II. *On the Histology of* Uredo dispersa, *Erikss., and the "Mycoplasm" Hypothesis.*

By H. Marshall Ward, Sc.D., F.R.S., *Professor of Botany in the University of Cambridge.*

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[Plates 4–6.]

There are still so many and such important gaps in our knowledge of the biology of the Uredineæ, that no one will expect an apology for a serious attempt to fill any of them, and the less so in the particular case of the Rust-fungi of the Gramineæ and cereals, because, notwithstanding the numerous researches which have succeeded each other since De Bary's classical investigations, the whole question of the origin and spread of epidemics of rust has been raised again owing to the remarkable discovery of the specialised parasitism exhibited by certain adapted races of these fungi, and to the still more remarkable assumptions published by Eriksson in what is known as the "Mycoplasm" hypothesis.

I have from time to time brought before the notice of the Royal Society and elsewhere, the results of my own investigations into the biology of that particular form of Rust-fungus known as *Puccinia dispersa*, Erikss., more especially with reference to its peculiar parasitism on the grasses of the genus *Bromus*, and have treated successively of the questions of predisposition and immunity,* of the relations between the host and the parasite,† of the method of pure cultures of such a Uredine‡ as the one in question, and of the effects of mineral starvation on its parasitism.§ In all these cases, however, the studies were chiefly experimental, dealing with the physiology of infection, and the publication of the results of the extensive and far more laborious microscopic investigations was reserved, a few remarks only being made here and there showing the directions in which these were leading from time to time.‖

* 'Camb. Philos. Soc. Proc.,' vol. 11, 1901, p. 307.
† 'Ann. of Bot.,' vol. 16, 1902, p. 233.
‡ 'Roy. Soc. Proc.,' vol. 69, 1901, p. 451.
§ 'Roy. Soc. Proc.,' vol. 71, 1902, p. 138.
‖ *E.g.* 'Ann. of Bot.,' *loc. cit.*, pp. 275—277.

I have now so far completed the microscopical examination of the Uredo-stage in *Bromus*, and compared the results with what happens in wheat and other cereals, and with the behaviour of other adapted races or special forms of the fungus, notably *P. graminis*, PERS. and *P. glumarum*, ERIKSS. and HENN., that I am able to generalise regarding the life-history of this Uredo with some confidence, and since the results obtained appear to be quite decisive so far as the assumed existence of any mysterious "mycoplasm" is concerned—or, at any rate, as decisive as histological evidence is likely to be—it seems advisable to publish them now.

I should like it to be understood that the results here put forward are based on microscopic work extending over more than a year and a half, and involving the cutting and preparation of many thousands of sections in all stages of development. This labour has been greatly reduced in its later stages during the present winter owing to the kindness of Miss E. Dale, of Girton College, who was so good as to volunteer her aid in embedding and cutting the sections of material I had put up under the necessary conditions during the past summer and autumn. My thanks for this valuable assistance in the somewhat dreary details of technique are the more emphatic, in that it would have been impossible to complete the task so quickly without this aid.

So far as the present publication is concerned all the figures are from my own drawings of preparations, for the staining and control of which I am also entirely responsible.

In 1897, ERIKSSON, of Stockholm, apparently overwhelmed by the numerous difficulties in detail which he met with in his field experiments on the rusts of wheat, &c., found himself driven to the conclusion that the sudden and wide-spread epidemics of these rusts of cereals and other grasses are not to be explained without the assumption of an invisible infective substance, handed on from plant to seed and growing up with the seedling until a certain stage in the development of the latter, when it bursts forth in epidemic form, and at innumerable spots simultaneously.

ERIKSSON supposes that this invisible state of the parasite is such that minute portions of naked fungus-protoplasm are mingled with the normal cell-protoplasm of the host, living with the latter in a state of temporary harmony, and being handed on from cell to cell of the embryo and plant at each division, so that, when the favourable season arrives the grass plant is more or less saturated, as it were, with the invisible infective substance, which then suddenly takes on the mycelial form, pierces the cell-walls, and grows out into the intercellular spaces as the well-known hyphæ which rapidly reach the epidermis and form the characteristic spores.

ERIKSSON'S first definite statement of his hypothesis appeared in the 'Comptes Rendus' for 1897,* under the title "Vie latente et plasmatique de certaines Urédinées," and he proposes to term this hypothetical state of affairs "mycoplasma-

* 'Comptes Rendus,' vol. 124, No. 9, 1897, p. 475.

symbiose"; the hypothetical mixture of cell-protoplasm and infective substance he terms "mycoplasma."* Although there is at first sight a suspicion of mediævalism about this hypothesis, ERIKSSON expressly repudiates any intention to arouse the ghost of spontaneous generation. It need hardly be stated that anything on the subject of Rust-fungi coming from ERIKSSON was bound to attract attention, and this remarkable hypothesis has probably caused more flutterings in the dove-cotes of mycological science than any pronouncement since DE BARY'S classical memoir on parasitic fungi in 1863, or KOCH'S paper on Anthrax in 1876, and at first sight it appeared to be intended to supplant the firmly-established theory of infection by means of the Uredo-spores which, germinating on the leaf, send their infecting tubes *viâ* the stomata into the inter-cellular spaces of the host, where they spread as a mycelium which shortly puts forth spores again. But ERIKSSON does not intend to deny that this normal infection by means of the Uredo-spores occurs, exactly as DE BARY† described it; and, indeed, in a previous work‡ he and HENNING had already given excellent drawings once more confirming, as have so many other observers, the scrupulous accuracy of DE BARY'S work.

ERIKSSON'S chief argument is, that numerous as are the Uredo-spores, the sudden epidemic outbursts of rust over large areas of country are not to be explained by the theory of infection by wind-blown or otherwise dispersed spores, or by other circumstances of external infection, and that some other mode of infection must be assumed to occur.

In ERIKSSON'S first announcement of his hypothesis, there occurs a statement which I must quote in full, because it contains the gist of the only positive observations, so far as I can discover, that he has made on the subject. He states:—"En examinant ces taches de rouille très jeunes sur les feuilles de froment, j'ai découvert, à l'aide d'un grossissement puissant, que les cellules chlorophylliennes renferment des *corpuscules spéciaux*. Ces corpuscules, mélangés aux autres éléments des cellules, sont plasmatiques, d'une forme oblongue le plus souvent, un peu recourbés; ils sont solitaires ou réunis dans chaque cellule. Quelques-uns paraissent flotter librement dans le protoplasma; d'autres ont atteint la paroi; d'autres enfin se sont ramifiés, ont perforé cette paroi et ont émis au dehors un filament mycélien intercellulaire, en laissant un suçoir à l'intérieur de la cellule. À une distance plus grande de la tache, on n'observe plus ni filament ni corpuscule."

It will readily be understood that we awaited with great interest further particulars regarding these "*corpuscules spéciaux*," the first visible materialization, as it were, of the hitherto invisible "mycoplasm" assumed in ERIKSSON'S hypothesis, since the original paper contained no figures.

