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On the Tubercular Swellings on the Roots of Vicia faba

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XVIII. *On the Tubercular Swellings on the Roots of* Vicia Faba.

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[Plates 32, 33.]

It has long been known that the roots of the Leguminosæ are commonly provided with peculiar tubercle-like swellings of various sizes, from that of a mustard-seed to that of a hazel-nut, and attention has been repeatedly directed to them of late years. They were observed by Malpighi, who seems to have looked on them as of the nature of Galls,* and Treviranus regarded them as undeveloped buds, while A. P. DeCandolle considered them as diseased structures. Since that time very various ideas have been published with respect to them, and as to their origin and relation to the roots which bear them. One of the most curious facts about them is that, although it is very difficult to find a specimen of our ordinary Leguminosæ (Clover, Lucerne, Beans, Peas, Vetches, &c.) the roots of which are free from the swellings, no one has succeeded in showing that they do any injury to the plant: this has been repeatedly employed as an argument against their being due to the influence of any parasite. The contrary opinion has gradually gained ground, however, and I am now in a position to prove conclusively that it is the correct one.

The first close investigation of these root-tubercles (as they may be shortly termed) is due to Woronin, who, in 1866,† examined in detail the structure and contents of the similar swellings which are to be found on nearly every Alder, as well as those on the roots of the Lupin. In the cells of the Alder tubercles Woronin found a curious little Fungus, which was referred to the genus *Schinzia*, founded by Naegeli on a form which he himself discovered in the roots of *Iris*‡ in 1842. In the cells of the tubercles of the Lupin, Woronin found multitudes of minute corpuscles, which he took to be Bacteria, or Vibrios, or organisms of that kind.

Similar swellings have from time to time been observed on the roots of various

* 'Botan. Zeitung,' 1874, p. 382; and Sorauer, 'Pflanzenkrankheiten,' vol. 1, p. 744.

† 'Mémoires de l'Académie des Sciences de St. Pétersbourg,' vol. 10 (May, 1866).

‡ 'Linnæa,' vol. 16, 1842 (Plate xi., figs. 1–10).

other plants, those on *Cyperus flavescens* and *Juncus bufonius** being the best-known. Those of *Juncus* are especially interesting, because they have lately been shown to contain a parasitic Fungus belonging to the group Ustilagineæ.† The swellings on the roots of *Orobanche* also contain a Fungus, referred to by SCHACHT‡: those of *Sonneratia* and *Taxodium* are not (so far as I can discover) so well known; although there is still so much that is doubtful about them, they probably differ in character from the others.

Mention may be made of the fungi long known in the roots of Orchids, but not causing tubercles of the kind referred to: some of them at least are now known to belong to the genus *Nectria*.§ These, and other root-fungi, need be no further regarded here, however; nor is it necessary to speak of other root-tubercles.||

In 1878 WORONIN published his celebrated memoir on *Plasmodiophora Brassicæ*,¶ a Myxomycete which causes the long-known hypertrophies (Club-foot, Hanbury, Fingers and Toes) on the roots of various species of Cruciferæ. It is important to notice this paper, because it seemed to throw an entirely new light on the general question as to the causes of swellings on roots, and at any rate it was the forerunner of several suggestions.

Confining our attention to the swellings or tubercles on the roots of the Leguminosæ (and particularly the Papilionaceæ), as already stated, their more exact study was initiated by WORONIN, who examined those of the Lupin, and found the cells in the interior of the swellings filled with a slimy colourless matrix in which Bacteria-like granules were embedded: the "Bacteria" were found to increase, in proportion to the rest of the matrix, as the section receded from the growing apex of the swelling. In a paragraph at the end of his paper on *Plasmodiophora*,** in 1878, WORONIN practically retracted his previous opinion that the above granular bodies are really Bacteria or Vibrios, and regarded it as highly probable that a *Plasmodiophora*, or similar parasite, would be found to be the exciting agent.

Meanwhile, in 1874, ERIKSSON had published a masterly investigation of the occurrence, form, structure, and growth of the swellings on the roots of Leguminosæ.†† Their forms differ on the roots of different species, but are remarkably constant in the case of any one species. Perhaps the most striking fact about them is their ubiquity, confirmed subsequently by FRANK.‡‡ They are found on the roots of species of all the

* MAGNUS in 'Hedwigia,' 1878.

† C. WEBER, 'Botan. Zeitung,' 1884 (No. 24).

‡ Reference in FRANK, 'Krankheiten der Pflanzen,' p. 653.

§ WAHRLICH in 'Botan. Zeitung,' 1886 (Nos. 28, 29).

|| Such as those in which nematoid worms, &c., are found, for instance, and the gall-structures caused by various insects.

¶ 'PRINGSHEIM, Jahrb. f. Botan.,' vol. 11, 1878, p. 548.

** *Loc. cit.*, p. 571.

†† "Studier öfver Leguminosernas rotknölar," Lund, 1874. See 'Botan. Zeitung,' 1874, p. 381.

‡‡ 'Botan. Zeitung,' 1879 (Nos. 24, 25).

genera, and in all parts of the world, as the examination of herbaria shows: on heights and in valleys, in poor soil and in good land, wet or dry, they may almost invariably be met with.

ERIKSSON found that in *Faba vulgaris* the young tubercle-like swellings arise in the cortical layer of the main root, or of a lateral rootlet, and without reference to the position or age of other parts. Although the development of the tubercle resembles that of a rootlet in many respects, its origin has no constant relation to the xylem and phloem strands—sometimes it begins to develop between the strands, or opposite one or other of them. Although the pericambium joins in the development subsequently, the origin of the swelling is outside the plerome cylinder, and is first evinced by active irregular divisions of the innermost cells of the periblem.

ERIKSSON observed that the inner cells of the swelling contained the vibrio-like bodies described by WORONIN: in the outer part of the swelling he occasionally noticed Fungus hyphæ proceeding radially inwards from cell to cell, and becoming branched and finer as they go inwards. ERIKSSON saw no hyphæ in the cells containing the vibrio-like bodies: he noticed also that the latter are not always simple rod-like bodies, but oftener forked, &c. He left it open whether any connection exists between the hyphæ and the vibrio-like bodies.

In 1878 KNY asserted* that the tubercular swellings on the roots of Leguminosæ do not appear on plants cultivated in nutritive fluids, and expressed the opinion that this was because some parasite which causes the tubercles is absent from the fluids—in fact, is evidence in favour of the parasitic nature of the swellings. KNY also stated that he found a *plasmodium* in the dividing cells of the tubercles, especially in *Cicer*: he states that the parasitic plasmodium can be followed from cell to cell in the form of delicate, sparsely branched strands. Where they traverse the dividing cellulose-walls the plasmodial strands are slightly thickened (*loc. cit.*, p. 55).

Then followed the Paper by FRANK,† in which he confirmed and extended ERIKSSON'S statements as to the ubiquity of the swellings on the roots. FRANK states that the greatest care is necessary to prevent the development of the tubercles, that they occasionally occur on water-cultures as well as on roots in any soil. They did not appear on roots in soil which had been heated, though every plant in soil not so treated had them. FRANK concluded that the roots are infected from outside by a "germ" which must be as ubiquitous as the germs of putrefactive and fermentative organisms.

In the cells of the swellings there are always‡ two elements to be found, (1) fine hyphæ running across the lumina of the cells and through their walls, (2) minute cell-like corpuscles suspended in the protoplasm of the cell as in an emulsion.

That the hyphæ are the same as KNY'S plasmodial strands is almost certain, and

* 'Sitzungsber. d. Botan. Vereins d. Prov. Brandenburg,' 1878 (April 26), p. 55.

† 'Botan. Zeitung,' 1879 (Nos. 24 and 25).

‡ Except in those of the Lupin, p. 396, *loc. cit.*

they are undoubtedly the same as those found by ERIKSSON. FRANK also found the hyphæ here and there in cells containing the minute corpuscles (these corpuscles are the "vibrios" or "bacteria" of WORONIN and ERIKSSON): these corpuscles are cells, and not mere granules; their form varies; some are rod-like or curved, or shaped like a finger-biscuit, others Y-shaped, and so on. They seem to increase by budding, but this is assumed only. They have no proper movement, as WORONIN supposed.

The hyphæ are thicker than the above corpuscles, and grow through from wall to wall across the lumina of the cells. They may end in the cavity, and in any case are rarely traced through more than two cells or so. FRANK and SCHENK both regarded certain short branches in the cells as haustoria.

FRANK then goes into the question as to the continuity of the hyphæ and the bacteria-like corpuscles. Are there two parasites present in the swellings? Without actually deciding the point, FRANK has little doubt that the corpuscles are budded off from branches of the hyphæ.

No results were obtained on trying to cultivate the corpuscles beyond the putting out of a fine hypha from either end in one or two unsatisfactory cases.

FRANK regarded the parasite as allied to that described by WORONIN in the Alder, and named it *Schinzia leguminosarum*.

KNY* replied to FRANK'S Paper that he regarded the corpuscles as the spores of a plasmodium; the strand-like extensions of the latter show no membrane, and abut on the wall with curious funnel-like widenings—this is clearly the case in FRANK'S figures. SCHWENDENER is also quoted as viewing the strands as membraneless plasmodial strands. KNY also states that DE VRIES and others have seen the tubercles on the roots of water-cultures.

This appears to be a satisfactory account of the position of our knowledge of these curious structures up to date: it resolves itself into the following. While it is certain that some organism or other exists in the tubercles, no observer has cleared up the question as to whether there is one constant Fungus present, or whether it is a plasmodium which causes the hypertrophy, or whether two forms coexist in the cells: it is also uncertain how the parasite (if there is only one) enters the tissues—whether from the soil into the root, as seems so probable from ERIKSSON'S and FRANK'S researches, or whether it is already present in the seed. The latter is a more improbable suggestion, and we may dismiss the idea that no parasite exists at all, in spite of the doubts implied in SORAUER'S statement on p. 744 of his book, and of the very general belief to the contrary which exists among agriculturists especially. In any case, however, no one had as yet succeeded in infecting the roots, and producing the tubercles artificially. I have done this, and may now proceed to describe my own observations and experiments.

I first commenced the microscopic examination of the tubercles in 1883, but, although the investigation was renewed each year since, the really important additional

* 'Sitzungsber. d. Botan. Vereins d. Prov. Brandenburg,' 1879 (June 27), p. 115.

results were only obtained recently. The chief investigation has been almost entirely confined to the tubercles on *Vicia Faba* (L.), the common Broad Bean, no particular regard being paid to the garden varieties employed.

