MEMOIRS.

On the Morphology *of* Hemileia vastatrix, Berk. and Br. (*the* Fungus *of the* Coffee Disease *of* Ceylon). By H. Marshall Ward, B.A., on special duty as Cryptogamist to the Ceylon Government. (With Plates I, II, III.)

During the past twelve months the progress of my investigations into the life history of *Hemileia vastatrix*, the fungus so prominent in the so-called "coffee-leaf disease" of Ceylon, has resulted in the accumulation of a series of facts concerning its structure and development which have been hitherto undiscovered, or, in some cases, misunderstood. Sufficient material being now at hand to throw light upon the morphology of this remarkable parasite, I purpose bringing together shortly the main points which have been established.[1]

Since it is not intended to enter upon any speculations, and none of the numerous physiological and pathological phenomena of "leaf disease" can be introduced here, it appears unnecessary to discuss at length the previous publications on the subject of "coffee-leaf disease."[2] In so shortly referring to them no inattention is implied, but it will be impossible to clear up points of difference without numerous figures in addition to those hereto annexed. Nor is it necessary to enlarge upon the history, so far known, of this serious pest to one of our most important cultivations. I shall therefore proceed at once to the immediate object of the present paper.

[1] Mr. Marshall Ward has reviewed the whole course of his investigations, especially in relation to the economic aspect of the subject, and the possibility of remedial measures, in a final report to the Ceylon Government (Colombo, Sessional Paper XVII, 1881).—[Ed. 'Q. J. M. S.']

[2] They are chiefly—*Abbay*, 'Journ. Linn. Soc.,' Dec., 1878; *Morris*, 'Journ. Linn. Soc.,' March, 1880; and a valuable summary of the whole question up to 1880, by *W. T. Thiselton Dyer*, 'Quart. Journ. of Mic. Sci.,' April, 1880. Smaller papers by Cooke, Berkeley, Thwaites and Morris, are referred to in these.

The external appearance of a leaf severely affected with the "disease" is characterised as follows:—Small, cloudy yellow spots appear on the under side of the leaf; any one of these may be observed to increase in area and depth of colour, spreading centrifugally from a point in a more or less circular manner. Sections of such a spot show that a young mycelium is spreading in the lacunæ between the cells of the leaf, and that the discoloured area corresponds to that occupied by the mycelium. In a few days small groups of orange-coloured, granular bodies, appear externally, and, rapidly increasing in numbers, soon form an orange-red powder on the under side of the leaf; this pulverulent "rust" consists of the *spores*[1] developed by the internal mycelium. They arise in rosette-like groups from the stomata to which the mycelial branches have direct access from within. As age progresses the yellow colour of the "disease patch" becomes darker, and at length brown in the centre; the brown colour, which is due to destroyed leaf cells, &c., spreads centrifugally as before, and at length a shrivelled, dark-brown blotch of dead tissue is all that remains of the affected area.

This is the typical mode of development of the "disease spot," and there are several points of importance regarding it. The oldest part is always the centre, and as we proceed outwards from this, each successive phase is younger than the last. The oldest part appears to be always on the *under* side of the leaf; the discoloration of the upper side and the corresponding appearance of the mycelium there occur later. The appearance of various saprophytic fungi on the old shrivelled spot indicates the completion of the destruction. With these and other phenomena which vary the described course of the "disease spot" we are not at present concerned.

The orange-coloured "rust" consists chiefly of small, somewhat reniform bodies, which, from their structure, behaviour towards reagents, &c., and especially from their manner of germination, I have called *Uredospores*, to distinguish them from a second, less common, napiform spore, which presents sufficient analogies to the typical *Teleutospore*[2] of the *Uredineæ* to warrant the adoption of that name also.

The "Teleutospores" were discovered in Ceylon in March, 1880;[3] they occur mingled with the much more numerous Uredospores on the same "rust" patch, and, indeed, spring from the same spore-group. Mr. Abbay seems to have incompletely figured similar bodies, without understanding their nature,

[1] The evidence which proves this will be found below.

[2] *Vide* 'Second Report to the Ceylon Government,' l, 1880.

[3] *Vide* 'Preliminary Report to Ceylon Government,' June, 1880.

on coffee from Sumatra.[1] Such are, shortly, the external features of the "disease spot," and we may now pass on to the details of form and structure of the parasite itself.

Since the main facts of development are now discovered, it will be perhaps the simplest plan to trace the history of the adult fungus from the *Uredospore*—to relate, in fact, what occurs after sowing the spores on coffee, giving the details of structure as we proceed.