For these, however, we had to wait until 1902, when ERIKSSON published an

* *E.g.* 'Ann. des Sci. Nat.,' 8 Sér. (Botanique), vol. 15, 1902, p. 193 (separate).

† 'Ann. des Sci. Nat.,' Sér. 4, vol. 20, 1863.

‡ ERIKSSON and HENNING, 'Die Getreideroste,' 1896.

exhaustive paper* on the subject, and devoted one plate to illustrations of these *corpuscules spéciaux*. Now, it may be at once stated that these "corpuscules" occur exactly as, and where, ERIKSSON says they occur, but to my astonishment I found the figures to represent certain bodies in the cells of infected plants, with which I was—so I thought—perfectly familiar, and had figured so long ago as 1881 in another genus of Rust-fungi.† ERIKSSON'S figures represent such organs in an imperfect form, the necks connecting them with the hyphæ, as well as the latter themselves, being wanting, as indeed are all details regarding the cell-contents also.

In other words, ERIKSSON'S figures conveyed to my mind the impression of haustoria, the attaching necks of which had been cut off, or hidden, or not seen by him. If this is so, he has entirely misunderstood the significance of the facts, and has read the sequence of events backwards. His whole contention turns upon this.

Before passing to the discussion of this question, however, it will be advisable to examine ERIKSSON'S position somewhat more in detail.

In his later paper, he gives the following instructions for observing the so-called first appearance of the hitherto invisible and undiscoverable "mycoplasm" as it assumes the visible mycelial condition.

He takes the young pustules, at as early a stage as possible, and proceeds as follows.

I quote ERIKSSON'S exact words, because, as will be seen later, something depends on his procedure.

"Le traitement que j'ai trouve le meilleur pour les rendre bien visibles est celui qui suit. Des sections très minces, faites à la main dans de la moelle de sureau, sont d'abord plongées, durant cinq minutes, dans de l'alcool, et ensuite pendant deux à cinq minutes—à varier selon l'épaisseur des sections—dans une solution d'hématoxyline alcoolique de 3·5 p. 100. Après cela elles sont lavées, durant trois à cinq minutes, dans une solution d'alun de 2 p. 100 et sont enfin mises dans une goutte de glycérine pour être ensuite examinées au microscope."‡

Now ERIKSSON expressly states that no attempts to make visible his hypothetical "mycoplasm" in the cells of the seed, the embryo or the plant at any stage of its existence, have been successful, and he assumes that these curious *corpuscules spéciaux* are the first visible signs of the hitherto latent and invisible "mycoplasm," caught in the act of separating itself from the normal cell-protoplasm, to pierce the cell-walls of the host and march out on a triumphant career as an intercellular mycelium, which then branches and extends throughout the disease-spot.

Several reflections occur to an attentive observer at this stage.

If we really have here an erumpent and aggressive stage of the fungus, suddenly manifesting itself—ERIKSSON (*loc. cit.* p. 193) says, "Le temps qui s'écoule entre la

* "Sur l'Origine et la Propagation de la Rouille des Céréales par la Semence," 'Ann. des Sci. Nat.,' 8 Sér. (Botanique), 1902, vols. 14 and 15.

† "On the Morphology of *Hemileia vastatrix*," 'Qu. Jl. Micro. Sci.,' vol. 21, 1881, Pl. 2, figs. 29, 30, &c.

‡ *Loc. cit.*, p. 192 (separate).

séparation du germe mycélien d'un côté et la formation proprement dite du mycélium de l'autre est peut-être à compter en heures . . . ," is it not remarkable that the contents of the delicate cells of the host, with their nucleus, chlorophyll-granules, &c., suffer no apparent injury! It would seem almost incomprehensible that the hitherto dormant or latent "mycoplasm," already inside the cell in the best possible position for attack, should take the trouble (so to speak) to emerge into the intercellular spaces, where they are at a far greater disadvantage, and then turn round and besiege the very fortresses they have just abandoned and left uninjured; and when we add that the relatively enormous expenditure of energy thus represented in the growth of the "mycoplasm" into an abundant mycelium, is nevertheless unattended by any discoverable disturbance in the cell-contents of the abandoned cells, the apparent inconsequence of the phenomena seems overwhelming.

Again, let it be noticed that ERIKSSON'S *corpuscules spéciaux* are to be looked for, according to him, at the *margins* of the young disease spots. His words (*loc. cit.* p. 191*) are, "Nous voyons alors que *dans la continuation immédiate des raies de pustules*, à une distance de 5 à 10 millimètres de la pustule extrême, il y a, surtout sous l'épiderme supérieur, des cellules chlorophylliennes qui renferment—excepté les éléments ordinaires, comme par exemple le protoplasma, le noyau et la chlorophylle—*une sorte de corpuscules spéciaux*." Now, if each of these *corpuscules spéciaux* is the commencing centre of a new disease pustule, then ERIKSSON'S own admirable descriptions of the distribution and extension of the disease-pustules on the leaves are inconsistent with his hypothesis of their origin. If each of these *corpuscules spéciaux* is the centre of a disease-pustule, from which young hyphæ are radiating outwards, then we ought not to have to seek for the "corpuscules" in a zone of cells just marginal to the disease-pustule and its radiating mycelium. Yet this is what we have to do.

But this is not all. Let us examine ERIKSSON'S description of the *corpuscules spéciaux* a little more closely. He says (*loc. cit.*, p. 193, separate) "nous voulons faire remarquer assez souvent des cellules, contenant des germes mycéliens libres, contiguës à des cellules où ces germes ont atteint la paroi, l'ont perforée et ont émis au dehors un filament mycélien," and, further on (p. 194), "Aussitôt que le germe mycélien a atteint la paroi de la cellule, l'a perforée et a émis au dehors un filament mycélien intercellulaire, le champignon est entré dans son *état mycélien*. Une fois qu'il y est entré les pustules ne tardent pas à apparaître."

And, further (p. 194), "Suivant cette manière d'envisager la chose, on devrait dans les formations que depuis longtemps on connaît sous le nom de *suçoirs*, et qui dans ces taches de pustules primaires se recontrent en si grande abondance, voir, tout simplement, des restes du germe mycélien laissés dans le lumen de la cellule après la perforation de la paroi par le germe mycélien." . . .

* The italics are in the original.

"Dans ce cas il faudrait ainsi considérer ces suçoirs *comme des restes de germes mycéliens et nullement comme des formations secondaires ayant pénétré du dehors.** Comment il en est avec des suçoirs d'un mycélium né d'une autre manière, c'est-à-dire d'une inoculation au moyen d'æcidiospores ou d'uredospores, si après tout, il y a des suçoirs pour un tel mycélium, c'est là une tout autre question."

Here then the crux of the "mycoplasm," hypothesis is reached, in so far as histological investigation is concerned, and I may now pass to my own observations.