At the same time it should be stated that I have seen and to a certain extent examined them on the roots of several Clovers,* Peas, Vetches, and the Scarlet Runner in this country, on Broad Beans in Germany, and on the roots of several Papilionaceæ in Ceylon, and can to a certain extent confirm the much more extensive observations of FRANK as to their ubiquity and general resemblance. In Manchester it was by no means rare to find the tubercles on the roots of *Vicia Faba* as large as a small Hazel-nut, and I have had specimens even larger from the damp heavy soil of some districts. In the light sandy soil of this part of Surrey I have not seen them much larger than a fair-sized pea, though they are often very numerous and crowded on the roots.

I have examined many hundreds of roots of *Vicia Faba* during the last four years, and have only once or twice failed to find the tubercles on plants in fruit, and even in the case of younger plants the percentage of failures has been small.

In 1884, at the Owens College, Manchester, several beans grown in pots of burnt soil, and watered with solutions of nutritive salts, developed no tubercles on the roots; and in the majority of cases, then and since, beans grown for laboratory purposes in carefully-prepared nutritive solutions have been devoid of the tubercles. Nevertheless, this has not always happened, and in some instances the water-cultures have developed excellent specimens of the tubercles. Considering that these were growing in solutions of chemically pure salts in distilled water,† it is hardly to be wondered at if one sometimes doubted the existence of an external cause for the swellings, and felt tempted to believe that either the tubercles were really due to the roots themselves, or that if a parasite exists it is present in the seed from the first. The possible sources of infection in these experiments were (1) germs in the air, (2) germs attached to the testa of the Bean, (3) the medium (damp sand or sawdust) in which germination was commenced. Although it was not demonstrated, I thought the second a more probable source of error than the others. I have this year found the tubercles on a bean which was germinated in "clean river-sand" not heated, and then grown as a water-culture—everything hereafter chemically pure, and in a new building. Of course it is not impossible that a germ might fall into the culture; but it seems much more likely that the infecting agent was attached to the seedling before its roots were placed in the solution of nutritive salts, and possibly came from the sand. In any case, whether the cause of infection is in the medium or on the Bean, it must be very minute and ubiquitous, and the results quite bear out FRANK's comparison with the minuteness and ubiquity of putrefactive and yeast germs. In

* Those of the Lupin may be distinct.

† It should be stated that they were exposed to the air, however, and that no regard was then paid to the medium in which they were allowed to commence to germinate.

any case, again, it seems clear from further research that the infecting agent is present in the soil practically everywhere, and it is by no means difficult to suppose that it might attach itself to a seed harvested in the ordinary manner if the roots and soil are disturbed so that germs could be scattered.

The tubercles are formed without any order on the tap-root and lateral roots, the only apparent rule being that they are not developed until the young root-system is fairly advanced,* and they do not arise close to the apex of the root: they seem to especially affect the region where the root-hairs are in full vigour. There may be only a few, or very many, crowded in groups or scattered (fig. 1).

They are at first very small, and much like young rootlets in appearance; but they soon swell, often very irregularly, and may become lobed in various ways. Their colour and texture are quite like those of the rest of the root.

A longitudinal section (figs. 2, 3, 7) through the tubercle shows the structures described by ERIKSSON. The axial chief mass of the tubercle consists of rather large polyhedral parenchymatous cells, passing at the apex into smaller, closely packed, thin-walled cells which constitute a meristem, which would be homologous with the growing-point of a rootlet: several layers of more compressed parenchymatous cells envelope the above tissues, and may be looked upon as a periblem (fig. 7). There is no root-cap.

A short distance from the meristem, rows of cells at the boundary between this periblem and the axial (plerome) cylinder gradually pass over into vascular strands. Perhaps the outer layer of the periblem mantle may be regarded as the homologue of an epidermis and root-cap, but the resemblance is not very obvious. In the lobed or convoluted older tubercles (figs. 1, 2) all these tissues take part in forming the lobes.

It is in the large-celled axial tissue that the parasite is rampant (fig. 3), and the cells which contain the densely crowded corpuscles (figs. 4, 5, 12) are seen on the section as slightly pinkish or buff-coloured masses sending ramifications into the various lobes (fig. 2).

Any cell at the base of this mass may be seen to contain a densely granulated mass of substance (figs. 4, 5), which swells in water, and allows the corpuscles to escape passively, but with the well-known dancing Brownian movement. The separated corpuscles are very brilliant, and vary in size and shape; some are rod-shaped, others have the form of a Y or V, and others are still more branched, as described by FRANK. (Fig. 6.)

These corpuscles are deeply stained by hæmatoxylin, and become yellow-brown in iodine; in chlor-zinc iodine the mass of corpuscles turns bright golden-yellow, the cell-walls of the parenchyma containing them turn blue. All their reactions point to the accuracy of the previous ideas as to their nature; they are unquestionably organised bodies.

I have repeatedly examined them in sections of old dried tubercles gathered the year before. If kept dry during the winter, the tubercle shrinks considerably, and

* Usually when the seedling is about three to four weeks old, if growing luxuriantly.

slowly becomes very hard and wrinkled, turning dark-brown or almost black in the process; it is not easy to cut such tubercles. Their consistency is that of stiff horn, and the razor "drags" unpleasantly in the section. Thin sections placed in water swell for several days, and the contents of the cells are, as before, densely crowded brilliant corpuscles in a matrix, which is bright yellow in chlor-zinc iodine. These corpuscles are particularly minute, and like mere points in the section.

In the cells containing these corpuscles the presence of hyphæ is to be observed (figs. 12 and 15–18); even in the cells of tubercles which have been dried for a year, and are hard as horn, a few minutes' maceration in very dilute ammonia enables one to detect these hyphæ (fig. 18), which are obviously those described by FRANK. These hyphæ are very curious. In the cells filled with corpuscles they are short, often much branched, extremely delicate, and apparently springing from the cell-walls, though really coming from hyphæ running in and through the substance of the cell-walls (figs. 12, 17).

Sections through young and actively growing tubercles show that the hyphæ branch and pass from cell to cell throughout the meristem of the interior (figs. 7–18). In very young tubercles the cells contain only these hyphæ; subsequently, when the tubercle reaches the dimensions of a mustard-seed, the tiny bacterium-like bodies begin to accumulate.

In sections through very young tubercles, made transversely to the long axis of the root, and passing axially through the tubercle, I have observed the following facts. A fairly strong hypha, several times thicker than the cell-walls in many cases, can be traced through from the epidermis to the origin of the young tubercle (figs. 7, 8, 9); *the tip of the tubercle is always directed so as to meet this hypha.*

I had frequently satisfied myself of this fact, before more fortunate preparations showed the facts explained by figs. 9 and 13. Here, as is seen, the hypha referred to passes down the cavity of a root-hair, and thence traverses the cortex of the root, cell by cell, beginning to branch when it enters the mass of tissue which constitutes the young tubercle. It is more difficult to see the branches in the meristem of the tubercle, for two chief reasons: the cells are smaller and more numerous, and their walls are very thin. Moreover, their protoplasm and nuclei are very bright. Nevertheless the difficulty is only relative, and, as already stated, FRANK had already seen the hyphæ passing from cell to cell inside the tubercle, though he did not trace them far.

The isolated thicker hypha in the epidermis and cortex (figs. 8, 9) offers more distinct characters than the finer ramifications which it makes further inwards. The hypha is without septa, so far as can be made out by reagents of all kinds. It has a very delicate membrane, which is quite distinct in specimens treated with osmic acid or with chloral hydrate, or hardened in alcohol, &c. It passes straight across the lumina of the cells, through wall after wall, on its way towards the centre of the root; but a curious and very characteristic feature is the trumpet-like enlargement of the hyphæ at the spots where they pierce the walls (figs. 9–14). This has been noticed before and is figured by FRANK, and is also a character of the mycelium found in *Juncus bufonius*, and named by WEBER* *Entorrhiza*. In suitable preparations the hypha may be seen to swell up inside the substance of the cell-wall, and it looks as if the widening was due to the cellulose wall itself (fig. 14). Two possibilities suggest themselves: the swelling might be due to increased nutrition—a less probable view; or, as I think more probably, the cellulose wall extends by the growth of the cell after the hypha has pierced it, carrying the insertion of the hypha with it as its area increases. As the tubercle becomes older the hyphæ in the cortex of the root turn yellowish and gradually decompose, so that no trace of them can be detected in the larger tubercles which have broken through the cortex of the root.

In the cells of the very young tubercle the finer branches of the above hypha can be seen behaving similarly as regards their passage through the walls and across the lumina; and, although they become too fine to enable the observer to decide as to the presence or absence of a cell-wall and septa, it may no doubt be assumed that the characters are essentially the same. It is often possible to see the hyphæ running in the substance of the cell-wall (figs. 9 and 9A). In one respect these more ultimate hyphæ differ, however; they send out branches which end blindly in the cavities of the cells (figs. 15–18). These branches may be simple, or they may have several rounded or tufted bodies projecting from them, and looking like haustoria. The surface of these haustorium-like projections is often found presenting the appearance shown in figs. 16 and 17. Numerous very minute protuberances stand off from the rest of the mass.

In very thin fresh sections of tubercles, about the size of a mustard-seed or smaller, and which are only just beginning to project markedly from the root, the projections just referred to are very numerous, and every cell of the inner meristematic mass of the tubercle seems to be provided with the branches bearing them. A change is also noted in the cell-contents in these cases. In place of the normal-looking protoplasm of the cells in the earlier stages, the protoplasm now becomes extremely vacuolated and frothy, and the tiny bacterium-like corpuscles referred to are found to be gradually increasing in number in the cells (fig. 12). At a slightly later stage these bacterium-like corpuscles have become distributed in dense crowds throughout the frothing mass of protoplasm, and they become so numerous that they obscure the hyphæ, and the appearance is that of a plasmodium, gradually becoming more and more densely filled with granules. At length, the enlarged cell is seen to contain a dense mass of the granules arranged around a large central vacuole (figs. 12 and 4). The nucleus remains in the protoplasm. So dense is the mass of granules in the protoplasm at last, and so sharply defined the vacuole, that extremely thin preparations hardened in osmic acid or picric acid, and then in absolute alcohol, can be washed and stained with hæmatoxylin and mounted in Canada balsam in the usual way, without destroying this arrangement (fig. 4).