The *Uredospore* ("Sporange" of Abbay and Morris) is figured on Plate I, fig, 1, in various positions, and is seen to be a somewhat kidney-shaped body, broader, and rounded at the free end, and slightly tapering at the other, where it is attached by a very short pedicel to the spore-bearing structure hereafter described (Pl. III, fig. 40, *e* and *f*). The free upper surface is convex from before backwards and from side to side, and is studded with small solid papillæ. The remainder of the surface forms two converging, slightly flattened sides, which gradually meet below in a broad, rounded, saddle-like ridge. This is quite smooth, concave from before backwards, and convex from side to side. The vertical transverse section of such a body is somewhat triangular, with rounded corners; but various figures are obtained by projecting the several oblique optical sections as it rolls over (Pl. I, figs. 1—3). The upper side alone is normally ornamented, though the papillæ at times occur on the upper portions of the otherwise smooth sides; these papillæ are outgrowths of the thick *exospore*, and are usually pointed and regularly distributed on its free surface (fig. 6 *d*).

The granular protoplasmic contents of the spore are enclosed by a delicate hyaline *endospore*, which becomes readily seen on germination, or may be detected by such reagents as sodic-chloride, sugar solution, &c. (figs. 6 and 7), which cause it to contract away from the exospore, to the inner surface of which it was before applied (fig. 4). The contents are usually coloured orange red, and at times contain oil drops of an intense orange-red tint (fig. 5); as a rule, however, the granular matrix is uniform throughout in the fresh spore. Under certain conditions the orange tinge is lost, and the contents of the spore become grey and cloudy. With these and other abnormal changes we are not here concerned. The size of these Uredospores averages $\frac{1}{600}$ inch long by $\frac{1}{900}$ broad and deep.

After lying in water for some hours it commonly happens that many of these spores become filled with spherical vacuoles, closely packed in the granular matrix, of equal or unequal size, and varying in number accordingly. A common appearance is that figured in fig. 7, and the impression of a sac filled with

[1] Loc. cit., Pl. 13, figs. 10, 11 and 12.

spore-like bodies is suggested These spherical bodies are, however, not solid, but cavities excavated, so to speak, in the protoplasm, and filled with watery liquid. In the first place they are not constant, but may be seen to change their position, size, &c., very slowly; moreover, they eventually disappear, not by escaping bodily from the sac, but slowly, and this is accompanied by other changes. On crushing the whole carefully, these spheres are no longer seen in the extruded mass of protoplasm. Staining reagents do not colour them darker than the matrix, and they are not rendered clearer by such fluids as would cause contraction of denser protoplasm; on the contrary, solutions of sugar, salt, &c., and such reagents as iodine, glycerine, &c., make them disappear, evidently by the abstraction of water.

With careful treatment I have caused these vacuoles to reappear, after applying weak sugar solution, on washing in pure water. What is the nature of this vacuolisation? How far is it normal, and how far connected with other phenomena, must be left for future consideration. The proofs that these bodies are vacuoles must, however, I think, be admitted.

The strongest evidence that the papillate body is itself a spore, however, and the basis on which I have chiefly proceeded, is afforded by its germination. This occurs in pure water on glass typically as follows. At about two to five, frequently three nearly equidistant spots, the exospore becomes thinner, and pushed aside by the swelling endospore, and a delicate finger-like tube is protruded from each (fig. 8). This tube has very thin cellulose walls and a blunt, rounded apex. It may dilate slightly just beyond the place of exit, and the constriction at that spot is rendered conspicuous in contrast; otherwise the diameter of the tube is equal, and measures about one fifth of the narrow diameter of the spore (fig. 9). This germinal tube rapidly grows forwards, extending, by apical growth, as a simple unbranched cylinder for some time. As it elongates its diameter remains uniform, and its cavity continuous with that of the spore. The orange-coloured granular spore-contents meanwhile pass along the germinal tube, often presenting a most beautiful streaming motion here and there along their course; vacuoles and oil drops form both in spore and tube, and branches are soon put forth at various points, to remain short or become extended, in the same manner as before (fig. 9). This process is, however, limited, and the amount of growth is clearly dependent upon the quantity of food material originally present in the spore.[1]

[1] Attempts to grow a more extensive mycelium in nutritive fluids of various kinds have utterly failed; this is not surprising in the light of what follows.

After growing thus for some time with a sinuous course and uniform diameter the germinal tube dilates, at some place, usually near the growing end, into an ovoid or pyriform sac-like vesicle, into which all, or nearly all, the coloured contents soon pass (figs. 12 and 13), leaving the rest of the tube and spore empty of everything, except a few granules and frothy vacuoles. Fig. 11 *f* shows in outline what an extensive growth may take place before this sacculation occurs; it usually occurs sooner, however.

The pyriform dilation may remain simple, or put forth branching processes here and there from any point (fig. 14). Sometimes it grows forwards as a simple tube (fig. 15), on which a similar dilatation may arise afterwards, and in this case the coloured contents pass forwards into the new vesicle. This forward growth is very rapid, and accompanied by vigorous streaming of the protoplasmic contents. It sometimes happens that a septum is formed across the neck of this swelling between it and the rest of the tube (fig. 14).

Where the branching is vigorous these vesicular bodies may become very complicated, and assume the most grotesque figures; huge vacuoles, streaming, &c., arise as the growth continues (fig. 14). This is not for long, however, and though the swellings may remain some hours after the remainder of the tube and spore have rotted, they and their contents become at length the prey of Bacteria, Torulæ, &c.