For the present, I propose to confine myself especially to the questions raised in what precedes, and to pass over the evidence accumulated which suggests that ERIKSSON has laid too much stress on negative results. I have shown elsewhere† that we have still much to learn concerning infection and the predisposition or immunity of host plants, as also concerning the peculiar behaviour of spores and mycelia towards different hosts, the action of the environment on germination, &c.; and in a forthcoming paper‡ I have shown that Uredo-spores can retain their germinative power longer than is usually supposed, and that a certain species of grass (A) may be capable of infection by means of spores from two other host-plants (B and C), neither of which is predisposed to reciprocal infection, though both may be infected from such a "bridging" species (A) as is referred to above.

That the mycelium of a Uredine, in the intercellular spaces of the host, may sometimes grow very rapidly and extensively, and at others slowly or not at all, was also pointed out by me some time ago,§ and I was for a long time suspicious that certain dormant conditions of the mycelium must be the important factors with which ERIKSSON was concerned.

It will be noticed that ERIKSSON's figures of the "*corpuscules spéciaux*" are unaccompanied by any details as to the other cell-contents. I have observed these "corpuscules" in abundance, and just in the positions he indicates—at the margins of young pustules—and they occur in all the species and varieties of Uredo and of host-plant examined.

Like ERIKSSON, I also have failed to discover any trace of visible parasitic cell-contents in the seeds, embryos or seedlings of even the most predisposed species of host. It is true, my work has been chiefly confined to the Bromes, but by no means entirely so, and it is almost inconceivable that if any such "infective substance" as the hypothetical "mycoplasm" existed in the cells, the methods adopted would have been entirely without positive results.

Having concluded that no hope of discovering any such body could be entertained so long as one merely investigated the organs of the host in stages chosen at

* The italics are here mine.

† 'Camb. Phil. Soc. Proc.,' vol. 11 (1901), p. 307; 'Roy. Soc. Proc.,' vol. 69 (1901), p. 451; 'Ann. Bot.,' vol. 16 (1902), p. 233; 'Roy. Soc. Proc.,' vol. 71 (1902), p. 138.

‡ 'Annales Mycologici,' 1903, vol. 1, pp. 132—151.

§ 'Ann. Bot.,' *loc. cit.*, p. 237.

haphazard, as it were, and since no seeds, however shrivelled from disease, appeared to yield any signs of "mycoplasm," any more than did portions of the leaves of the most "predisposed" seedlings I could find, I abandoned all such procedures, and confined my attention to the detailed life-history of the fungus, from the moment it enters the leaf to the period of spore formation, and this with such success that it is now possible to say with almost absolute certainty, what this particular fungus is doing on any given day, in the tissues in which the mycelium is growing.

In this part of the work the methods and procedure adopted were as follows:—

During the spring and summer I grew a number of seedlings of various species of Bromes in the closed tubes adapted for the pure culture of Uredines as described in 1901,* and when the plants had developed the second and third leaves I carefully infected these, at definite spots, with Uredo-spores from a definite host—sometimes the same species, sometimes another. In other tubes, highly predisposed species of the grass were grown, but were not infected, the object being to see if any difference in the cells could be detected. The latter, in fact, served as control plants.

The spores placed on the leaves rapidly germinated and commenced to infect the latter at the spots marked.

Some of the specimens in course of being infected were removed from the tubes after the first 24 hours, and the spore-laden spots cut off and preserved. Others were removed after 48 hours, or on the 3rd, 4th, 5th, or 6th day respectively, and in each the infected area was at once cut out and dropped into a fixing solution, and suitably hardened and prepared for future examination, each bottle being properly labelled and notes kept of essential correlative phenomena. The material was then put aside for examination in the autumn and winter.

This method ensures that when sections are cut—and the research of course involved the examination of thousands of such—no time need be wasted in examining, without knowing it, parts of the leaf on which no spores had been placed.

The properly hardened material was then embedded, and cut in the usual way, the sections being examined in series so that all sequential phenomena could be traced. Moreover, I examined such series of sections cut in all three planes—transverse, longitudinal, and tangential—and compared non-infected sections at every stage.

The results were beyond all expectation, for I found that such carefully hardened material enables one to obtain sections of practically any degree of thickness, and which can be stained so sharply and differentiated so exquisitely that not only can one trace the germination of the spore on the epidermis on tangential sections, and trace the entry of the germ-tube through the stomata in sections cut in all three planes, but the details of growth of all the hyphæ developed from the latter can be followed, their septa, nuclei, and branches observed plainly, and all stages in the development of the haustoria and in their growth, branching, &c., in the host-cells

* 'Roy. Soc. Proc.,' vol. 69, p. 451.

can be clearly traced, as well as their behaviour towards the cell-nucleus and other cell-contents of the host. This method has, moreover, the important advantage that all the cut pieces of hyphæ lying loose in the lacunæ are fixed *in situ*, and not washed out as in loose sections such as ERIKSSON used.

The germination of the Uredo-spore on the epidermis is usually effected during the first 24 hours, and the tip of the germ-tube can be seen preparing to enter the stomata in abundance during the second day. I have, moreover, succeeded in fixing, hardening and staining the germ-tubes and their nuclei, the preparations being then mounted in balsam.

The young germ-tube grows very rapidly, and the nucleus of the spore soon passes into it, and by suitable methods of hardening and staining can be detected either somewhere along its course or near the tip as it approaches a stoma to enter it. (Fig. 2, *bis.*) In some cases two or more nuclei are seen in the tube, and the resemblance to a germinating pollen-grain is striking. The first stage in inoculation is evident in the swelling of the tip of the germ-tube over the stoma into a thin vesicle, into which all the nucleated protoplasm derived from the spore passes. This vesicular swelling—the so-called *appressorium*—then puts a thin process down through the stomatal slit, which process again swells out into a vesicle on the inner face of the stoma and projecting into the respiratory cavity, and the protoplasmic contents are passed through from the external *appressorium* to the internal vesicle (Plates 4–6, figs. 3, 21–23 and 40.)

The latter quickly bulges out at one or more points in the form of a tube—the true infecting hypha—into which the whole, or part of, the vesicular protoplasm passes and its nucleus soon divides therein. (Fig. 3.)

In figs. 1 and 2 I have shown a stoma from above and in longitudinal vertical section, and in fig. 21 the transverse section can be made out clearly.

In fig. 21 a portion of the *appressorium* is clearly visible over the transverse section of the stoma: its stomatal process, and the internal vesicle, still retain some protoplasm, though the greater part has passed into the infecting hypha, in which two nuclei are plainly visible.

In fig. 22 two germ-tubes have attacked the stoma, which is cut in the longitudinal median plane, and have formed internal vesicles and infecting tubes. The remains of the germ-tube are still visible attached to the right-hand *appressorium.*

In fig. 20 are drawn three successive sections of one and the same stoma in transverse section, showing, in addition to the phenomena already described, the formation of the first haustorium from the vacuolated and nucleated, but as yet unseptate and unbranched infecting tube: this haustorium penetrates the second epidermal cell counting from the stoma, showing at what an early stage the direct attack on the host cells is initiated.

