, &c., or are not easily accessible to students.

The text, it will be noticed, applies to the drawings throughout, any theoretical matters touched upon being referred to incidentally and in connection with points of structure, &c., in question. It has not been my intention to discuss hypothetical matters at any length, and hence the form of the descriptive letterpress.

Fig. 1 was drawn from a preparation made by cutting off the epidermis of the leaf at the margin of a disease-spot, where the tissues are as yet green, and the white aërial hyphæ of the fungus form the well-known velvety "bloom." Two stomata are shown, and a number of intervening cells of the epidermis. The stomata are very wide open, and each guard-cell contains chlorophyll-corpuscles and a nucleus (see the stoma on the right). The vertical boundary walls of the epidermis-cells are sinuous. From each stoma emerge branched aërial hyphæ of Phytophthora infestans, and their continuation into the leaf can be traced for a short distance. The aërial hyphæ branch considerably and produce the ovoid "conidia" at the tips of the branches, the tip often growing forwards and developing another "conidium" before the first one has fallen, and so on repeatedly, as indicated by the peculiar joint-like swellings (see fig. 3 for further explanation of this process). Septa are found here and there below the joint-like swellings; otherwise the hyphæ are not segmented. Conidia in various stages are still attached to the hyphæ, others have fallen or been knocked off during manipulation; such a fallen conidium is seen lying on the epidermis. The preparation was made from a perfectly fresh leaf, and examined in water.

Fig. 2.—This transverse section of a potato leaf was made across a similar part of the disease-spot, but passed somewhat closer to the dark brown part of the spot, where the cells are already dead; this is evident at the right hand of the drawing. The epidermis is very similar on both sides, the stomata being less numerous on the proper upper surface of the leaf. The mesophyll to the left of the preparation is still quite healthy and free from the mycelium of the fungus; the cells (seen in

optical section and in plan) are turgid, and the chlorophyll-corpuscles bright green and sharply defined. A fibro-vascular bundle consisting of a few vessels, wood cells, and soft bast, and surrounded by a sheath, has been cut across transversely; the palisade cells on its dorsal surface are arranged in a peculiar manner, and a depression in the epidermis of the upper side of the leaf corresponds with its course. To the right hand of the section the parasitic mycelium of Phytophthora infestans is rampant, though not as yet abundant in this younger portion of the disease-spot. The tubular, non-septate hyphæ, are seen in the intercellular spaces, but not in the cells themselves. Many of the cells are already losing their turgescence and the chlorophyll-corpuscles are becoming discoloured and misshapen, the protoplasm and cell walls eventually turning brown and becoming disorganised. The hyphæ in the intercellular spaces send branches through the stomata, especially on the lower side of the leaf, partly because that is more sheltered, and there is more moisture, and these branches become aërial hyphæ which again ramify and produce the conidia, as seen in the centre of the figure. The stoma to the extreme right is cut through transversely, but not in the median plane, and the tip of a hypha is already protruding through it; one hypha in the lacuna below has been cut across obliquely, and the extreme tip of another is just making its way outwards towards the orifice of the stoma. The stoma to the left of this is cut longitudinally, and the tips of two hyphæ are about to protrude through to the exterior; the stoma to the left, again, is also in longitudinal section, and two branched conidiophores have passed through and developed their conidia outside. The details of these are similar to those in the preceding figure. To the left of this stoma is one of the numerous capitate hairs, with yellow, oily-looking contents; and still further to the left one of the longer pointed hairs, the outer cell walls of which are dotted with minute papillæ. Similar minute papillæ exist on the outer walls of the epidermis-cells; the enlarged epidermis-cells surrounding the base of the hair are often much larger than in the specimen drawn. A stray hypha is to be seen between the cells of the spongy mesophyll close to the epidermis at this part: it is cut through. The dark grey cell below the smaller hair is filled with fine granular particles of calcium oxalate. Below and to the right of this cell a hypha is making its way in the spaces between the palisade cells to the stoma (in oblique longitudinal section) on the morphologically upper surface of the leaf. It will be noticed that the cell walls of the epidermis turn brown over the badly infected portions, but, as elsewhere, the discolouration and disorganisation do not result immediately on the access of the mycelium; the process is accelerated or retarded by wet or dry weather—possibly apart from the mere quantity of mycelium, though certainly not independent of its presence.