* 'Botan. Zeitung,' 1884 (No. 24).

The next question is, what are these bacterium-like corpuscles, or granules, and how do they arise and thus crowd the protoplasm? They seem to be budded off from the projections of the hypha in the cells: although I have failed to see an undoubted case of the actual formation and separation of one of these minute corpuscles, still there is not much room for any other view of their origin in the face of preparations such as yielded figs. 12; 15, 16, and 17. These extremely minute bodies are, according to my view, budded off in large quantities from the hypha.* They then multiply further *by budding in the cells*. This explains their shapes, which are Y and V-shaped, or branched still more, in the younger states, but more uniformly ellipsoidal when older, and also accounts for their rapid increase in numbers.

The bacterium-like corpuscles are thus to be looked upon as extremely minute, elongated, yeast-like cells or gemmules, and it is not difficult to suppose that their sudden and rapid increase in the juices of the cell would give rise to the vacuolation of the protoplasm. It seems impossible to doubt that the so-called plasmodium seen by various observers in these tubercles is really the protoplasm of the cells themselves, *stimulated into increased activity by these parasitic gemmules*.† In any case this seems a fair explanation of the fact that the protoplasm of the cell becomes exceptionally frothy and vacuolated, and the cell itself enlarges quickly, coincidently with the appearance and rapid increase of the tiny gemmules. At this time, also, vigorous specimens have these cells abundantly supplied with starch at the period when the above increase in size and coincident multiplication of gemmules occur (fig. 12). The only explanation seems to be that the parasitic gemmules and hyphæ are stimulating the protoplasm to greater activity, and thus making the cell act as a centre of attraction for the plentiful supplies of carbo-hydrates sent down from the leaves, and other substances coming up from the root-hairs.

Tracing the hyphæ from the cortex into the young tubercle, then, and the changes which go on in the cells of the latter, the following facts are to be noted. The single stout hypha (fig. 9A) first breaks up into numerous branches (figs. 8–12) which distribute themselves in all the cells of the dividing mass of tissue—the incipient tubercle. While the younger branches continue this process the older ones form the short branches, and begin to bud off numerous tiny gemmules into the cell-contents (figs. 12–17): the struggle between the protoplasm and these gemmules—which themselves go on budding—is evinced by the frothing and activity of the protoplasm, which thus comes to resemble a plasmodium, and by the accumulation of starch, and perhaps other substances also. This causes the cells to grow (hypertrophy), and by the time the hypertrophy has ceased the gemmules in that particular cell have ceased

* Frank was also inclined to the same view ('Botan. Zeitung,' 1879, p. 385), but he has since retracted his opinions on the whole subject in a most unaccountable manner. See 'Berichte d. Deutschen Botan. Gesellschaft,' 1887, pp. 56 and 57.

† In order to avoid the inference that I suppose these cells to be true Saccharomycetes, they may be conveniently termed *gemmules*.

to multiply. The ferment is now over. The gemmules come to rest in the matrix, which they have first stimulated and then exhausted, and a large vacuole may be formed (figs. 4 and 12), in which lie the remains of the nucleus. It should be noted that at this stage the nucleus is often very bright and fatty-looking, and stains black in osmic acid. By this time the filaments which budded off the tiny yeast-like gemmules are so obscured by their progeny that they are almost certain to be overlooked, and special methods are necessary to detect them. They are there, however—at least, recognisable remains of them are (fig. 18).

The tubercle, when all its cells have undergone the above changes, now passes into a state of rest: it is a mass of cells full of yeast-cells—gemmules, germs—so tiny* that they might well be, as they were, mistaken for Bacteria. The rotting of the root and tubercle liberates these into the soil, and an extended acquaintance with these "germs" and their numbers leads one to feel no surprise if they turn out to be the ubiquitous germs which it has been suggested must exist to account for the universality of the root tubercles.

Speculation apart, however, I have the following facts to offer. I made several attempts last year to infect the roots of the Bean by laying pieces of the tubers on the young rootlets of water-cultures. The success was only partial and doubtful, and the results did not seem sufficiently satisfactory to be worth recording. At the end of the summer I collected a number of the Bean-roots which had tubercles on them, and dried them; these I kept through the autumn and winter, and made further experiments in January, February, and March of this spring (1887). Several of the infected plants had already developed several typical tubercles in March and April, and indeed the preparations from which figs. 7–9 and 11–13 were made have been obtained from these artificially infected tubercles. I have since repeated these experiments with marked success.

The most curious feature about the matter is, perhaps, the long "period of incubation" (as the doctors would term it). I infected the roots by placing very thin sections of the dried tubercles on the young tap-root; but no signs of the tubercles were noticed before the root was five weeks or so old, and had developed an abundant outgrowth of lateral rootlets.† It was from a section of one of the younger of these tubercles that the preparation of the root-hair in fig. 13 came. It will be noticed that the hypha running down the root-hair starts from a minute bright dot; unless this dot is one of the above-named "germs," I do not know what it can be. I have now seen *the root-hair thus containing a hypha starting from a mere bright point many times*, and have several permanent preparations of such infected hairs with the hypha passing down the cavity and across into the root. It very commonly happens that the

* FRANK gives their measurement as about 0·001 mm. thick. WORONIN gives length ·0016 to ·0028 mm. Many Bacteria are larger.

† They were, probably, at least a week or ten days old, however, and I now know that this specimen (growing slowly in January and February) furnished late examples.

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root-hair thus infected is curiously coiled and twisted at the spot whence the hypha takes its origin—*i.e.*, at the point of infection—looking as if the growth of the cell-wall had continued after the attack, but was profoundly affected at the point of entry (fig. 13A). It is sufficient to germinate a Bean in ordinary garden soil for a week or ten days, and then place the seedling as a water-culture, to obtain such preparations in nearly every case, provided the *very young* tubercles are examined.

It is now necessary to say a few words as to the systematic position of the above Fungus. The behaviour of the mycelium is so like that of some Ustilagineæ (especially the *Entorrhiza* found in *Juncus bufonius*) that an alliance to that group might readily be claimed; the difficulty of determining the membrane of the hypha, the glistening character of the protoplasm, the course straight through the cell-walls and across the lumina of the cells, are all points which remind one of the Ustilagineæ. The dilated abutments on the cell-walls I have explained as due to the extension of the cell-walls by growth after the perforation.

On the other hand, there are no septa discoverable in the hyphæ; nor can I detect anything of the nature of a true spore like the resting-spores typical for the ordinary Ustilagineæ, unless the haustorium-like branches are to be regarded as aborted spores. The "yeast-like" minute corpuscles (gemmules) which are sprouted off from the short branches in the cells, and which then go on budding until they fill up the space, appear to be the only reproductive organs which exist.

But, in view of BREFELD's recent discoveries as to the extensive "yeast" formation which goes on in the Ustilagineæ,* it seems not at all improbable that this character may be in favour of, rather than against, the alliance of this Fungus to the Ustilagineæ.

In fact, I regard the Fungus as one of the Ustilagineæ, which has become so closely adapted to its life as a parasite in the roots of the Leguminosæ that it has come to stimulate and tax its host in an exquisitely well-balanced manner, and has lost its needless true resting-spores because the more numerous and tiny sprouting yeast-cells (gemmules) are kept in the cells of the tubercle through the dry summer and autumn, and freed during the rotting in the soil in the winter and spring. Their very minuteness and numbers enable these "germs" to become as ubiquitous as "Bacteria" or ordinary "Yeast" forms, thus explaining the ubiquity of the tubercles.

BREFELD has shown that the ordinary resting-spores of the Ustilagineæ, which usually take a long time to germinate in pure water, will germinate much more rapidly in nutritive solutions, and that several species can be made to develope their promycelia in nutritive solutions which had hitherto been cultivated in vain. The curious fact comes out, however, that when thus germinating in nutritive solutions the promycelia go on budding off yeast-like cells, which multiply still further by budding, and thus extend the parasite outside the plant enormously. According to BREFELD's discoveries,

* BREFELD, 'Botanische Untersuchungen,' Heft 5, 1883.

whereas some forms, such as *Ustilago destruens*,* first develope a definite mycelium from the resting-spore, and the yeast-like cells are budded off from this into the air, in other cases the budding commences at once on the germination of the spore. Thus, in *Ustilago antherarum*,† the budding begins direct from the spore, or immediately the promycelium is protruded. Similarly with *Ustilago intermedia*,‡ *U. Maydis*, and others. Now, if we supposed the spore to germinate in this manner while still attached to the mycelium inside the plant, we should have something very like what happens in the Fungus I have described. In fact, I regard the swollen parts of the mycelium which give rise to the gemmules as the homologues of the spores proper (*i.e.*, resting-spores) in the ordinary Ustilagineæ. Anyone who compares the development of the spores in those Ustilagineæ which have been sufficiently studied§ cannot fail to notice the remarkable similarity to the development of the branches which bud off the gemmules in this Fungus. If, for any reason, the future resting-spore of an *Ustilago* began to put forth buds (gemmules) before it developed its thick coats, we should have the very process I have been describing. In the case of the Fungus causing the tubercles on the roots of Leguminosæ it is not difficult to suggest that it might obviously be a disadvantage to the parasite to develope resting-spores, of (comparatively) large size, which should be set free on the rotting of the root, since they would not easily be disseminated in the soil; on the other hand, it would seem to be a great step gained if the habit of forming yeast-like gemmules no larger than Bacteria was thrown back, as it were, earlier into the life-history. There is no lack of equally curious adaptations in the life-history of Fungi. In fact, steps towards such retrogression are already found in the asexual development of the resting-spores of the Ustilagineæ if we accept DE BARY's views as to their homologies; and it does not seem possible to refute them.

These extremely minute gemmules will be disseminated between the interstices of the soil as easily as the well-known Schizomycetes found everywhere; every stream, every puff of wind, and many slighter movements will carry them from place to place, and hence their ubiquity.

I have been much exercised with the question as to whether these gemmules bud and multiply in the soil, *i.e.*, in artificial nutritive solutions, and have repeatedly made efforts to cultivate them in hanging drops of pure water, PASTEUR's solution, and other nutritive media. The general result has been disappointment. In some cases I have thought that certain dense flocculent groups which develope in PASTEUR's solution were clouds of the buds, but they never form rapidly, and in the interval (four to eight days, or longer) Bacteria have always made their appearance. The suspicion that these flocculent clouds might be colonies of budding gemmules is not an absurd one: very similar, I believe identical, clouds form on the root-hairs of my water-

* *Loc. cit.*, Plate VII., fig. 24.