Even more complete is the preparation drawn in fig. 40. The stoma is in slightly oblique vertical longitudinal section, and shows the germ-tube and *appressorium*

outside, connected through the slit of the stoma with the internal vesicle; the latter has emitted an infecting hypha to which it has yielded all its protoplasm, in which vacuoles and nuclei are discernible. This hypha has glided between the surfaces of the epidermal cell and the chlorophyll-cell nearest the stoma, and has formed a septum and a branch below it just beneath its tip. The branch has dipped down behind the next chlorophyll-cell, while the tip has put forth a distinct haustorium into the latter cell.

It will thus be seen that infection may be fully consummated by the fourth day, and the further extension of the mycelium between the cells of the host now follow rapidly.

The vesicles or appressoria, infection tubes, &c., may be visible much later than this, however, and it seems probable that infection can occur after delay, at least up to the sixth or eighth day (see fig. 4), a point worth investigating. When the infecting tube has been developed, it usually plunges directly across the intercellular spaces beneath the stoma, and if very vigorous may give rise at once to numerous branches (fig. 23), the principal members of which are stout and much nucleated (fig. 24). But in other cases the infecting tube, or a branch of it, passes laterally beneath the epidermis, and at once forms a haustorium (figs. 4, 20 and 40).

I am inclined to suspect that subtle relations between cell and hypha are concerned in these matters, or that the relative vigour of the spore and its resulting germ-tube, &c., may be the important factors. Here, at any rate, we see the first signs of power to truly infect.

The examination of many hundreds, or even thousands, of sections has convinced me that this extension never proceeds far from the centre of infection marked by the stomatal vesicle, and I have never been able to detect the slightest signs of the fungus in any area not infected from outside.

It may here be repeated that I have examined numerous diseased grains, and seedlings grown from them, as well as seedlings and leaves of species so predisposed to rust that it is with great difficulty they can be grown free from it, and have fully confirmed ERIKSSON'S experience that no trace of mycelium can be discovered in the apparently healthy leaves or in the embryo. ERIKSSON, it is true, assumes that it is there—or may be there—though invisible, in the hypothetical condition of mycoplasma, but that it is non-discoverable by microscopic methods.

During the fourth to the sixth day the mycelium extends rapidly, and soon attains the maximum of its development. In this period two chief phenomena are to be noted, namely, the lateral spread across the breadth and depth of the leaf by means of short branches of the hyphæ, which rapidly occupy the intercellular spaces between the parenchyma cells, and put forth haustoria into them and into the epidermal cells, and into the cells sheathing the vascular bundles, and, secondly, the rapid extension up and down the leaf by means of long, almost straight hyphæ, which grow so quickly and have such long segments and so few branches that they remind

one of runners in higher plants. Indeed they may for purposes of description be shortly so termed (*e.g.*, figs. 23, 24 and 27).

Neither the runners nor the transverse hyphæ invade the vascular bundles, while the latter gradually fill up all the interstices of the infected area, the runners quickly reach the limits of extension, which are co-incident with the longitudinal extension of the disease-spot. It is in the area last occupied by these hyphæ that the "*corpuscules spéciaux*" of ERIKSSON are most easily found, partly because these "corpuscules"—*i.e.*, haustoria—are here younger and less numerous and voluminous in the cells, the contents of which they have not yet had time to injure, and partly because the hyphæ in the intercellular spaces are not yet so abundant as to obscure the view. The dimensions of these hyphæ vary considerably, not only in different hosts and species of Uredo, but even in the same disease spot. That the hyphæ contain nuclei has already been stated, and is indeed well known. These nuclei are small in the more elongated and older hyphæ, but in the young terminal segments may be relatively large, and two, three or four in number. (See figs. 5–8.)

In properly stained specimens they are frequently very conspicuous, as the figures show, and are oval or spheroidal, and may exhibit a distinction into nucleus and nucleolus (fig. 9). I have been unable to satisfy myself of the existence of definite chromosomes, but the fact that nuclear division may be accompanied by the well-known figures (see fig. 25) suggests their existence. Delicate staining with alum hæmatoxylin, saffranin, or diamant-fuchsin and licht-grün always bring these nuclei out sharply, and their regular co-existence in pairs in the terminal clavate segments of the older hyphæ, which are mobilising beneath the epidermis to form spore-layers, is very striking (fig. 10).

It may be added that the variations in colour produced by the fuchsin-green method, seen on comparing, *e.g.*, figs. 5–10, are due partly to the developmental state of the hyphæ and nuclei, and partly to the control of the successive stains; and the same is true of the difference in the colour brought out in haustoria and cell-nuclei, &c.—*cf.* figs. 10–16 for instance.

Too prolonged action of the green removes too much of the fuchsin, as a rule, a fact of which considerable use may be made in producing sharp contrasts. Thus, the cell-nuclei in figs. 1–3 and 15 are intense red; whereas in figs. 16 and 16*a* they are vivid green; in figs. 11 and 16 the haustoria are bright green, but in fig. 16*a* they are nearly pure red. In other cases intermediate hues due to less perfect replacement of the fuchsin by the green are observable, *e.g.*, figs. 4, 7, 11, and 12, &c. Beyond the general statement that the more nuclear substance, or dense protoplasm there is in the organ, the more tenacious it is of the fuchsin, I do not propose to lay stress on these matters.

Haustoria are to be found throughout the diseased area, *and nowhere else.* They are not developed by the quickly extending runners, but are abundantly formed by the short branches occupied in filling up the intermediate lacunæ between the cells.

They are put forth by these hyphæ, generally from the extreme tip, into the cells of the epidermis (figs, 4, 16, 20, 34, 37–39) and even into the guard cells or companion cells of stomata (figs. 14 and 15), as well as into the chlorophyll-cells of the mesophyll (figs. 12, 13, 30, 40–43, &c.) and into the cells bounding the vascular bundles (figs. 16*a*, 31 and 33), but never into any element of the bundle itself. In fact they accompany the filling up hyphæ in all the extra-stelar tissues, but nowhere else. In no case—a conclusion based on months of search for them—can they be found beyond the limits mentioned.

I have already said that "corpuscules" such as ERIKSSON figures occur in the positions he indicates, both with and without the little protuberance which connects the "corpuscule" with the cell-wall. For instance, in fig. 27 the upper cell contains one "corpuscule" without and one with the attachment to the wall, while the lower cell has a much curved one close to the cell-nucleus. In fig. 31 the upper complete cell contains a "corpuscule" in contact with the cell-nucleus, showing the narrow neck of attachment to the cell-wall, as well as a more elongated one with the neck attaching it to a hypha outside the cell. The cell to the left above, again, shows a characteristic "corpuscule" in contact with the cell-nucleus, as also does the cell below one free from the nucleus.

The definite point at issue is, What are these "corpuscules," and in what relation do they stand as regards the fungus?

Perhaps the best form of exposition I can adopt is to describe their development, and the methods I have adopted for investigating them.

The development of these haustoria has been studied very carefully, since it is clear that the crux of ERIKSSON'S hypothesis resides in the relations of these haustoria —his so-called *corpuscules spéciaux*—to the rest of the fungus and to the contents of the cells of the host.