Fig. 3.—Portion of the tip of a branch of one of the conidiophores which had grown out from a piece of potato-tuber, lying in a drop of water beneath the microscope. The order of development is from *a* to *e*; the conidium marked * is the same throughout. In *a* the tip of the hypha is commencing to swell up, and soon becomes an ovoid body full of fine-grained protoplasm (*b*), still continuous with that of the conidiophore. Soon afterwards the now larger conidium presents a more granular clouded appearance, and its proximal or basal end is cut off from the hypha below by a septum, At its distal end a colourless papilla has appeared, due to the deliquescence and swelling of the cellulose wall at this point. In *d* this conidium is nearly ripe, and in *e* it has just fallen away by the rupture of the short, slender pedicle. Meanwhile, as the conidium referred to was approaching maturity, the hypha continued to grow by bulging out below the septum in *c*, and (in this case) to the right. This pushed the point of union between the above conidium and its hypha over to the left, and thus caused the displacement of the conidium in *d*. In the latter figure the new growing point of the hypha has already swollen up to form a new terminal conidium, which was in its turn displaced (to the left also), as shown in *e*, by a renewed growth, and so on. In each case a flask-like dilation of the hypha

marks the previous place of origin of a conidium, which had been thus developed and displaced.

Fig. 4.—In this drawing the germination of the conidium and of the zoospores to which it gives origin are represented. The conidium (*g*) was one of five which had been isolated in a drop of water, suspended in a damp chamber beneath the microscope. The sowing of conidia was taken from fresh, damp, diseased leaves, and made at 11.35 a.m., and the conidium (*g*) was then drawn. The contents were densely granular, and looked dark grey. The cellulose wall was sharply marked, and the pale papilla at the anterior end distinct, but not prominent. The short, broken-off pedicel was also evident. The first changes of note occurred at about 2 p.m., when the contents appeared much more translucent and watery, the dark, coarse granules being no longer obvious. This seemed to be owing to their having altered in character and become more translucent. The cell wall was also paler, perhaps because the contrast between it and the closely fitting contents was less marked. The apical papilla was thicker, and slightly more prominent. During the next two hours or so (2 p.m. to 4 p.m.) the peculiar hyaline granular contents were obviously undergoing changes, and very slow movements of the translucent granules occurred, producing variations in the faint cloudy markings shown in the drawings (*h* and *i*). Towards 4 p.m. certain vacuoles appeared, and at length became sufficiently distinct to draw (*i*). There were about nine of these altogether. The papilla also became more prominent, as if it was swelling up. At 4.50 it was obvious that each of the vacuoles formed the centre of an angular block of protoplasm, as seen in *k*. These angular blocks were separated by thin, sharply marked plates, and the whole mass was no longer close to the outer wall of the conidium, but a pale, watery looking line lay between them. The apical papilla was still more gelatinous in appearance. That these angular blocks were incipient zoospores was proved by what followed. Their arrangement appeared to be as follows—one at the apex, three in the next tier (two visible in the drawing), four in the next tier (three visible

in the drawing), and one at the base. The drawing was made at the higher focus.

Four minutes after the drawing of *k* was completed the papilla at the apex gave way, and the zoospores glided out as shown at *l*. They appeared to be quite passive, as if being pushed out from behind. Special attention was paid to the fact that they were squeezed through the narrow aperture. On reaching the exterior the zoospores did not immediately move away, but remained some seconds, as if hesitating, as it were; possibly the shock of meeting the water outside affected them. Two of the zoospores remained united for several seconds, presenting a superficial resemblance to conjugating amœbæ; after slight amœboid writhings they separated, but did not move out of the field. The zoospore (*n*) moved away briskly, but came to rest in less than a minute, and close to the now empty conidium, or zoosporangium, as it may be called.