† *Loc. cit.*, Plate I., especially figs. 14–17.

‡ *Loc. cit.*, Plate VI. and Plate IV.

§ *E.g.*, DE BARY, 'Morphol. und Biol.,' fig. 82, p. 189 (Engl. Edition, p. 175).

cultures, and even close to where a hypha enters the root-hair. However, the whole of this matter is as yet too uncertain for me to say more than that it seems a point well worth investigation to determine whether the gemmules will go on budding in the soil or on root-hairs. It may, perhaps, be permitted to make another suggestion here. Even if, as is usually accepted, the protoplasm of the root-hairs of plants cultivated in nutritive solutions—and, if so, presumably of plants in soil—is confined to the interior of the cell-walls, these cell-walls are very thin and delicate. There are facts which seem to support the view that the germinal hypha starts its course at the root-hairs for this reason, and it is not impossible that the gemmules will only germinate on the root-hairs of the host-plants. At any rate, I never find them elsewhere.

It is a well-known and very popular view that the Leguminosæ enrich the soil in nitrogenous substances. There is no doubt that the plants of this natural order contain abundance of nitrogenous substances, and it is a fact that the roots of our ordinary field and garden Papilionaceæ go deep, and thus bring up nitrogenous compounds from below to enrich the surface soil when the plants are turned into it by the spade or plough. It is also unquestionable that the root-tubercles which have been described above are extraordinarily rich in nitrogenous substances. Apart from the evidence already given, analyses of the tubercles of the Lupin show an enormous excess of proteids as compared with the other parts of the roots,* and, since these tubercles are apt† to be on the upper portions of the root, it is not improbable that these circumstances have contributed to the views so favourable among agriculturists. Of course there may be more in this matter than meets the eye, if future investigations demonstrate that the gemmules can live as saprophytes or ferment-organisms in the soil outside the plant.

The above results were already on paper, and had been shown to friends, when the 'Berichte der Deutschen Botanischen Gesellschaft,' Heft 2, 1887, came to hand, containing a paper by TSCHIRCH on the root-tubercles of the Leguminosæ.‡ The Paper is somewhat long, and not always clear, but, since it contains important and sweeping statements as to the biological significance of these root-tubercles, it will be necessary to devote some time to its examination. TSCHIRCH has investigated chiefly the anatomy of the tubercles on the roots of the Lupine and *Robinia*, and expressly states that the research was undertaken at an unfavourable season (October to December), a statement which makes it the more surprising that his generalisations should be so sweeping in character. He does not seem to have examined *Vicia Faba* particularly, and the following criticisms must be regarded as bearing only on the points common to *Vicia Faba* and other Leguminosæ; these are no doubt numerous, but it

* TROSCHKE, cited by SORAUER, 'Pflanzenkrankheiten,' vol. 1, p. 746.

† Not necessarily so, as my cultures prove.

‡ 'Beiträge zur Kenntniss der Wurzel-Knöllchen der Leguminosen, (1),' pp. 58–98. The same also contains a paper by FRANK, advocating similar views to those of BRUNCHORST and TSCHIRCH.

is as well to bear in mind that until other forms have been investigated on the basis of the knowledge now to hand, there is always a certain amount of assumption in supposing that the tubercles of the roots of Leguminosæ are all due to the Fungus I have described. TSCHIRCH, like others, has by no means hesitated at assuming a common cause for these structures, however, and I am strongly of opinion that they are so far right, but that the causal agent is in all cases a Fungus.

TSCHIRCH regards the tubercles as normal structures, differing in form and anatomy according to two types. In *Robinia* and the majority of the Leguminosæ the anatomy, &c., is so like what occurs in *Vicia Faba* that we need not dwell on the details. He finds the development of the tubercles on the roots begins with that of the first leaves above ground, and traces coincidence of developments of tubercles and leaves onwards: at the close of the period of vegetation the tubercles become emptied, in whole or in part, as the seeds ripen.

This emptying is considered to be due to the dissolution (and absorption into other parts of the plant) of the inner core of tissue containing the gemmules. Here comes in an important difference between our descriptions. TSCHIRCH accepts a view, already propounded by BRUNCHORST,* that the gemmules belong to the root, and are formed by the protoplasm of the cells; as will be shown shortly, the two writers differ somewhat in their interpretations of these bodies, but both employ the same name for them—*Bacteroids.*

In *Robinia* TSCHIRCH finds that the "bacteroid tissue" is partially or nearly wholly emptied in autumn, and states that this is the rule. The phenomenon thus assumed to be a resorption of the bacteroids I take to be the escape of the gemmules from the tubercles; they are not absorbed, they are set free.

TSCHIRCH finds much starch in the young bacteroid tissue, but less as the cells become older; this is in accordance with my observations, but is subsequently explained differently.

In annuals the tubers are found to attain a maximum of development, and then become emptied as the seeds ripen.

TSCHIRCH agrees with BRUNCHORST that the gemmules or "bacteroids" are not organisms, but bodies which arise by differentiation from the protoplasm of the cell. This view he bases on their "development" (though no account of their development appears), their fate (the assumed resorption into the plant), and the normal occurrence of the tubers in all genera of the strictly defined group Leguminosæ. TSCHIRCH also adds that every attempt to cultivate the "bacteroids" has failed, and that they could not enter the tubercles, because the latter are covered by cork.

Reference is made in a footnote (p. 67) to the observations of WORONIN and FRANK as to doubtful development of the gemmules (bacteroids) in artificial cultures. Why it should be assumed (p. 67) that "im vorliegenden Falle hat man es, wenn

* 'Berichte der Deutschen Botanischen Gesellschaft, 1885,' p. 241; and 'Untersuchungen aus dem Botanischen Institut zu Tübingen,' vol. 2, Heft 1, 1886, p. 151.

überhaupt mit einem Pilze, mit einem Schizomyceten zu thun" is not clear, but the author admits no other alternative.

BRUNCHORST supposes the "bacteroids" to arise by differentiation from the protoplasm of the cell, and TSCHIRCH accepts this view; the "bacteroids" (*i.e.*, gemmules) are supposed to be remnants of a protoplasmic network (p. 68). Insuperable difficulties against accepting the view that the tubercles arise by infection from without are found in that it involves the assumption that the germs must be in all soils and in water, and TSCHIRCH states (p. 69), "Thatsächlich sind derartige geformte Pilze aber gar nicht im Boden vorhanden." The "bacteroids" are thus denied autonomy as parasitic bodies, and are relegated by TSCHIRCH and BRUNCHORST to the protoplasm of the root as "geformte Eiweisskörper."

TSCHIRCH then goes further, and classifies these bodies with the vegetable caseïns related to Legumin.

The filaments (hyphæ) observed by ERIKSSON and FRANK, and which I have traced from the exterior, through the root-hairs and into the tubercle in *Vicia Faba*, are said to be absent from the cortex and outer tissues. "Ich habe in der äusseren Rinde niemals Fäden gefunden" (p. 72). They were observed in abundance, however, in the outer limits of younger "bacteroid tissue."

TSCHIRCH denies their fungoid nature entirely, and refuses to see in them either hyphæ or plasmodial strands. His account of their "development" (pp. 73, 74) is quite unintelligible: among other things he regards the cell-walls of the tubercle as cutting these filaments (hyphæ) in two. He thinks that no connection exists between the "bacteroids" and the filaments, but this is admitted to be doubtful. The view accepted—so far as any definite idea about the hyphæ is accepted—is that the filaments dissolve, and the protoplasm of the cell then gives rise to the "bacteroids" by differentiation.

Looking at TSCHIRCH's statements so far, it seems clear that, apart from the small amount of direct observation which has been brought to bear on these bodies, two chief points come in to explain the mistakes. In the first place, he has not examined tubercles sufficiently young to determine the course of the hyphæ from without inwards; and, secondly, he has failed to observe the relations of the hyphæ to the cells and protoplasm of the interior of the tubercle.

Apart from the dying-off of the older hypha which enters the young root-tubercle—often at a time when root-hairs are still present—it is, of course, obvious that many sections will not take in the point of entrance and the course of the hypha shown in my figures 8 and 9. TSCHIRCH's figures 1, 2, 4, and 44 show that the sections were made in the wrong direction to give the required information, and, although the tubercles examined are described as "young," they are far too old for the purpose referred to. Again, in the absence of some intelligible suggestion as to what the filaments are, if not hyphæ, there is nothing gained by reiterating that "mit einer Pilzhyphe haben sie also nichts gemein." The assumption that they are cut in two by the cell-walls required some evidence. Anyone who has worked with such mycelia as those of the Ustilagineæ must have seen hyphæ as bright and delicate as these.

The point as to the budding of the gemmules from the hyphæ is a more difficult one, because it seems impossible to witness the process of budding off itself. The view proposed by BRUNCHORST, and accepted by TSCHIRCH, that the gemmules (" bacteroids") are remnants of a broken-up protoplasmic network, is quite untenable in the cases examined, and TSCHIRCH gives no figures illustrating such a process. I have shown that the gemmules arise in connection with the hyphæ in the cells, and not as points scattered in a matrix. BRUNCHORST himself seems to have held at one time the idea that these "bacteroids" escape from the interior of the hyphæ like minute sporules, but the account is by no means clear. It seems impossible to explain the frequent cases shown in my figures 15, 16, and 17 on any other view than that they are the gemmules budding off from the hyphæ, and it ought to be noticed that others have seen similar cases, but without properly explaining them. For instance, TSCHIRCH himself says (p. 76), " In einem Falle nur schien der Kopf eines Fadens mit unzähligen behäuften rundlichen Ausstülpungen besetzt," and in a foot-note he remarks, " Diese Bildung ist vielleicht mit der von PRILLIEUX beschriebenen Auflösung der Fäden der warzigen Massen identisch" (p. 76).* Again, it is difficult to attach any other meaning to FRANK's figures ('Botan. Zeitung,' 1879, Plate V., esp. fig. 11) than the one he then ascribed to them, though he has since retracted his explanation in a most unaccountable way—erroneously, I am convinced.