The development of the haustorium begins as a small and extremely delicate peg-like process from the hypha (fig. 14). This pierces the cell-wall and at once swells up at its distal end inside the cell into a minute spherical head, which, in good preparations, *e.g.*, stained with diamant-fuchsin and licht-grün, or with saffranin and licht-grün—is seen to be already provided with a nucleus (figs. 12 and 15). In many cases the young haustorium is surrounded with a sort of halo, of some substance which may be gelatinous in nature (figs. 20 and 40), and is possibly due to alterations in the protoplasm of the cell pushed before it. Very often the point of entry is in the neighbourhood of the cell-nucleus (figs. 16, 16*a*), but this is by no means always the case (figs. 12, 15, 30, 32, 35, &c.). Soon after its entry into the cell, the head of the haustorium grows out, often irregularly, into a vermiform (fig. 44) curved (fig. 30), or irregularly branched or lobed (fig. 42) or pyriform body (figs. 13, 16*a*, 43, &c.), or may be shaped like a hammer-head (fig. 33) or a sausage (figs. 16*a*, 31, 32). In many cases the growth is directed towards the nucleus, even in cases where the point of entry was far distant in the cell (figs. 12 and 32). But here again there seems to be

no constancy in the procedure, at any rate at first, though it should be observed that one is not always so fortunate as to secure both haustorium and nucleus in the same cell.

When young, the haustorium stains deeply, but almost always differently from the cell-nucleus, from which it is easily distinguished, moreover, by its less granular contents, as the figures on Plate 4 show.

Even older haustoria show a well-marked nucleus in properly prepared sections (figs. 13, 30, 32, 43, &c.), and, as they enlarge, the contents usually show well-developed vacuoles, sometimes very large (*cf.* figs. 30, 32, &c.).

In certain stages the older haustoria swell up during preparation, and may even assume a globular distended form, well seen in fig. 12. Finally, young haustoria occasionally show a curious toothed outline due to minute stainable papillæ on the surface. This is just visible in fig. 16, but is often much more pronounced. I cannot explain these papillæ, unless they are the remains of the investing gelatinous halo seen on haustoria which have just penetrated the cell (figs. 37 and 41).

One of the most surprising features of the invaded cells is the longevity of their cell-contents. Even in preparations of tissue thoroughly infested for some days, and in which the mycelium has already burst through the epidermis as spore-bearing pustules, the nucleus, chlorophyll-corpuscles and cytoplasm may retain their form and colour, and even their normal capacity for staining (see fig. 10 for instance); eventually, however, they are destroyed, but, as I am still occupied with these matters, their discussion is best postponed, as also the details concerning spore formation.

Returning to the discussion regarding the bearing of these observations on ERIKSSON'S hypothesis, it will doubtless be admitted that they place his facts in a totally new light, and remove the difficulties incident to any attempts to deal with negative assertions. So long as ERIKSSON merely assumed the existence of the invisible "mycoplasm" it was practically impossible to refute his hypothesis; all that one could say was that since the assumed "mycoplasm" could not be found, no direct proof was forthcoming, and the matter lay outside the region of science, except in so far as indirect evidence could be adduced.

The decisive observation, without which it would be impossible to accept ERIKSSON'S interpretation of his *corpuscules spéciaux*, would be the discovery of a young "corpuscule" *inside the cell*, and which has given rise to a *young vesicle-like rudiment of a hypha outside*. This I have sought for in vain. Hundreds of cases have been observed where a very small haustorium is attached by a distinct neck, passing through the cell-wall, to a hypha in an advanced stage of development, clearly showing that the latter had given origin to the former (*e.g.*, figs. 14–16), but the converse has never been seen, nor does ERIKSSON figure it, and the whole history of development is entirely against the assumption that it can occur.

Again, if ERIKSSON'S view were admissible, we ought to see the "corpuscules" commencing as minute vesicles and growing larger and larger before any neck or

connection with hyphæ outside the cell is formed; but exactly the converse is true. It is comparatively easy to observe hyphæ in contact with cells containing no "corpuscles," and others, fully developed, which have put forth the minute haustorium through the wall into the cell-cavity, and so on; and it seems almost inconceivable that, such being the case, the converse process would be missed if it occurred.

As before said, ERIKSSON has entirely reversed the true sequence of events. So far from his "corpuscules" arising from germs in the cells, growing therein, and then piercing the cell-wall to produce the hyphæ in the intercellular spaces, it is only when the hypha in the latter has attained an advanced stage of development that it is capable of piercing a cell and putting forth a haustorium into it.

Moreover, ERIKSSON's own words (see p. 33) suggest that he may not have been entirely convinced in his own mind of the accuracy of his own reading of the phenomena, or at least that he regards haustoria as possible. I think the history of their development here given decides the doubt.

To my mind the preparations shown in figs. 4, 20, and 40 are in themselves completely destructive of ERIKSSON's interpretation of these matters, for they show that the first thing an infecting tube may do is to put forth a haustorium into a cell close to the stoma, through which the germ-tube entered the leaf.

How comes it that ERIKSSON's figures show no hyphæ? Not only are there no hyphæ shown in connection with his "corpuscules," but none are visible in the figures he has given, even in contact with the cells containing them.

Two possibilities suggest themselves in this connection. It may, of course, occur that a section, especially if transverse, may be so cut that the hypha, which has pierced the cell-wall and is attached by a thin long neck to the body of the haustorium, lies below or above the plane of section, and in that case the body of the haustorium would appear isolated in the cell as a so-called "corpuscule." Such has in fact occurred in the preparations shown in my figs. 11, 16*a*, 27, 28, and 44, but I have so rarely failed to observe the hyphæ, or at least traces of them, in the immediate neighbourhood, that I hesitate to put such a suggestion forward in explanation of ERIKSSON's figures.

The second, and more probable, possible explanation is that ERIKSSON's method of preparation of his sections is responsible for his failure to trace his isolated "corpuscules" to their parent hyphæ.

Even when the section—which must be thin—is cut so that the neck of the haustorium lies in the plane of section, as in my figs. 4, 13, 16*a*, 33, 34, &c., it is extremely difficult to see the true state of affairs unless the staining is delicate and sharp, and I have found it almost impossible to make out details in preparations in glycerine and treated as ERIKSSON recommends. That the necks would often be invisible in glycerine may be readily supposed, though it does seem somewhat extraordinary that hand-cut sections, unless they were extremely thin, should fail to show

the hyphæ in the neighbourhood of the cells. Still, even thick sections in glycerine might fail to show the connecting hypha if the latter were cut transversely, as in my figs. 16*a* and 31, where the most perfect staining only brings them out as delicate rings.