The movements and changes of the other zoospores could not be followed, as attention was devoted to the specimen *n*, the further fate of which was followed, and noted as accurately as possible (see *o* to *z*). Owing to its movements being nearly circular, and its coming to rest close to the empty zoosporangium, it was easy to see the two cilia and two vacuoles (*o*) as the movements ceased. The zoospore then became rounded off and seemed to throw off its cilia—at least I saw one detached (*r*, *s*), and lost sight of the other one. The two vacuoles became smaller and soon disappeared, as if the zoospore in diminishing its volume squeezed out water. As far as it was possible to judge, these processes occupied one minute, and the zoospore had then come to rest as a spherical mass (*s*), which soon clothed itself with a recognisable but thin membrane.

It should be pointed out that the zoospore here followed moved for a very short time compared with others. I have frequently seen the zoospores still active twenty minutes or more after emergence, and they are said to move even longer. At the same time they often come to rest in from one to five minutes, and sometimes only give one little flirt and then come to rest.

The spherical resting zoospore remained unaltered, to all appearance, from 4.56 till 6 o'clock, but soon after that was seen to be putting forth a protuberance (*t*) (6.14 p.m.), which soon elongated into a hypha (*v*) (6.34) as long as the diameter of the zoospore. At 7.10 this germinal hypha was twice the diameter of the zoospore in length, and a large vacuole had formed in the rapidly emptying zoospore (*w*). This vacuole occupied nearly the whole of the cavity at 7.50—in other words, the protoplasm had nearly all passed into the developing germinal hypha (*x*). A tiny protuberance on the hypha also indicated an incipient branch, which, however, did not attain any considerable length, and soon became emptied. At 11.20 p.m. the state of affairs was as shown in the drawing (*z*). The whole of the protoplasm was in the apical one fourth or one fifth of the germinal tube, the rest being empty like the zoospore, and having three very thin septa across at pretty equal distances. Whether these septa are really cellulose walls it was impossible to determine. Next morning there was no appreciable change, and the protoplasm seemed to be dying towards evening.

The other four of the five conidia sown did not develope zoospores; two of them germinated directly in the manner shown in fig. 7. The development of the zoospores is delayed or even arrested by direct daylight, even if not very strong, and it is not improbable that in the present case the formation of the zoospores was arrested in the other conidia by the repeated and continued exposure of the preparation during the observations.

Fig. 5.—In this drawing two zoospores are represented germinating on the epidermis of a potato leaf, and one has become rounded off, but has not yet put out a germinal tube. The preparation was obtained by painting the lower surface of a fresh leaf with a camel-hair pencil dipped in rainwater and then passed over freshly-developed conidia at the margins of disease-spots on other leaves. At the end of twenty hours the leaf was examined, and numerous zoospores were found on the epidermis, many of which had put forth germinal tubes which

were entering the leaf. The drawing is combined from two preparations to save space, the zoospore which is putting forth a tube through the stoma having been observed on a different part from the other—in other words, instead of only one cell intervening between the stoma and the lower of the two germinating zoospores, there were very many. The zoospore close to the stoma simply protrudes its tube into the orifice. The one lower down has germinated on an epidermis-cell, and the tip of the tube at once commenced to bore through the outer wall; once inside, the germinal tube swells up and has in this case branched. The empty remains of the zoospore and part of the tube are left outside. The chlorophyll-corpuscles are shown in the guard-cells, but the nuclei are omitted.

The germination of the conidia on the living leaf is often very rapid, and may certainly take place in two hours after the sowing was made. I could not satisfy myself that it is affected by light, as is that on glass slides. It is accomplished readily during the night, on leaves kept wet under bell-jars. The results seem to be more satisfactory if rainwater is employed, in preference to well-water; and the same is true of experiments on glass slips. It is improbable that temperature was the important factor in these differences, possibly the oxygen present in the rainwater was of more significance.

It occasionally happens that a zoospore germinates in an angle of the venation of the leaf, and sends its germinal hypha through the epidermal cells of the rib or "vein" lying at its side; in such cases an optical section of the tube and zoospore can be obtained, but the best proof of the entry of the germinal tube through the wall of the epidermis-cell is obtained as follows.