It is still more difficult to accept TSCHIRCH's assumptions as to the " bacteroids" (gemmules) themselves. It may be true that their variable shapes militate against their being *bacteria*, but bacteria are not the only alternative, as he assumes, and these very shapes are fully in accordance with their being gemmules—tiny, yeast-like, budding organisms—as a comparison of FRANK's figures and my own with those of TSCHIRCH's amply testify. TSCHIRCH's assumption that the filaments (hyphæ) dissolve, and the protoplasm then breaks up into " bacteroids," is certainly not true for the tubercles of *Vicia Faba*. In fact, the whole of this purely hypothetical explanation of the nature of these bodies is contradicted by the facts observed in *Vicia Faba*; whereas the phenomena are simply and naturally explained when we recognise that the hyphæ bud off the gemmules, which then multiply further by budding like tiny yeast-cells. These then pass into a dormant state in the matrix, and escape into the surrounding soil when the tubercles decompose at the end of the season of growth, and are there distributed in readiness for contact with root-hairs of other seedlings in the following season.

It now remains to examine the rest of TSCHIRCH's paper. He expressly states, and repeats, that water-cultures are useless in the investigation, though no satisfactory reasons whatever are given in support of this view. He admits that the

* PRILLIEUX ('Bull. Soc. Botan. de France,' 1879, p. 98) regards the cause of the tubercles as a plasmodium.

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tubercles occur on water-cultures, and I have now shown that most satisfactory results can be obtained by means of water-cultures. TSCHIRCH also states that they are commoner on the roots of plants growing in poor soil (as regards nitrogen) than on those in rich humus-soils, a statement I fail to confirm. Darkness, drought, and other disturbing influences of the kind hinder the proper development of the tubercles; and the rule is that the tubercles flourish in proportion to the thriving of the plant.

It is obvious, on a little reflection, that there would be nothing in these facts which contradicts the explanation I have given. Since the gemmules stimulate the protoplasm of the cells to greater activity—as indicated by the increased supplies of starch and the hypertrophied growth—it is clear that the needs for the increased activity will be satisfied only in proportion to the ability of the host-plant to furnish supplies: darkness would cut off the supplies of carbo-hydrates, and drought those of water and minerals, and thence the parasite and protoplasm in the tubercles suffer. This can be directly shown in water-cultures, as well as in the case of plants growing in the open. As regards the generalisation that the tubercles are commoner in soils which are poor in nitrogenous substances, this might be because there is more oxygen supplied to the very large active root-systems in such soils; a view, moreover, which is in accordance with the well-known fact that the tubercles are apt to be more abundant and larger on those parts of the roots which are nearer the surface of the soil, though this is not necessarily the case. There is another point to note here. TSCHIRCH himself observes (p. 90) that the Leguminosæ are deep-rooted plants which do not thrive well in rich humus-soils, abounding in nitrogenous materials. Now, without agreeing with him to the extent of his generalisations (for it is by no means established that the Leguminosæ as a class are dependent on one kind of soil), it is obvious that nothing in this contradicts the explanation of the tubercles as hypertrophies due to the stimulating action of a symbiotic Fungus. TSCHIRCH lays great stress on the facts that the analysis of the tubercles shows them to be relatively very rich in potassium, phosphorus, and nitrogen, and claims that the abundance of nitrogen is due to the "bacteroids": no doubt this is the case, since the "bacteroids" are of the nature of Fungi—yeast-cells—structures known to be particularly rich in potassium and phosphorus as well as nitrogen.* TSCHIRCH employs the evidence of the analysis in support of the hypothesis that the tubercles are normal organs used as storehouses for reserve materials: the evidence, however, distinctly supports the view that the protoplasm becomes surcharged with materials employed in the hypertrophy and for the good of the parasite. Other facts, quoted by TSCHIRCH in a different sense, are equally in favour of this: for instance, the absence of sugar and the presence of fatty substances in the winter.

At the end of his memoir TSCHIRCH gives a summary substantially as follows:—

The Leguminoseæ are plants which require much nitrogen, but only towards the

* *Cf.* HUSEMANN, 'Die Pflanzenstoffe,' 1882, vol. 1, p. 279.

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close of their period of vegetation, and especially as the seeds ripen: in accordance with this, their root-system is abundant and far-reaching in the soil poor in nitrogen. The nitrogenous materials not employed at the time are stored up in the root-tubercles, which are emptied as the seeds begin to ripen, the nitrogenous contents being absorbed for the good of the latter.

As I have shown, this hypothesis is utterly untenable with regard to the tubercles on the roots of *Vicia Faba*, and everything points to its being equally so for the other Leguminosæ: not only is TSCHIRCH's attempt to explain away the hyphæ and the gemmules a failure, but his drawings indicate that he is not in possession of the histological facts necessary to constitute him an authority on the subject of the development and physiology of the root tubercles of the Leguminosæ, whence his repeated assumptions lose in value.

It now remains to give some account of the experimental cultures which have had so much importance in leading to the discovery of the infecting hypha, and to the general results given above. I found some time ago that Beans grown in a soil which had been burnt did not develope the tubercles, and that the same results followed as a rule when the cultivations were made in solutions of nutritive materials. I have since observed that if cultures in burnt soil are watered with washings of common garden soil, or of compost heaps, the tubercles are almost sure to be developed in the course of a few weeks; even river or pond water is dangerous—at least, in one case I traced the infection to the water with which I had watered the plants. Moreover, as I have shown, no experiment can be trusted if the seedling has been in contact with ordinary sand, turf, or leaf-mould.

I find that a very convenient process for obtaining the tubercles is to allow the seed to germinate for a week or ten days in common garden soil, and then carefully lift the seedling, wash its root very gently, and place it in a split cork so that its root goes on developing in a nutritive solution. In the majority of cases the young tubercles are quite evident some time during the fourth week following, and a little experience enables one to detect them at least a week sooner than that.

Now, seedlings carefully germinated in the same garden soil, previously sterilised by being burnt, and subsequently treated similarly, do not yield tubercles at all.* This fact is in itself strong evidence of the infection coming from the soil.

It may be well to state the composition of the nutritive solutions I have employed, and the mode of treating the plants grown in them.

In all the cases referred to the solution employed was that given by SACHS in his 'Vorlesungen über Pflanzenphysiologie,'† care being taken that all vessels and utensils were clean, and the water and reagents pure.

* Or only sporadically—a fact explained by chance infection during the culture.

† P. 342. English edition, p. 284.

Water	1000	c. cm.
KNO_3	1	grm.
NaCl	0·5	,,
$CaSO_4$	0·5	,,
$MgSO_4$	0·5	,,
$Ca_3(PO_4)_2$	0·5	,,

The seedlings were placed in a slit cork, so arranged that the whole of the root was submerged in the solution, and that the shoot could grow up freely into the light and air, which, together with the temperature and other conditions, were carefully regulated.

I followed SACHS' plan also in placing the cultures in new solutions when necessary, and in some cases of allowing their roots to remain for some hours in a solution of gypsum or salt between the changes. However, these precautions are scarcely needed if the roots are kept darkened and the solution changed once a fortnight or so. In cases where chlorosis was beginning to set in, traces of a salt of iron were added as necessary.

It may remove all cause for doubt to state that *it is just as easy to obtain the tubercles in the roots of these water-cultures* as it is on the roots of the plants growing in soil, in pots, or in the open; and, conversely, it is in both cases possible to prevent their formation by removing the infecting germs (by heating the medium, &c.). There are in my greenhouse and laboratory at the present hour no less than 32 water-cultures and 26 cultures in pots, growing under conditions so well controlled that it is possible to predict with great accuracy when and where tubercles will be developed.

I will confine myself to the following inferences from my Table of 81 experiments made this year, merely reserving the opinion that the ambiguous results in six or seven cases were due to faulty sterilisation.

The chief points to notice are (1) the all but invariable development of the tubercles within a month, when the Beans were germinated in sand or soil not previously heated; (2) their non-development when the medium was sterilised by being heated; (3) the number of times I succeeded in infecting the roots by means of pieces of old tubercles placed among the root-hairs; and (4) the number of times the infecting hypha was discovered entering the cortex by means of the root-hairs.

It is partly on these grounds that I infer that the tubercles so common on the roots of the Bean are due to the action of the Fungus, the very minute germs of which are all but universally distributed in the soil; and it will be conceded that there is evidence for believing that germ to be the gemmule developed in the cells of the tubercle. The further physiological effects of the symbiosis must be reserved for future discussion.

No. of Bean.	Date of sowing.	Medium in which Bean germinated.	Date when put as water-culture.	Date when infected.	Date when tubercles were seen.	No. of days occupied in infection.	Remarks.
1	Jan. 26	River sand	Feb.	..	Feb. 28	..	No record of date when put as a water-culture.
2	Feb. 28	Heated sand	..	..	..	..	Dug up on March 31; showed no signs of tubercles. (Nos. 2–7)
3	,,	,,	..	..	..	..	
4	,,	,,	..	..	..	..	
5	,,	,,	..	..	..	..	
6	,,	,,	..	..	..	..	
7	,,	,,	..	..	..	..	
8	March 7	Heated sand	March 14	March 14	March 31	17	
9	,,	,,	,,	,,	,,	17	In Nos. 9, 10, and 11 I found the hypha passing down the root-hair; the tubercles were already several days old.
10	,,	,,	,,	,,	,,	17	
11	,,	,,	,,	,,	,,	17	
12	,,	,,	,,	,,	April 2	19	
13	,,	,,	,,	,,	..	..	Bean No. 13 rotted away in a few days.
14	,,	,,	,,	,,	April 2	19	
15	,,	,,	,,	,,	..	..	No tubercles developed on No. 15, and the Bean rotted off.
16	March 16	Washed sand	March 30	March 30	April 9	10	Traced hypha down root-hair; more than 50 tubercles found.
17	March 31	Garden soil	April 11	..	April 25	..	
18	,,	,,	,,	..	April 18	..	Traced hypha down root-hair of No. 18.
19	,,	,,	,,	..	April 19	..	
20	,,	,,	,,	..	,,	..	Traced hypha down root-hair of No. 20.
21	,,	,,	April 20	..	April 20	..	The 8 Beans had tubercles when dug up, except No. 24, which developed them 4 days later. (The tap-root of this specimen was injured, and growth delayed.) I traced the hypha down the root-hairs in every case. (Nos. 21–28)
22	,,	,,	,,	..	,,	..	
23	,,	,,	,,	..	,,	..	
24	,,	,,	,,	..	April 24	..	
25	,,	,,	,,	..	April 20	..	
26	,,	,,	,,	..	,,	..	
27	,,	,,	,,	..	,,	..	
28	,,	,,	,,	..	,,	..	
29	April 2	Compost soil	..	..	,,	..	These 15 plants, on being dug up on April 20, all showed tubercles on the tap-roots, and some on the lateral roots as well. (Nos. 29–43)
30	,,	,,	..	..	,,	..	
31	,,	,,	..	..	,,	..	
32	,,	,,	..	..	,,	..	
33	,,	,,	..	..	,,	..	
34	,,	,,	..	..	,,	..	
35	,,	,,	..	..	,,	..	
36	,,	,,	..	..	,,	..	
37	,,	,,	..	..	,,	..	
38	,,	,,	..	..	,,	..	
39	,,	,,	..	..	,,	..	
40	,,	,,	..	..	,,	..	
41	,,	,,	..	..	,,	..	
42	,,	,,	..	..	,,	..	
43	,,	,,	..	..	,,	..	