It only remains to add that even the support which ERIKSSON attempts to derive from other examples of symbiosis fails him in all the cases which have been critically examined. For instance, he adduces as evidence of similar significance to his "mycoplasm" hypothesis, cases where other fungi lie latent in seeds or other organs of plants; but it must be remembered that the instance of *Lolium temulentum*, where the grains are almost invariably infected by a fungus, is a particularly unfortunate example, because FREEMAN, working in my laboratory, has shown* not only that the latent fungus in the seed of Lolium can be readily detected by existing methods, but that it occurs in the form of an extra-cellular mycelium. One of the best cases to all appearance in support of ERIKSSON'S contention would have been WORONIN'S *Plasmodiophora brassicæ*, where the parasite does actually invade the cells of the host as naked protoplasm living in the protoplasm of these cells; but NAWASCHIN has so clearly shown that refined laboratory methods are perfectly adapted to demonstrating the distinction between the plasmodium and the cytoplasm in which it lives,† that this important instance also fails us.

EXPLANATION OF PLATES 4–6.

Unless otherwise specified, all the figures have been drawn from preparations mounted in balsam, and examined with Zeiss's $\frac{1}{12}$th oil immersion, 0·25, or the dry homogeneous immersion 0·4 mm. and the compensating ocular No. 12 or No. 18.

PLATE 4.

All figures on Plate 4 are from preparations stained with Diamant-fuchsin and Licht-grün.

Fig. 1. A stoma of *Bromus secalinus*, L., seen from above. The nuclei of the companion and guard-cells red, the chlorophyll corpuscles of the latter and the protoplasm green. Ridges of guard-cells red.

Fig. 2. The same in vertical longitudinal section. The ridge bounding the aperture red; nuclei as before.

Fig. 2. *bis*. Two Uredo-spores (*a*) of *Puccinia glumarum* after 20 hours germination, hardened and stained *in situ*; one showing the nucleus in the body of the germ-tube, the other, having swollen at the tip in preparation to form

* 'Roy. Soc. Proc.,' vol. 71; 'Phil. Trans.,' B, vol. 196, 1903, p. 1.

† 'Flora,' vol. 86, 1899, p. 404.

the appressorium, has its nucleus in the swelling. (*b*) Pieces of similar tubes found in sections.

Fig. 3. A stoma seen from the inside at the moment of entry of a germ-tube. The internal vesicle has put forth an infecting tube containing two nuclei and has been cut by the razor. The external vesicle (*appressorium*) can be seen on the other (exterior) side of the stoma slightly out of focus, both stained green. The chlorophyll corpuscles of the guard-cells (stained red) are paler at the point of attack.

Fig. 4. Stoma in longitudinal vertical section, with the inner vesicle and infection tube: the latter has passed to the left and branched beyond its first septum, and one of the branches has put forth a nucleated haustorium into the epidermal cell. Nuclei and vacuoles occur in the hyphæ. From a six-day culture.

Fig. 5. End of a hypha showing septum and three nuclei. The latter and the younger segment containing them, red; the older parts of the hypha, green.

Fig. 6. Similar preparation showing a vacuole and red nuclei in the older portions.

Fig. 7. Similar preparation showing two nuclei in each terminal segment.

Fig. 8. Similar preparation showing four nuclei in the terminal segment.

Fig. 9. Similar preparation of older hypha, showing nuclei (green) and nucleoli (red).

Fig. 10. Portion of vertical section of leaf, showing two parenchyma cells, and parts of three others and of two epidermal cells. The two left-hand cells have "corpuscules" in them; these are really the heads of haustoria of which the connecting necks are not visible. A cell nucleus lies near the large branched and twisted haustorium. Numerous hyphal ends, clavate and each with two nuclei, are pressing close to the epidermal cells preparatory to the formation of spores. From an eight-day culture.

Fig. 11. Similiar preparation showing the nucleus and three "corpuscules" (haustoria) in an epidermal cell, and nucleated hyphæ in the neighbourhood. The haustorium in contact with the cell nucleus shows traces of the neck, pointing to the contiguous hyphal end. From a six-day culture.

Fig. 12. Portion of a section of an older leaf, on which pustules had already appeared. In addition to the old and swollen haustorium in contact with the cell-nucleus in the centre, note the young haustorium with its conspicuous red nucleus in the cell to the left above. The cell-contents are partially collapsed and the chlorophyll-corpuscles, &c., stain faintly.

Fig. 13. Terminal segment of a vigorous hypha, showing a nucleus beneath the septum, vacuoles and nuclear substance, and a distinct neck connecting it through the cell-wall with the large nucleated haustorium in the cell. From a six-day culture.

Fig. 14. Hyphal end which has just pierced the wall of a guard-cell of a stoma, and formed the earliest stage of a haustorium.

Fig. 15. Slightly more advanced stage in the development of a similar haustorium in the companion cell of a stoma. The hyphal end shows three nuclei, a fourth is visible in the young haustorium.

Fig. 16. Still more advanced haustorium in an epidermal cell, and in contact with its nucleus.

Fig. 16*a*. Part of a longitudinal section of a leaf of *Bromus mollis* attacked by the Uredo of *Puccinia glumarum*. One cell shows a nucleus and a haustorium of the pyriform type; another contains three elongated (sausage-shaped) haustoria—one of which is connected by its neck with a hypha—and the cell nucleus.

PLATE 5.

The preparations from which the figures in this and the next plate were taken were stained in various ways: principally by Hæmatoxylin, by Saffranin and Lichtgrün, or by the triple stain. Magnification as before.

Fig. 17. Stoma in vertical longitudinal section, with a germinating Uredo-spore, the germ-tube of which can be traced to the orifice. From a two-days culture.

Fig. 18. Similar preparation, in which the germ-tube can be traced to the vesicular swelling inside the stomatal cavity. The latter has given off an infecting hypha, part of which is cut away. Two-days culture.

Fig. 19. Similar preparation showing traces of the appressorium, and the whole of the internal vesicle and the nucleated young infecting tube. Four-days culture.

Fig. 20. Three successive transverse sections through a stoma, showing the remains of the appressorium and its connection through the stomatal aperture with the internal vesicle, which has given off at least two infecting tubes. In *a* the principal infecting tube, containing two nuclei, is putting forth its first haustorium into the epidermal cell next but one to the stoma. (Zeiss D. Hom. Oc. 12.) From a five-day culture stained with saffranin.

Fig. 21. Similar preparation showing the connection between appressorium and inner vesicle through the stoma, and the greater part of the infecting tube containing two nuclei. From a five-days culture.

Fig. 22. Vertical longitudinal section of a stoma, through which two germ-tubes have passed and formed internal vesicles and infecting tubes. Four-day culture.

Fig. 23. Similar section, showing the vesicle and infecting tube; the latter branching into a spreading system of hyphæ which are beginning to run between the parenchyma cells of the leaf. Five-day culture.

Fig. 24. Portion of section showing the branched, septate, vacuolated and nucleated hyphæ rapidly spreading in all directions from an infection system such as that shown in last figure. Five-day culture.

Fig. 25. Portion of a branched hypha of such a system, showing vacuole and two nuclei in process of division. Five-day culture.

Fig. 26. Similar preparation with one nucleus just divided.