On sowing the Uredospores on the lower surface of vigorous living coffee leaves, I obtained results in the main similar. The germinal tubes produced, however, are usually shorter and less branched, or quite simple, and the whole process is apparently carried on more energetically.

At fig. 16 is represented a piece of the lower epidermis of a cotyledon of *Coffea Arabica*, on which Uredospores had been sown some eighty hours; the spores germinated and put forth the tubes freely as described above. The pyriform vesicle appears very early, and receives the whole of the contents. And now the meaning of the vesicular swelling above described becomes clearer, for it is usually formed over the orifice of a stoma and sends its processes through this into the intercellular spaces of the leaf (Pl. II, fig. 18). This is, in fact, the act of "infection." The Uredospore on germinating produces a simple mycelium (the germinal tube), which grows rapidly at the expense of the reserve material in the spore, and is only capable of further progress on reaching the interior of the leaf in this manner.

The commencement of this further development is well shown in figs. 19 and 20. That the internal mycelium is simply an extension of the germinal tube is proved by such examples as fig. 21: the spore germinating close to the orifice of a stoma, has sent its tube directly through into the leaf without forming the preliminary vesicle. These and many similar preparations were obtained by sowing spores on living leaves kept damp in glass cells, and cutting vertical sections at periods varying from 24 to 100 hours afterwards.

I carried still further the proof of the fact that the internal mycelium is but an extension in the leaf of the germinal tube by sowing spores on the *upper* surface of leaves at places from which the epidermis had been removed; the result was a rapid growth of the germinal tube directly into the tissues, pushing its way between the palisade cells as it advanced (figs. 22 and 23). Here again no vesicle was formed. All attempts to infect by sowing on the uninjured upper surface have failed; the spores germinate, tubes and vesicular swellings form as on glass, but the whole soon shrivels and dies.

The mycelium within the leaf, then, the action and extension of which corresponds to the yellow discoloration seen externally, is clearly but a continuation of the germinal tube sent forth from the *Uredospore*, and which enters a stoma as described. Once established in the lacunæ of the leaf this soon branches, chiefly at first in the plane of the leaf, and feeding upon the products of the cells of its host, produces the injury.[1] At first the young mycelial tubes are very delicate, filled with fine-grained protoplasm, and somewhat stumpy; they soon become vacuolated, and more coarsely granular, and send out tufts of short, thick branches towards the cells bounding the intercellular spaces, while here and there longer "leaders" run out between the cells in various directions.

The main features of the internal mycelium thus produced are typically as follows. Its ramifications are confined to the intercellular spaces (fig. 24), except that at numerous points here and there very slender processes pierce the cell-walls to form haustoria. The mode of branching is extremely irregular, and influenced by the arrangement of the cells between which the branches run; the rate of growth, depending on several circumstances, also affects the length of the branches.

Transverse septa occur here and there, often separated by long intervals (fig. 28), especially at the peripheral parts. The diameter of a mycelial thread is about the same as that of the

[1] As further evidence, I may remark the success of infection experiments based on these observations. In one instance, I made sixteen separate sowings on healthy leaves of as many plants: *in fifteen cases* the "disease spot" appeared *where the sowing was made, and nowhere else on the plant.*

germinal tube, and, like that, generally preserves its uniform calibre throughout. In some cases the similarity between mycelium and germinal tube is rendered more striking from the contents of the former being also coloured orange-red; as a rule, however, the protoplasm of the internal mycelium is colourless. Vacuoles, granules, and oily drops occur commonly in fresh preparations (fig. 27), but in some cases the branch is filled with a dense, homogeneous protoplasm, shining with a grey, pearly lustre (fig. 26). The "coral-like" habit of the tufted, short-branched form is well seen in figs. 25 and 28.

The *haustorium* is a somewhat remarkable structure. It consists of a stiff, long neck, piercing the cell wall vertically from a branch of the mycelium (figs. 25, 29, 30); the distal end is expanded into an ovoid or pyriform body, suspended free in the cell cavity, and containing usually one or two brilliant granules surrounded by a cloudy matrix. In older specimens a distinct wall is evident. Spreading in all directions from the point of entry, the mycelial branches become applied to the exterior of the cells, and feed upon their contents by means of these haustoria, until a stage is reached when the well-nourished vegetative structure commences to form the spores, which appear externally as "rust."

This process is begun by a tuft of branches collecting in a lacuna, and growing towards the orifice of the stoma, close to which their apices remain in contact for a short time; the tightly-packed bundle then forces itself into the orifice, and pushes the common apex through to the exterior (figs. 32 and 33), where the spores are formed by budding.