No. of Bean.	Date of sowing.	Medium in which Bean germinated.	Date when put as water-culture.	Date when infected.	Date when tubercles were seen.	No. of days occupied in infection.	Remarks.
44	April 2	Old sand	April 9	..	May 11	..	
45	,,	,,	,,	April 9	April 22	13	Traced hypha down root-hair in 3 cases.
46	,,	,,	April 10	..	..	..	No traces to May 21.
47	,,	,,	April 20	..	..	..	
48	,,	,,	..	..	..	..	No. 48 failed to germinate.
49	April 2	Heated sand	April 9	..	..	..	No. 49 was lost by an accident on April 22.
50	,,	,,	,,	April 9	May 21	42	Small tubercles appearing all over the roots.
51	,,	,,	April 10	April 10	April 23	13	
52	,,	,,	,,	..	..	..	No traces visible to May 21.
53	,,	,,	,,	..	..	..	
54	April 5	Heated soil	April 23	..	..	..	
55	,,	,,	,,	..	..	..	
56	,,	,,	..	..	..	..	No. 56, dug up on May 4, showed no traces of tubercles.
57	,,	,,	..	..	..	..	Nos. 57, 58, dug up May 11, showed no signs of tubercles.
58	,,	,,	..	..	..	..	
59	,,	,,	April 13	..	May 12	..	
60	,,	,,	,,	..	,,	..	
61	,,	,,	..	..	May 14	..	No. 61, dug up May 14, had developed tubercles, but few and small.
62	,,	,,	..	..	..	..	Nos. 62, 63, dug up May 21, showed no traces.
63	,,	,,	..	..	..	..	
64	April 13	Garden soil	..	..	May 9	..	Nos. 64–69 were dug up on May 9, and had many well-developed tubercles.
65	,,	,,	..	..	,,	..	
66	,,	,,	..	..	,,	..	
67	,,	,,	..	..	,,	..	
68	,,	,,	..	..	,,	..	
69	,,	,,	..	..	,,	..	
70	April 14	Heated soil	..	..	..	..	Nos. 70, 71, dug up May 11, showed no traces of tubercles.
71	,,	,,	..	..	..	..	
72	,,	,,	..	..	..	..	No. 72, dug up May 17, showed no trace of tubercles.
73	,,	,,	..	..	..	..	Dug up May 21; showed no signs of tubercles.
74	,,	,,	..	..	..	..	
75	,,	,,	..	..	..	..	
76	April 15	Heated sand	April 22	..	May 14	..	Nos. 76–81 stood under the same bell-jar. The question is, was sterilisation effected, or did after-infection occur? In all cases the hypha was traced down the root-hair.
77	,,	,,	April 23	..	,,	..	
78	,,	,,	,,	..	,,	..	
79	,,	,,	,,	April 23	May 11	18	Tubercles on No. 79 very fine and numerous May 11.
80	,,	,,	,,	,,	May 21	28	
81	,,	,,	..	..	..	..	No. 81, dug up May 11, showed no traces of tubercles.

Explanation of Plates.

PLATE 32.

Fig. 1. Portion of Bean-root with the tubercles or root-swellings of various ages, showing the different stages of development; a very young one is seen on the rootlet to the left above. (Natural size.)

Fig. 2. One of the larger swellings cut in two. The buff-coloured portion is the part where the parasite is rampant: the paler portions towards the apex contain hyphæ only. The narrow zone *a* at the apices of the lobes consists of meristematic cells scarcely affected as yet by the hyphæ. (Natural size.)

Fig. 3. Half-diagrammatic section of a root, with tubercle and normal lateral rootlet. The latter arises opposite a xylem strand. The tubercle originates in the cortex, and also (in this case, but not always) opposite a xylem strand. The various tissues of the tubercle are indicated. The apical meristem contains very few hyphæ: numerous branched hyphæ are found in the cells lower down; the dark-shaded cells in the lowest central parts are hypertrophied and filled with the bacterium-like corpuscles—gemmules or yeast-cells.

Fig. 4. Three cells of the dark inner portion of the tubercle (fig. 3). The corpuscles (gemmules) have multiplied to an enormous extent, causing the protoplasm to become vacuolated and plasmodium-like, and the cells to enlarge: the dark body embedded in the mass of yeast-cells and protoplasm is the nucleus. E/4.

Fig. 5. Similar cells after the macerated tubercle has rotted: the cells are separating, and appear full of bright (dark) granules in a matrix. The granules are the now dormant yeast-corpuscles (gemmules); the dark body is the nucleus of the cell. E/4.

Fig. 6. The gemmules—granular bodies in figs. 4 and 5—more highly magnified. *a* and *b* are from still active tubercles, and are more highly magnified than *c*, which is from an old tubercle of the preceding year. a and $b = \text{L}/4$; $c = \text{J}/4$.

Fig. 7. Transverse section across a rootlet, and tubercle about the size of the smallest one in fig. 1. The tissues of the tubercle are seen to have hyphæ in them; these hyphæ are branches from the thicker hypha which passes down the root-hair *a* (to the left of the figure) and across the cortex of the rootlet. (B/3.)

Fig. 8. Portion of fig. 7 more highly magnified, and showing how the hypha from the root-hair commences to break up into branches as it passes into the young tubercle. (D/4.)

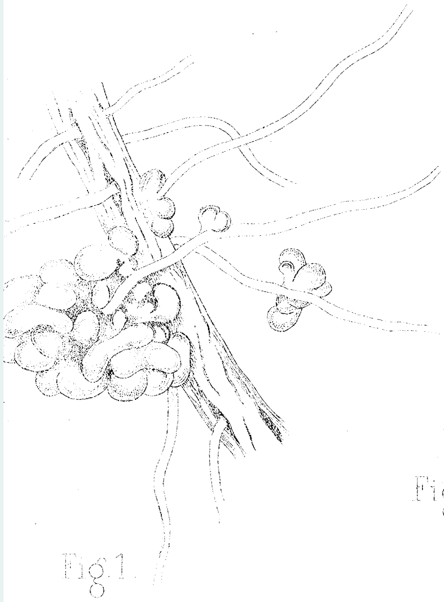
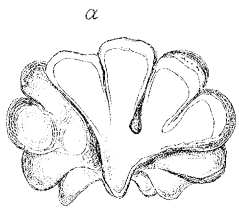


Fig. 1.



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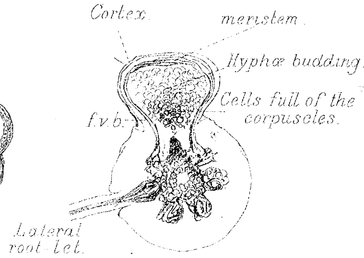

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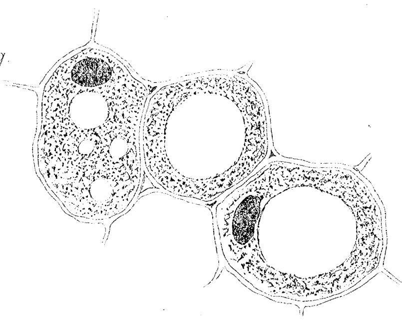

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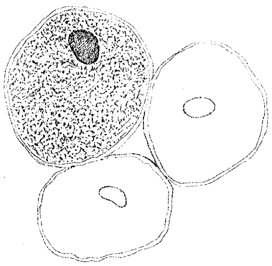
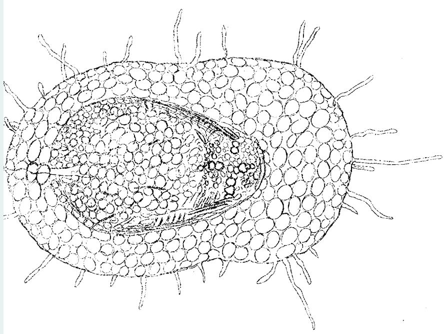


Fig. 5.

a b c

Fig. 6.



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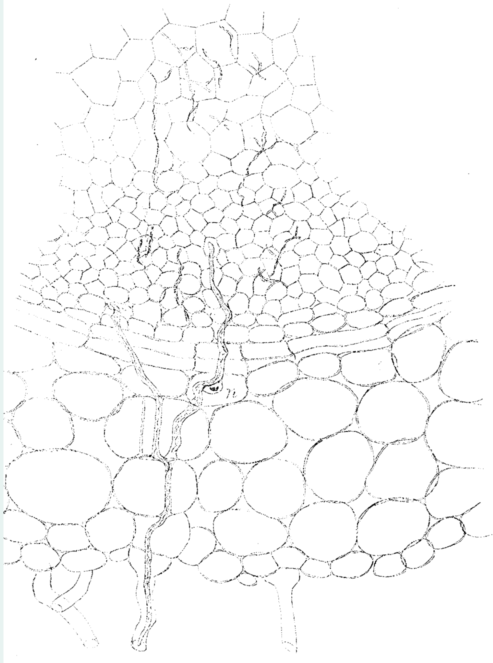

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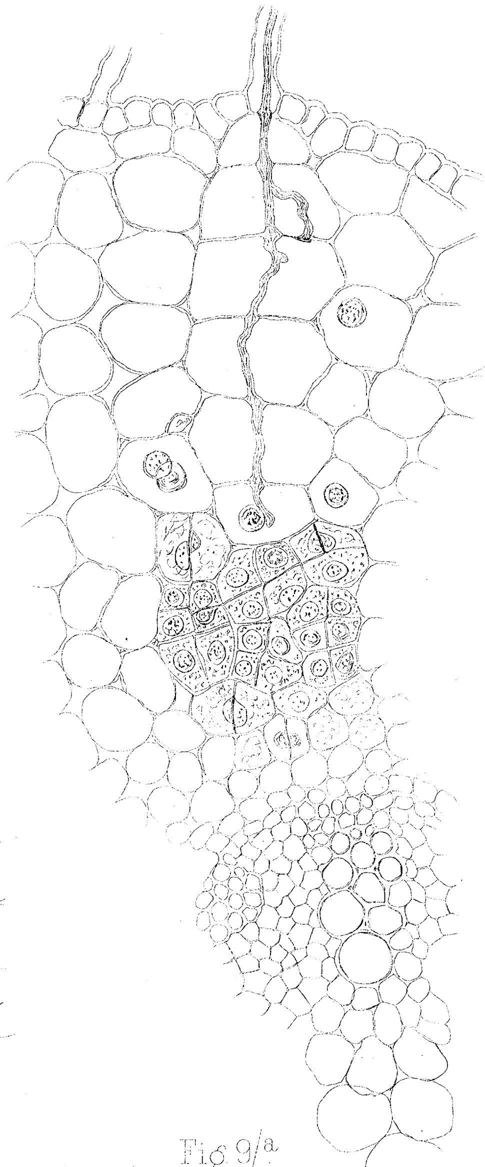

Fig. 9/a.