Fig. 27. Cells of the leaf-parenchyma of which one shows two "corpuscules" (haustoria), one with portion of its neck visible. In a lower cell the nucleus, and a branched haustorium. All three haustoria are nucleated, and nucleated hyphæ abound in the near neighbourhood. Six-day culture: Diam.-fuchsin and L. grün.

Fig. 28. Similar preparation showing the "corpuscule" (haustorium), closely investing the cell nucleus. Six-day culture.

Fig. 29. Similar preparation showing much branched "corpuscule," which exhibits traces of its haustorial neck, and nuclei. It is in contact with the cell nucleus.

PLATE 6.

Fig. 30. Similar preparation, but betraying clearly the haustorium nature of the "corpuscule," which is branched, vacuolated, and contains two nuclei, and is attached by a distinct neck passing through the cell-wall to the hyphæ which has given origin to it. The cell nucleus is to the left. Six-day culture; Diam.-fuchsin and L. grün.

Fig. 31. Section of parenchyma near a vascular bundle. In the lower and left-hand upper cells are "corpuscules" and cell-nuclei; in the upper median cell the haustorium-character of the former is clearly betrayed by the connecting neck, of which part only is seen in that next the cell-nucleus, but the whole is visible in the long vacuolated and nucleated haustorium in the left-hand lower corner of the cell. Hyphæ abound in the section. From an old disease-pustule.

Fig. 32. Similar preparation showing the long sausage-shaped, nucleated and vacuolated haustorium in contact with a pale and nearly exhausted cell-nucleus. Old pustule.

Fig. 33. Cells with "corpuscule" and with a complete haustorium of the "hammer-head" type in a cell sheathing the vascular bundle. From a six-day culture.

Fig. 34. Similar preparation from a five-day culture showing haustorium in an epidermal cell.

Fig. 35. Similar preparation from older leaf with haustorium in a parenchyma cell.

Fig. 36. Early stage in the formation of a haustorium, from the terminal segment of a hypha; note the four nuclei in the segment, and the gelatinous halo round the haustorium. Four-day culture.

Fig. 37. Slightly older haustorium with papillæ on it—probably remnants of the gelatinous halo. Five-day culture.

Fig. 38. Similar preparation of a slightly more advanced haustorium. Five-day culture.

Fig. 39. Somewhat older haustorium, in contact with the nucleus of an epidermal cell.

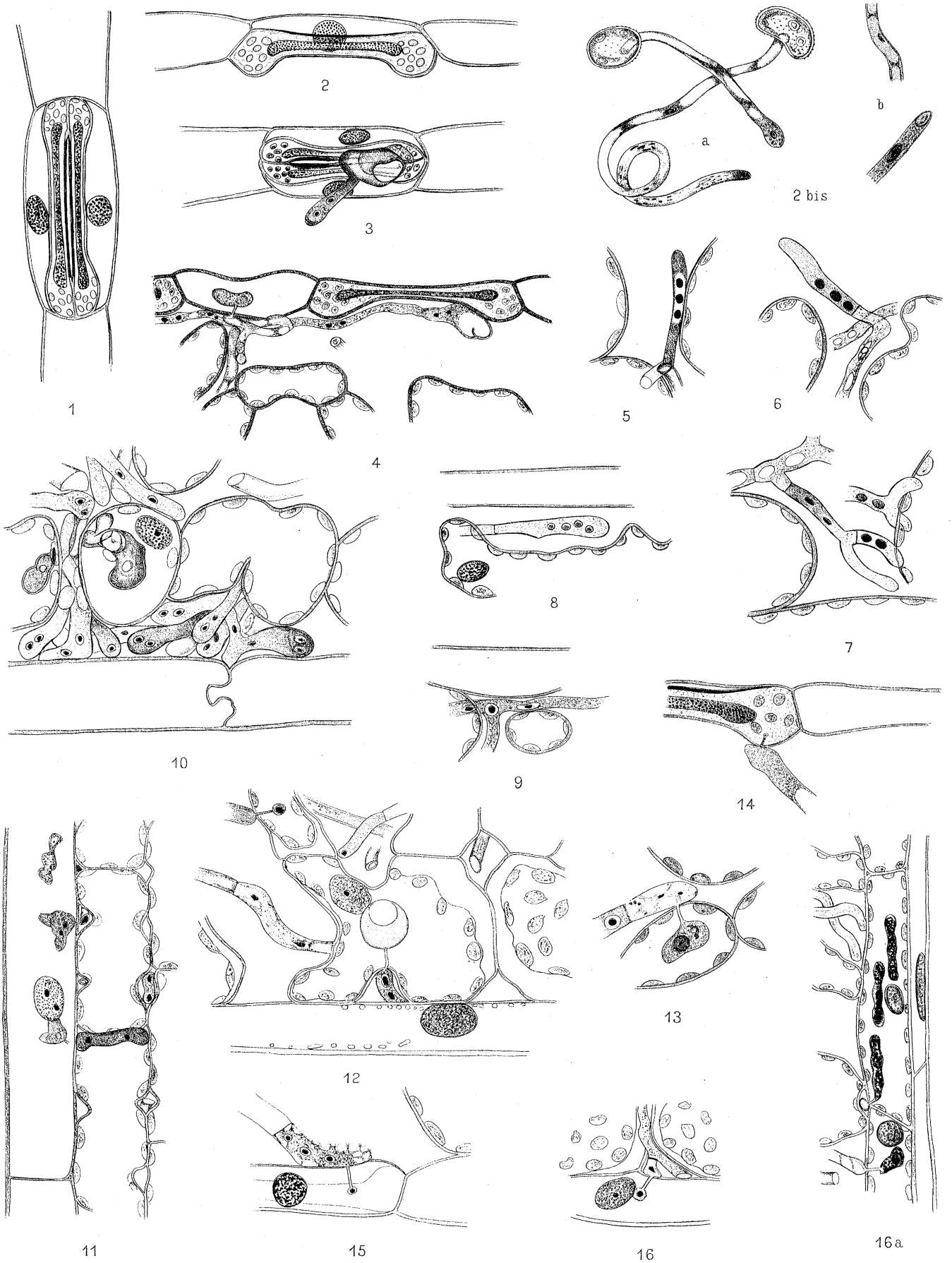
Fig. 40. Germ-tube and appressorium, which, passing through the stoma, has developed the internal vesicle and infecting hypha, and the latter is in process of forming the first haustorium enveloped in its gelatinous halo. Five-day culture.

Fig. 41. Older haustorium with prominences, and neck joined to hypha seen in transverse section.

Fig. 42. Still older haustorium, showing lobed type.

Fig. 43. Portion of hyphæ showing nuclei and vacuoles, and a typical nucleated and vacuolated haustorium. Five-day culture.

Fig. 44. Much coiled haustorium in contact with nucleus in a cell beneath a hair.