The first spores arise as follows :—The spore-bearing branches, ormed as above described, are filled with fine-grained grey protoplasm (fig. 33), and on reaching the exterior the apex of each expands into an ovoid sac (Pl. III, fig. 34), in which the protoplasm accumulates. A succession of other similar sacs arise by budding from the parts below this, and thus a tuft of young spores is formed (fig. 35 and fig. 40 *a*, *b*, *c*). Each of these spores arises by the protrusion of an ovoid sac, remaining narrow below, and becoming constricted off at the neck, where a septum also is formed separating the young spore from the spore-bearing branch (fig. 40). A small pedicel or stalk is usually formed, but the spore is readily detached from this, and it is rarely seen on adult specimens, though the circular place of attachment may frequently be detected.

Each spore consists at first of a simple, smooth, thin-walled sac, filled with fine-grained protoplasm, in which a nucleus-like body may be frequently observed (fig. 40, *c* and *e*). At a very early stage the existence of an endospore can be proved, but the exospore remains smooth and thin for some time. As the regular ovoid shape of the spore becomes altered by lateral and other pressures the thickening exospore develops the solid papillæ above, and the contents begin to assume the orange-red tinge.

As the spore-bearing branches (each of which forms spores as above at first) pass through the orifice of the stoma they are crowded together into a neck: below and above this constriction they expand again. As age advances, however, they are found to become coalesced into a kind of pseudo-parenchyma, and the later-formed spores arise from the sides and top of a compound body (figs. 39 and 40 *d*), produced by their union. This structure presents the form of an oval boss, with its lower side attached by a neck, which passes through the stoma to the mycelium within the leaf; its sloping sides are covered with crowds of short, stumpy processes (fig. 41), the remains of pedicels from which spores have fallen. The fusion of mycelial elements may even extend to the internal position close to the neck, and possibly the "dark body" figured by Abbay[1] is this structure, into which air had penetrated.

Viewed from above, the various stages of spore formation are easily discovered. The story is the same. A few ovoid young spores arise (fig. 41 *a*, *b*), and additional ones bud off from near their bases (*c*), until a rosette-like cluster is formed (*e*). The appearance of the old spore-bearing head, formed by the fusion of the spore-bearing branches, is figured at fig. 41 *f*. When the "disease spot" has ceased to spread, and all, or nearly all, the spore-bearing heads have become as advanced as this, the second form of spore is produced.

This *Teleutospore* is a very remarkable body, and it is only intended here to describe its morphological characteristics. It is at first indistinguishable from the young *Uredospore*, but, instead of developing into the reniform papillate structure, it remains somewhat smaller, quite smooth, and expands into a subglobular shape. When fully formed it is napiform, and situated on a short stalk (fig. 44) attached to the spore-bearing head already described (figs. 38 and 39). An endospore is early distinguishable, and the granular protoplasmic contents soon become coloured bright orange red.

Very soon after its complete formation the central portion of the free, slightly flattened end of the oblate spheroid protrudes as a rounded, blunt, boss-like eminence; this goes on until the whole structure assumes the shape of a flask (fig. 39). This outgrowth of the apex is the first indication of germination, and the free, straight, neck-like portion is the promycelium. The

[1] Loc. cit., p. 177, Plate 13, fig. 4, &c.

whole process normally advances to completion, while the Teleutospore is still attached to the spore-bearing head, though detached specimens germinate quite readily in water on glass slips.

When the promycelial tube has attained a length of about six to eight times that of the Teleutospore it becomes divided by transverse septa into four subequal cells (figs. 42—45), each of which receives its share of the orange-coloured contents, which have passed along the tube from the spore. In specimens grown on glass slips under cover the promycelium and chambers are much longer (figs. 45, &c.) than those found on the leaves, and the tube may be curved and delicate in the former case, whereas in the latter the promycelium stands up stiff and straight into the moist atmosphere. This may be compared with what occurs with Uredospores germinating on glass and leaves.

The promycelium fully formed, each of the four cells (normally) sends forth a slender process, into the cavity of which the coloured contents pass (fig. 45); the process from the upper cell is simply a continuation of its apex; those from the sides of the lower cells spring from beneath the septa. The free terminal portion of each of these four outgrowths now swells into the form of a small subglobular *conidium*, which receives the remaining contents, and at last is simply attached by one point to the constricted end of the branch which produced it (figs. 42—46), and may be detached with the greatest ease.

This *conidium*, abstricted in this manner from the promycelium, is much smaller than the *Teleutospore;* it is usually subglobular, but at times subreniform or ovoid in shape, and is filled with the usual orange-red, granular protoplasm (fig. 46), enclosed in a delicate, smooth envelope. During the formation of the structures just described—*i.e.* during the later stages of its germination—the walls of the Teleutospore and promycelium become collapsed (figs. 42—46, &c.), and, being very transparent, are not easily discovered.

The *Conidia* germinate readily in water (and, indeed, even while attached to the promycelium), and may produce a long delicate tube (fig. 46 *d*) very rapidly; as a rule, however, after forming a large central vacuole, the endospore protrudes slowly (fig. 46 *a*) as a blunt, thin-walled, finger-like process, which attains a length of some four times the diameter of the conidium, and then shrivels up and dies. This very simple and transient germination is all I have been able to induce, either on glass or living leaves.