Fig. 6.—The preparation is part of a vertical longitudinal section of a young internode of the potato plant, and shows a stoma in longitudinal but not quite median section, and to the right a germinating zoospore, the germinal hypha of which has pierced the cuticle and cell-wall and is growing on inside the epidermis-cell. The method adopted was to sow large quantities of the conidia on one of the flat sides of the tetra-

gonal, winged internodes of the potato plant; several such preparations were then laid with the sowings upwards, on damp blotting paper, in soup-plates covered with bell-jars, and the air kept damp. After twelve hours or longer, sections were cut longitudinally vertical to the flat places on which the sowings were made, and the section examined. It was not difficult to obtain evidence of the germination of the zoospores, and entry of the germinal hyphæ, but it was only after many weeks that I succeeded in preparing the really satisfactory case here drawn. The razor had passed close to but not through the germinal tube; the empty zoospore and first part of its germinal hypha are seen lying close on the exterior of the cell wall—the zoospore had come to rest in a slight depression at the junction of two cells—the very fine hole through which the germinal tube passed was clearly visible on focussing. On reaching the anterior of the cell the hypha thickened considerably, and passed along the roof of the cell, and was just about to turn and run down the vertical wall (or possibly to bore through it) where the section was made. The sub-epidermal cells frequently contain a crimson-coloured sap, but none of the cells in the preparation were so coloured.

Figs. 7 and 8.—When the conidia of Phytophthora infestans are sown in water on glass slips, they frequently assume the appearances figured at figs. 4, *g*, *h*, and *i*, after a few hours, and then cease to develope further in the direction of producing zoospores. Instead of doing that, the cloudy, vacuolated condition of the protoplasm which usually heralds the development of the zoospores (fig. 4, *i*) is again replaced by the more uniformly hyaline appearance of the earlier stage (fig. 4, *h*), and the papilla either commences to grow out as a hypha, or a protuberance from it (fig. 7) does so; occasionally, but rarely, this hypha branches, as in fig. 8. This germinal hypha, the development of which stamps the conidium as an ordinary spore (in contrast to its behaviour in other cases as a zoosporangium) elongates considerably, and in some specimens attains a length equal to ten times that of the conidium; its apex then dilates into an ovoid body much like the conidium from which it originated (fig. 7).

In one instance (fig. 8) this took place at the end of each of the two branches. The protoplasm of the original conidium passed entirely into the hypha, and along it (fig. 7) wholly into the new or secondary conidium, which is usually somewhat smaller and sometimes much smaller (fig. 8) than the primary conidium. One or two fine transverse septa may be formed in the germinal hypha (fig. 7). As a very general rule the secondary conidium is oblique or misshapen; fig. 7 was an exceptionally symmetrical one.

The conditions which determine this mode of germination, in preference to the formation of zoospores directly, are not quite clear; but they seem to be connected with the nutrition of the germinating conidium. When the sowings of conidia are exposed to light these secondary conidia are often formed; and I found it more difficult to obtain the zoospores in well-water than in rainwater. I here speak more especially of water from a particular well, which has proved to contain considerable quantities of organic matter. In large sowings, i. e. where eighty to hundred or more conidia existed in the drop of water, by far the majority of the conidia germinated in this manner; and wherever the germination was delayed from obscure causes beyond twelve hours, this was the prevalent form it assumed. Sowings in very dilute infusions of organic matter (jam and horsedung were tried) never yielded zoospores, whereas several conidia would germinate like this. When large quantities of the conidia were sown on a small area of the potato leaf or stem, a larger portion of them germinated thus (see fig. 9, below). No connection was established between differences of temperature and of mode of germination. Putting all these facts together, it seems not improbable that the difference is due to nutrition, and possibly three factors were concerned in affecting this, (1) the amount of free oxygen available, (2) the comparative maturity of the conidia themselves, and (3) the intensity of the light. It is not inconceivable that direct sunlight increases the oxidizing processes during the early stages of the germination; and I feel convinced that the presence of numerous competing conidia, or of organic matter generally, affects the germination by influencing the amount of oxygen available for any one conidium. I am satisfied that it is easier to obtain zoospores in dewdrops on the living leaf than in water on glass. Nevertheless, it must not be overlooked that the zoospores will develope on a wet leaf during the night, including the early hours of the morning. There can be little doubt that an interesting field of investigation in comparative physiology is here open.