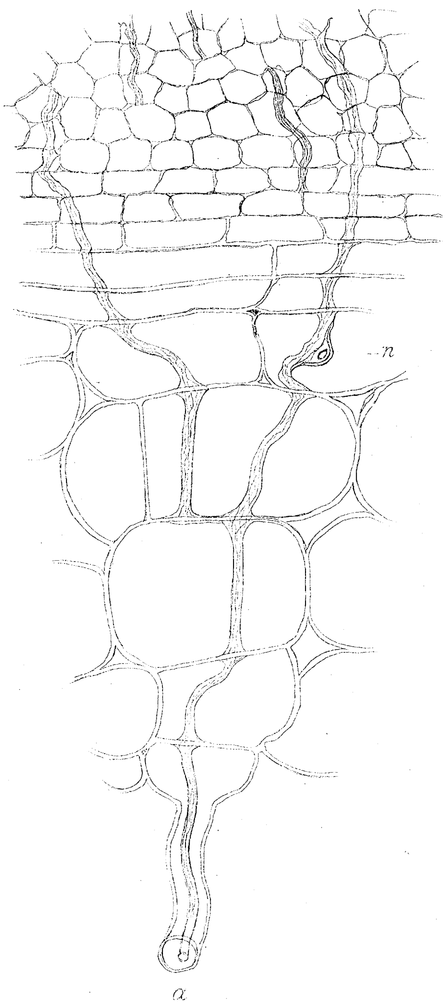

Fig. 9.

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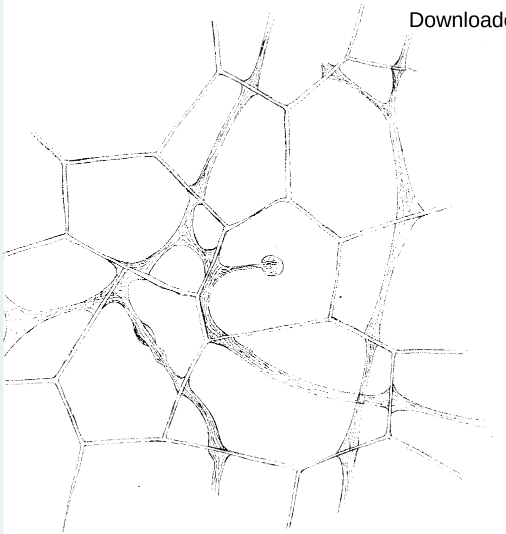
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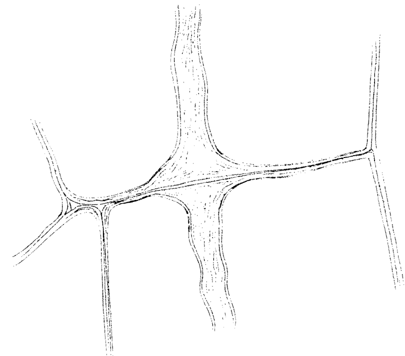
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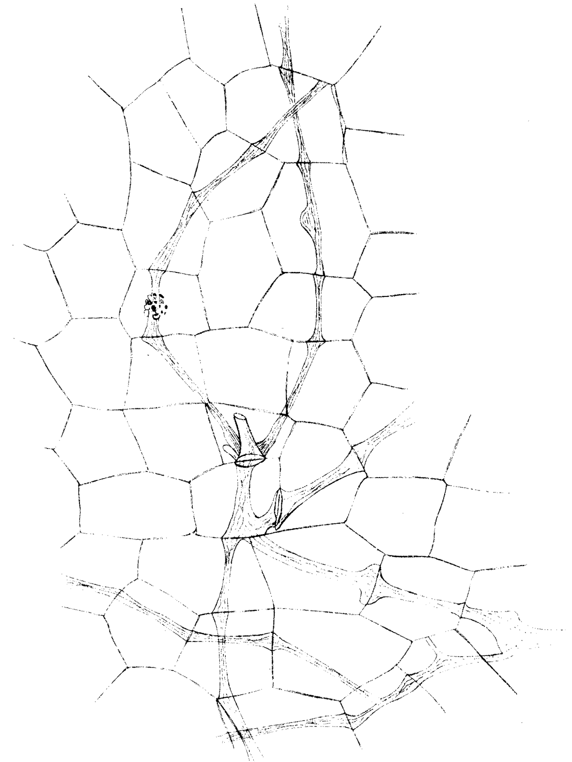
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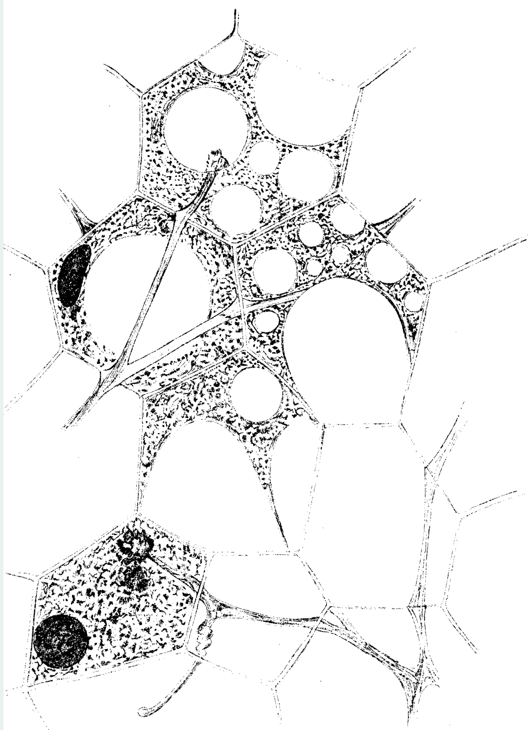
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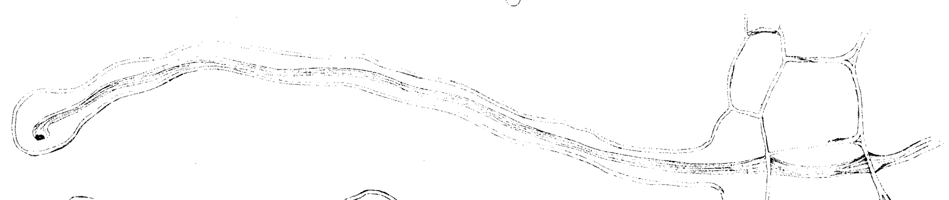
Fig 13.

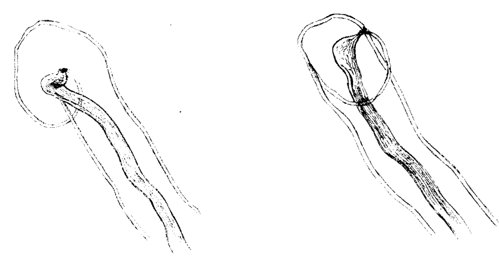
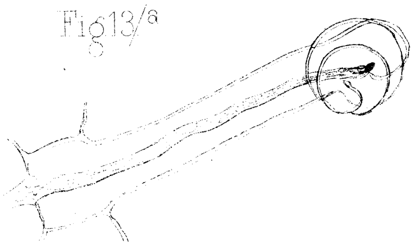
Fig 13/b


Fig 16.

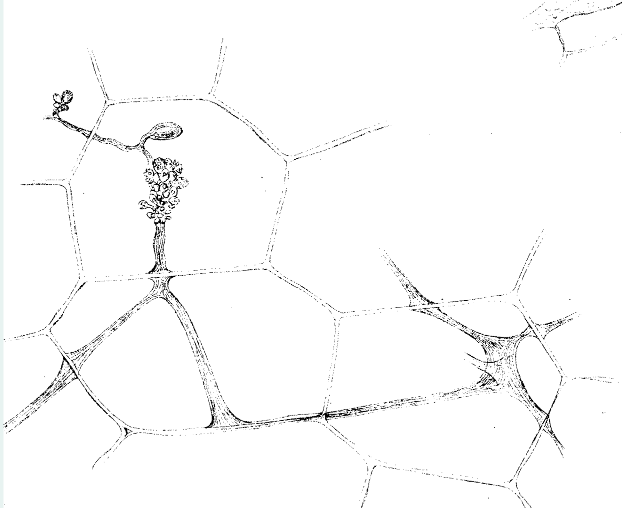
Fig 15


Fig 17.

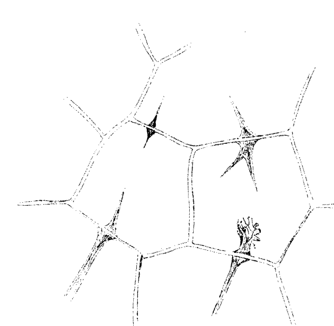
Fig 18.

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Fig. 9. A portion of the last figure still more in detail, and showing the curious trumpet-shaped widenings where the hyphæ pass through the cell-walls. One branch of the main hypha is attached to the nucleus *n* of one of the cells—this is not uncommon. The first branching of the main hypha clearly takes place in the cell-wall. The further branchings are not clear, because the course of the hyphæ varies in different planes. J/4.

PLATE 33.