The production of Teleutospores, &c., appears to be the last act of the mycelium within the leaf, and the brown, shrivelled remains of the "disease patch" soon becomes the prey of Bacteria, &c., which follow in the tracts of such parasites as Hemileia.

The foregoing description will naturally provoke the inquiry, to what group of fungi does Hemileia belong? Without insisting upon an answer to this question, I think it may be worth while to review some of the points in this connection. The general similarity of the "disease-spot" itself to the spots produced by many *Uredineæ* is somewhat striking, and the occurrence of the orange-red pigment in all the spore-structures, &c., vividly recals the same. The mycelium, ramifying in the lacuna and forming tufted groups here and there externally; the septa, sometimes separated by long intervals, sometimes more closely arranged; and again, the centrifugal spread of the fungus, are all points of analogy worth recording.

It seems impossible to overlook the resemblances of the two spores of Hemileia to the *Uredospores* and *Teleutospores* of an ordinary Uredine. In size, colour, ornamentation of the exospore, mode of germination, and entry of the germinal tube through the stoma after forming a vesicular swelling over its orifice, we have strong analogies, so far as the papillate spore is concerned.

The smooth, turnip-shaped spore, in its mode and time of origin, shape, structure and colour, and especially in its germination, so strongly recalls the *Uredineæ,* that I ventured to use the well known name Teleutospore. Indeed, the promycelium, with its four cells and conidia, might almost pass for that of *Uromyces* for instance.

Nevertheless, there are some difficulties in referring Hemileia to the ordinary Uredines. The curious spore-bearing head which protrudes through the stoma, and the long-necked haustoria, so numerous on the mycelium, are perhaps the chief. How much weight these difficulties carry may be an open question. In cases where two spore-bearing heads have passed through two closely adjacent stomata, it rarely occurs that the one or two intervening cells have become destroyed; the two heads here become one common, broad, and irregular receptacle, and very like an ordinary uredinous patch.

In conclusion, it appears necessary to make a few remarks on the other forms of fungi, believed by Messrs. Abbay and Morris to be phases in the life-history of *Hemileia vastatrix.* It is impossible to explain all the points raised without numerous drawings, for which there is not room here; at a future date I hope to illustrate more fully the following brief statements.

The forms figured by Abbay[1] are very common in germina-

[1] Loc. cit., Pl. 14, figs. 10 to 19.

tion experiments out here. I recognised them at an early date, cultivated them for several months, and through several generations; they produce mycelia and forms of fructification and spores, &c., which have nothing to do with Hemileia. Their connection with the spores of the latter fungus is not genetic. So with the forms illustrated by Morris;[1] they belong to saprophytic or epiphyllous forms, and can in no way be genetically connected with Hemileia. Of course in such statements I am not resting on the negative evidence that no connection has been traced, but upon the results of actual cultivation of these forms, as well as the successful propagation of Hemileia itself as above described. *May 1st*, 1881.

[1] 'Quart. Journ. Mic. Sci.,' April, 1880, Plates X, XI, XIII, and XIV.

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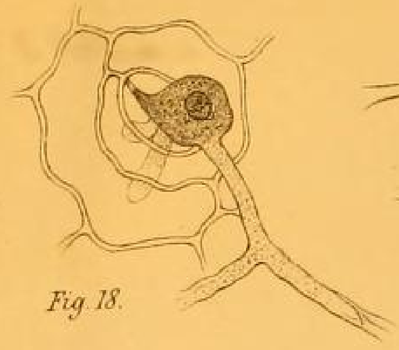
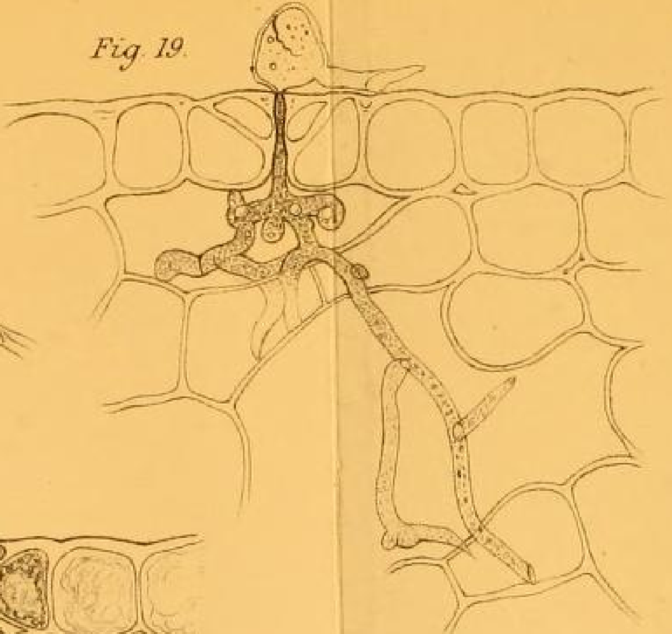


Fig. 18.