As to the significance of the secondary conidia and their formation, I have found them empty, as if they had developed zoospores which had escaped; but have never seen zoospores come from them. I have also seen a secondary conidium with what looked like a germinal hypha developed from it; but this died before developing very far, so that it was impossible to say whether a tertiary conidium could be developed. I have not seen these tubes enter a stoma. The secondary conidium would seem to be a second attempt on the part of the zoospore-producing protoplasm to prepare for the development of zoo-spores again; there is a loss of substance from respiration, and the cellulose of the hypha has to be formed, and the energy expended is no doubt evolved at the expense of materials in the protoplasm, and not replaced by nutrition.

Fig. 9.—This drawing was from a preparation of the epidermis of an internode on which several of the crowded conidia had germinated as above. The drawing is to scale. As usually happens in such cases as this, the germinal hypha is shorter than those developed in hanging drops, and the secondary conidium oblique.

Fig. 10.—The preparation shows hyphæ of the parasite in the cortical tissues of the internode three days after infection. The chief point of interest is the course of the hyphæ, they run between the thin-walled, closely-fitting cells, in the middle lamellæ, and even push aside the other layers of the cellulose walls; the diameter of the hypha is considerably greater than that of the walls they traverse. The same thing occurs in the tuber (see fig. 15, below). The hyphæ branch often; they are devoid of septa and have very thin walls and abundant finely

granular protoplasm. In the preparation drawn the hyphæ are particularly luxuriant and thick, the diameter varying, however, in different parts of their course. Several interesting questions here suggest themselves. How are these hyphæ nourished in the "middle lamella"? Is any protoplasmic substance present? Processes are very rarely sent into the cells of the cortex; the branches in the upper part of the drawing are running over the cells and in the middle lamella, between the cell which is drawn and one that would have lain nearer the observer. The longitudinal hyphæ often run for some distance in the line of junction between three cells. The cells contain pale chlorophyll-corpuscles; a nucleus is present in the one to the left, and a few crystals lie in the large cell to the right of it.

Fig. 11.—A potato-tuber was sliced across, and the mycelium of Phytophthora, from a diseased potato planted on the clean cut. The preparation was then put aside, and was not kept particularly damp. On examining the sections cut vertically to the cut surface, some time later, the mycelium was seen attacking the tuber, as in the drawing. The cut surface of the wound (at the top in the drawing) had undergone partial healing by means of tangential divisions of the exposed cells (the rotten remnants of the cut cells were destroyed), and the two or three tiers of cells thus cut off had become cork-like. The contents of the cells were removed to a large extent in making the preparation. The protoplasm or its remains turns coarsely granular, and eventually rusty brown; the starch-grains are dissolving, remains of the nuclei are found as opaque, coarsely granular masses, and here and there a crystal is noticed. The mycelium is strictly intercellular, the branches running between the cells in the narrow lacunæ, or in the substance of the walls. Eventually the cell walls soften and swell, and split apart, and finally turn rusty red and decay.

Figs. 12 and 13.—Cells of a diseased potato in a somewhat more advanced condition. In addition to the changes above referred to the cell walls are now swelling, and the cells separating from one another at the middle lamella. In the preparation from which fig. 13 was drawn it was easy to see the remains of the protoplasm as a sort of matrix in which the starch-grains (some partially eroded) were embedded. The nucleus and proteid seem to be destroyed long before the starch, and it is even doubtful whether the starch is directly attacked at all until bacteria gain access, and hasten the decomposition. In fig. 13 starch grains are seen to be displaced from the matrix in which they were lying.

Fig. 14.—Portion of mycelium from such a preparation as the last. The two branches running across from the vertical ones were passing along the surface of a cell, and a brownish tinge was given to the cell wall in the immediate neighbourhood. The same is seen in fig. 15, *a* and *b*. The hypha corrodes the walls, as it were, in its immediate neighbourhood: the rest of these cell walls were as yet not coloured.

Fig. 15.—Portions of hyphæ in the middle lamella between the cells of the potato tuber. The corrosive action of the hyphæ is indicated by the rusty hue which they cause the cell walls to assume.