Fig. 9A. A preparation similar to fig. 9, but showing a much younger stage of the development of the tubercle. The infecting hypha is crossing the cortex from cell to cell: soon after leaving the root-hair, it branches, the branch soon running in the cell-wall. The cells traversed by the hypha are hypertrophied. The young tubercle is arising by meristematic division of the innermost cortical cells opposite the entering tip of the infecting hypha, which is in contact with a nucleus. (E.)

Fig. 10. Part of the central tissue of a young tubercle (near the apex in fig. 3), showing the characters of the hyphæ, and their branching in the cells. The haustorium-like branch in the centre is very characteristic. E/4.

Fig. 11. Similar preparation from nearer the apex of the tubercle. The hyphæ are thicker, and their course more direct. This and the last figure from preparations treated with very dilute ammonia. E/4.

Fig. 12. Similar preparation (fresh) from the lower part of the central tissue of the tubercle, corresponding to the part shaded dark in fig. 3. The protoplasm of the cells is nearly filled with the budding gemmules, and has become vacuolated and plasmodium-like, the nucleus being driven to one side. Branches of the hyphæ are seen in the cells, and dense tufts of budding gemmules can be seen shining through the matrix (*e.g.*, the lower cell) in some cases : in others everything is obscured by the dense matrix. Starch often accumulates in the cells at this stage, and the nucleus in some cells undergoes a sort of fatty degeneration. (J/4.)

Fig. 13. Hypha traced down the root-hair into the cortex. It takes origin from a brilliant granule—presumably one of the gemmules. From a water-culture the root of which was infected by a section from a tubercle of the preceding year. The remainder of the course of the hypha is clear from figs. 7, 8, 9, 10, 11, 12. The hypha in the cortex subsequently turns yellow, and shrivels, and only remote traces are to be found in older tubercles. J/4.

Fig. 13A. Further preparations, resembling that from which fig. 13 was drawn. The

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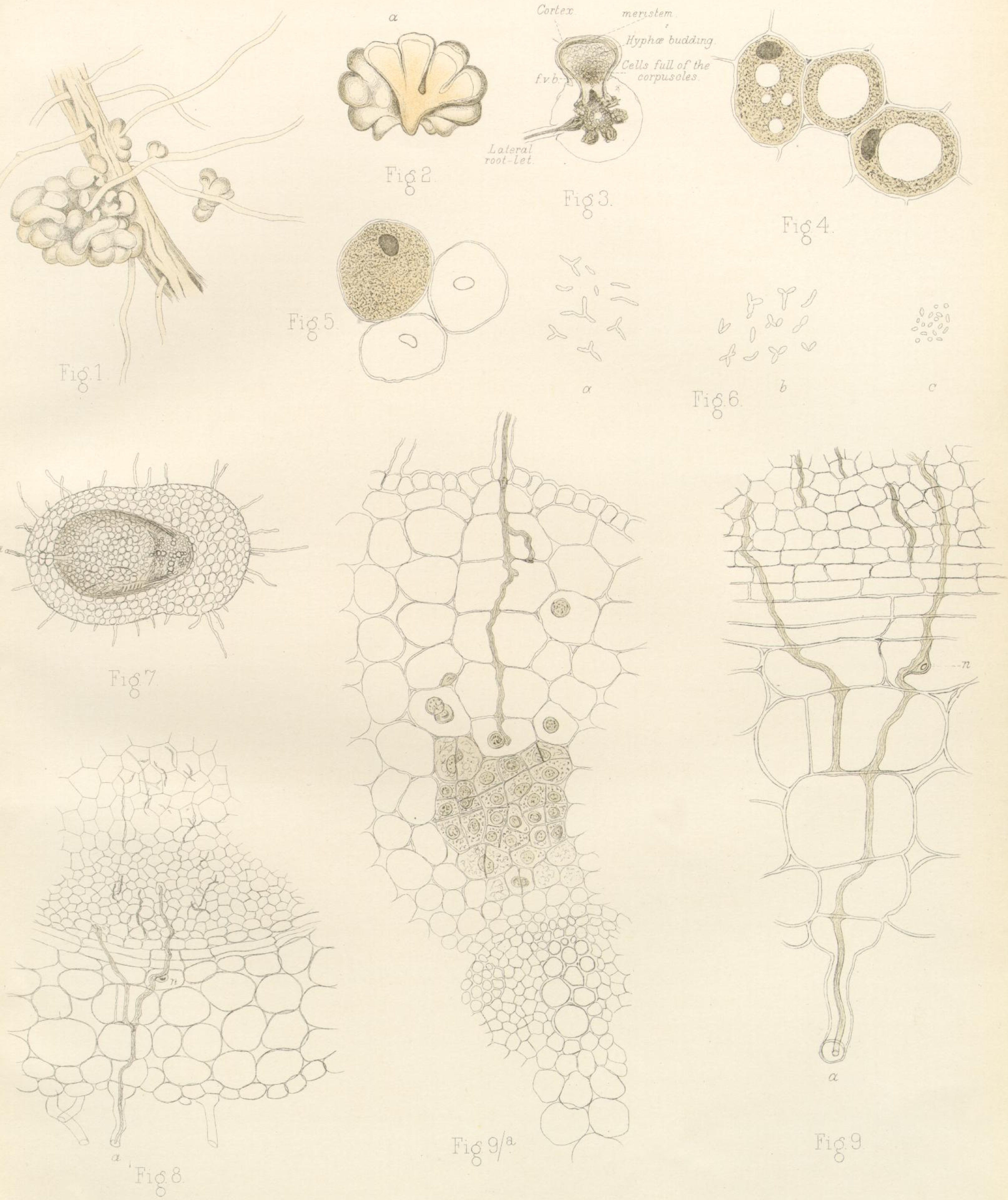
infecting hypha clearly arises from a brilliant point, around which the growth of the cell-wall of the root-hair is affected, causing distortions. L/4.

Fig. 14. Hypha passing through the cell-wall, showing the curious trumpet-shaped widening where the passage is effected. L/2.

Figs. 15, 16, and 17. Ultimate branches of the hyphæ in the cells of the tubercle, at the commencement of the budding process. The curious Y and V-shaped gemmules are well seen in fig. 17. The haustorium-like branches in figs. 15 and 16 are very like the incipient spores of an *Ustilago* (*e.g.*, DE BARY, fig. 82, p. 189), but they do not become spores, and in many cases seem to give off the gemmules. (J/4.)

Fig. 18. Part of a section of a last year's tubercle—very thin, and treated with dilute ammonia—showing that the remains of the mycelium are still there, though obscured by the dense aggregation of gemmules. (E/4.)

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



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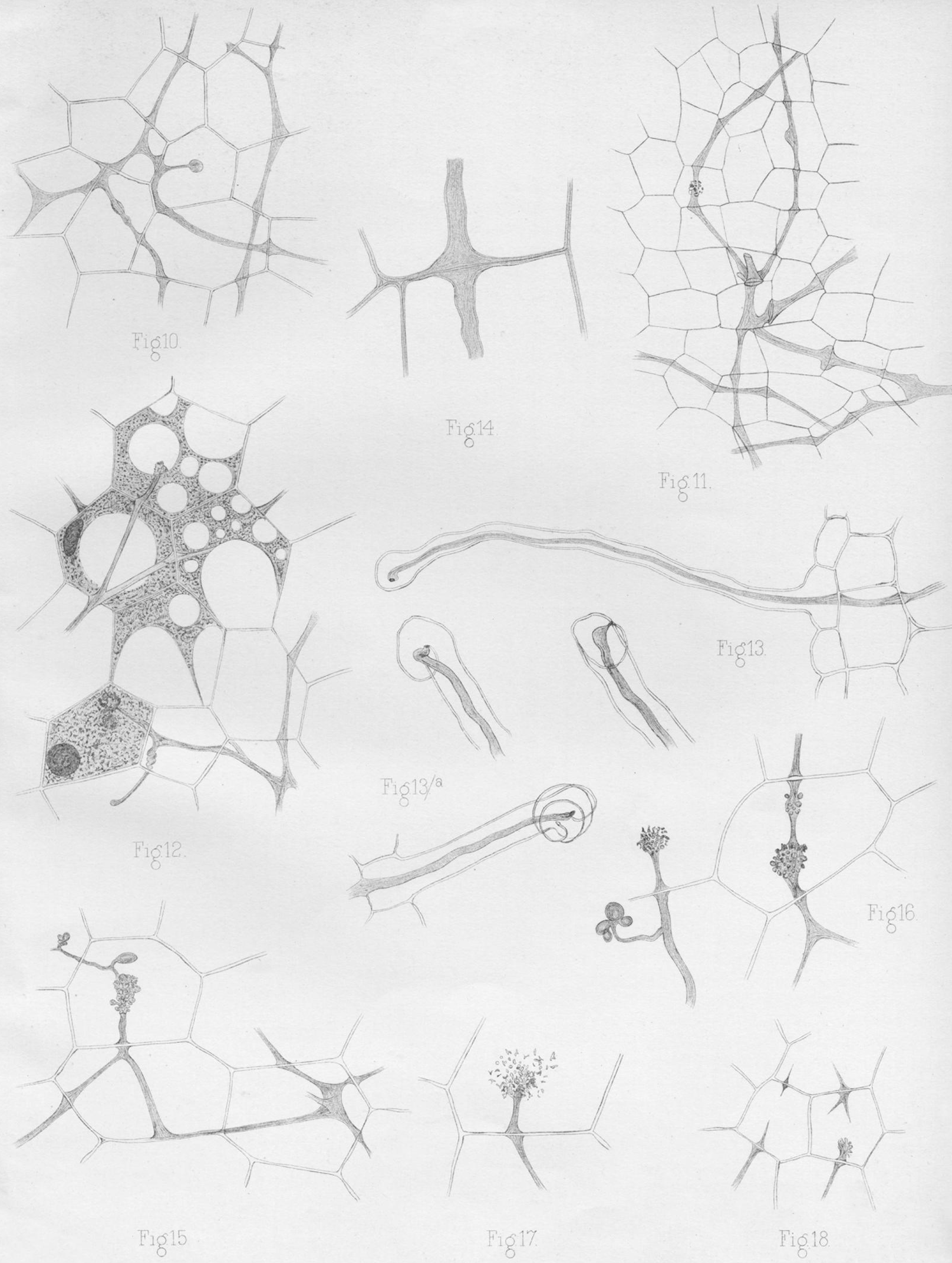

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