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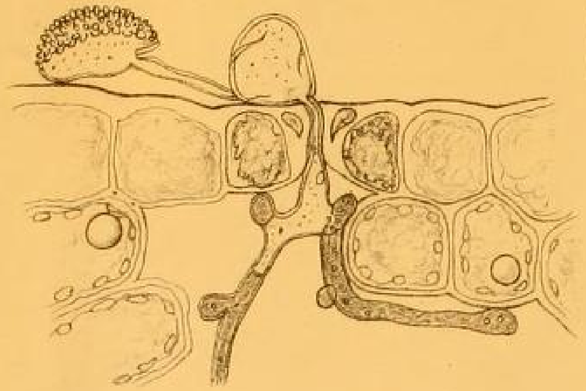

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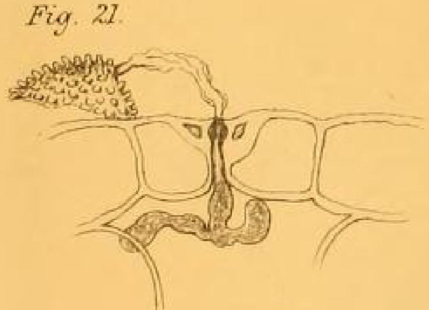

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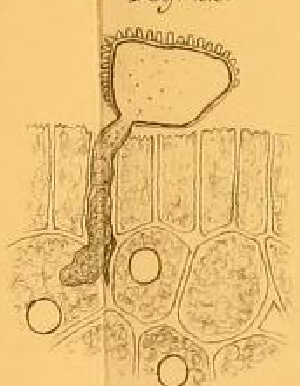

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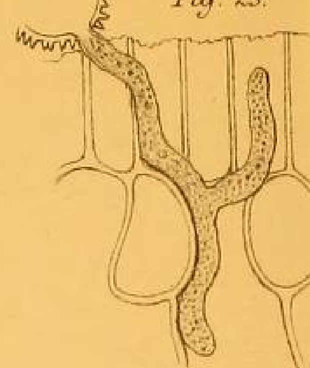

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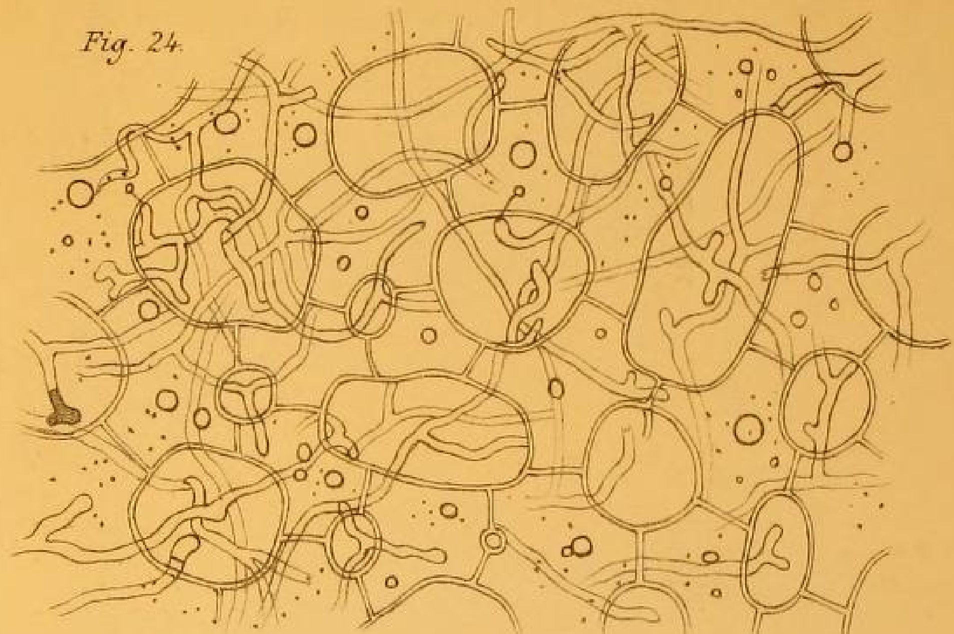


Fig. 24.

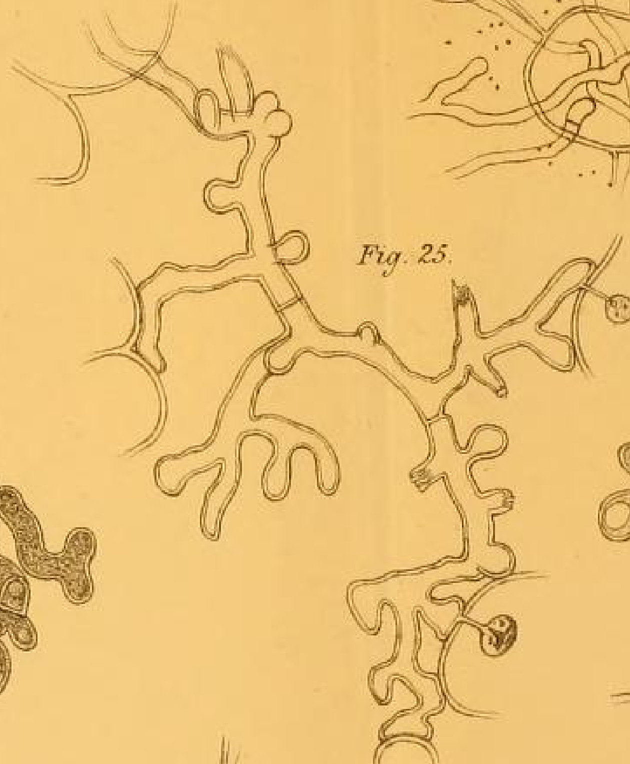
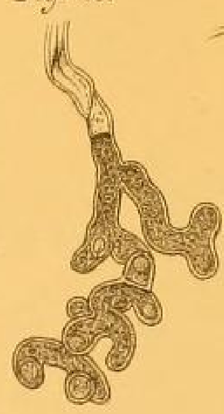


Fig. 25.



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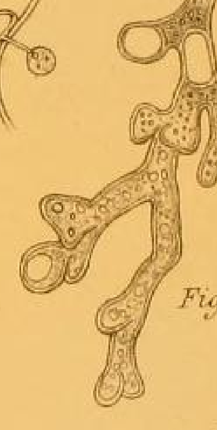

Fig. 27.

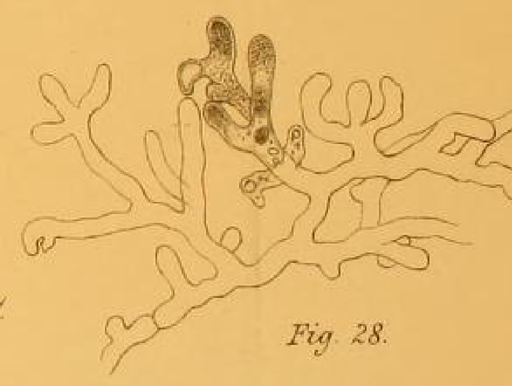

Fig. 28.

Fig. 29.

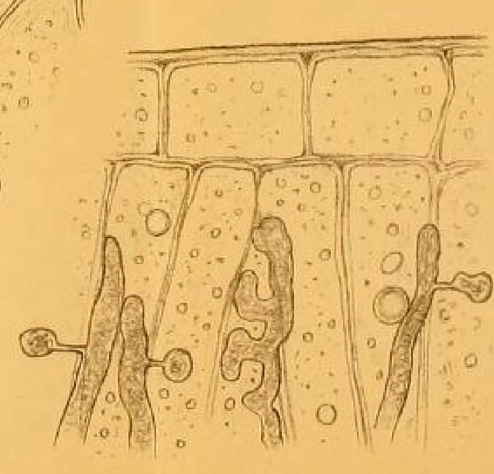

Fig. 30.

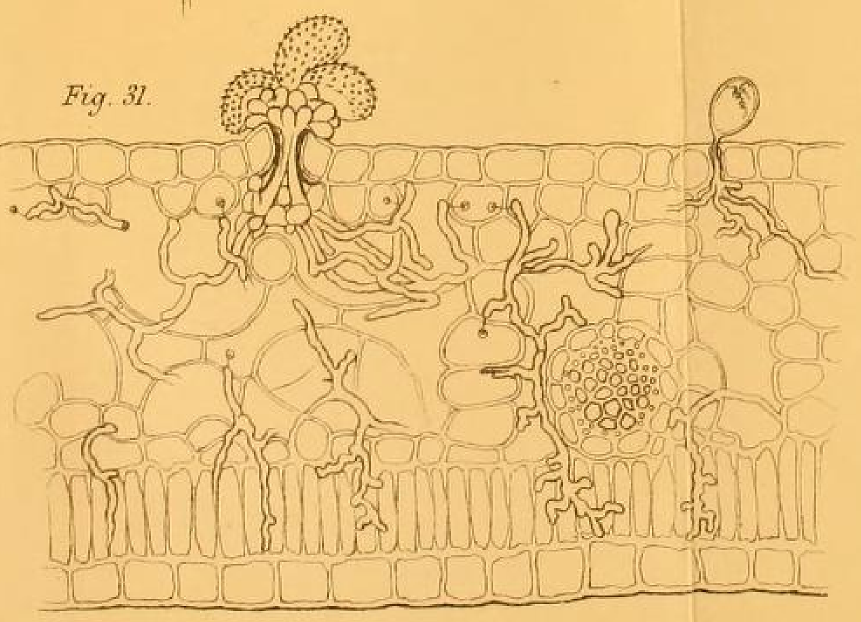

Fig. 31.

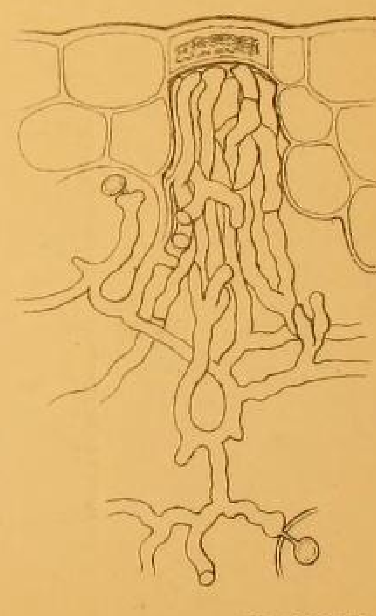

Fig. 32.

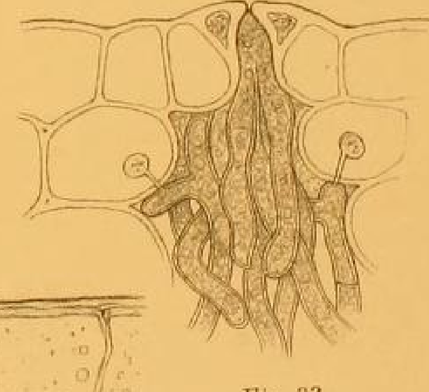

Fig. 33.

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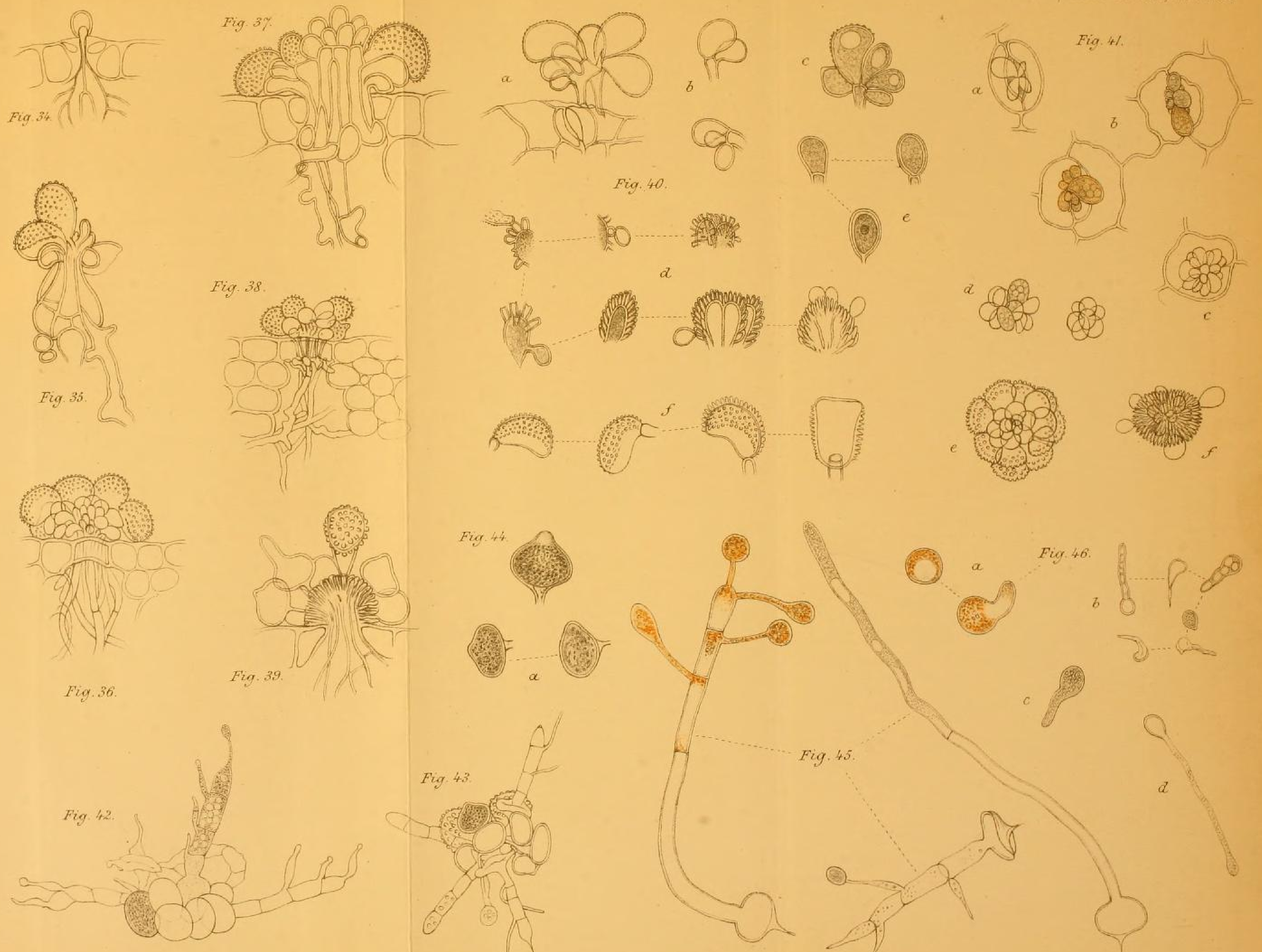

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