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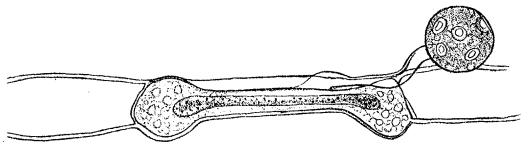

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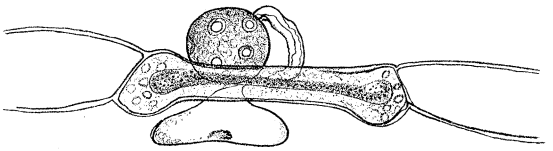

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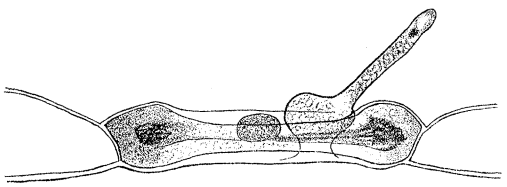

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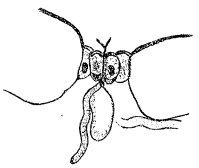

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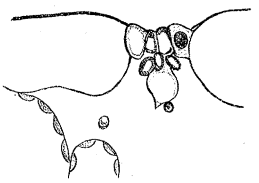

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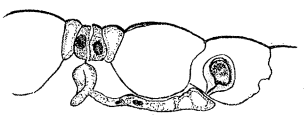

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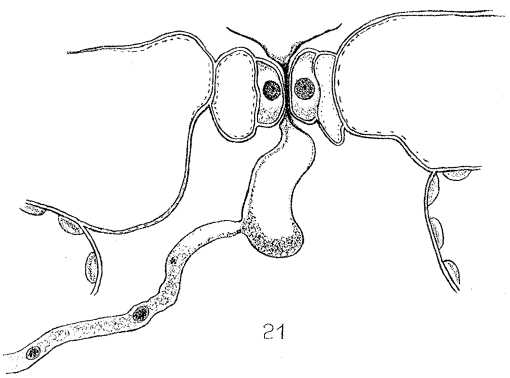

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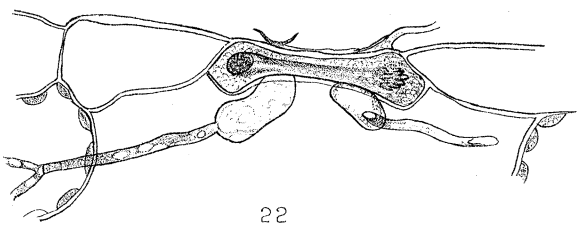

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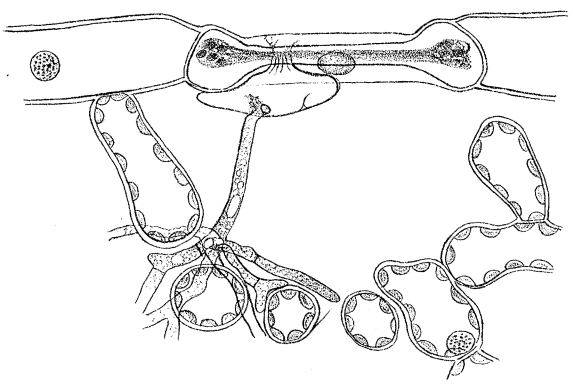

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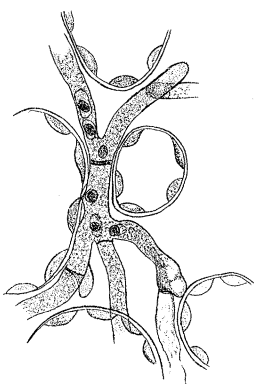

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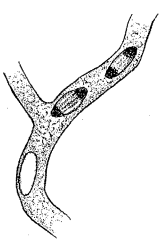

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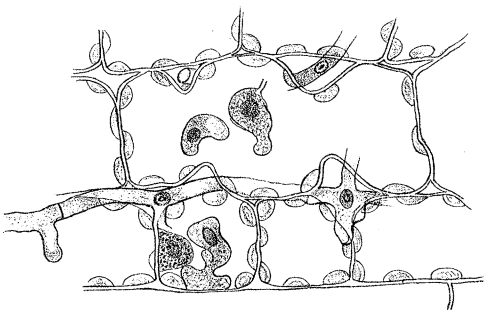

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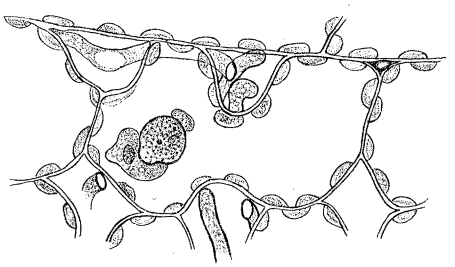

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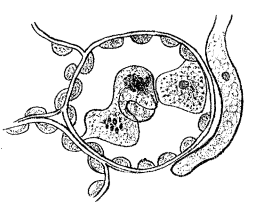

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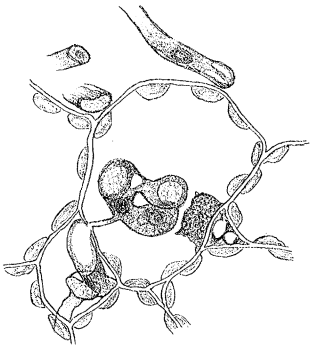

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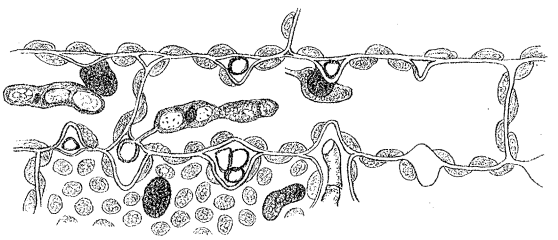

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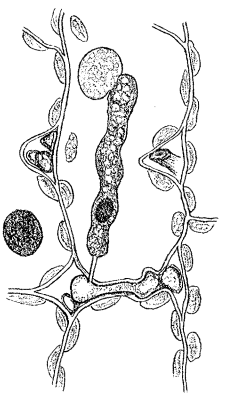

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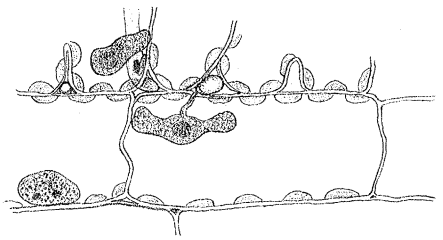

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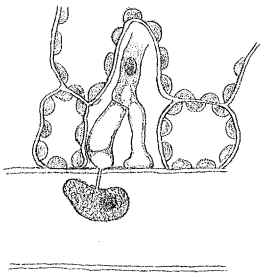

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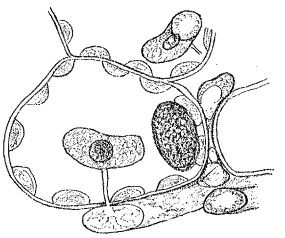

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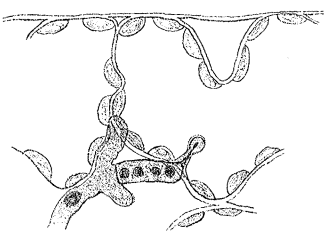

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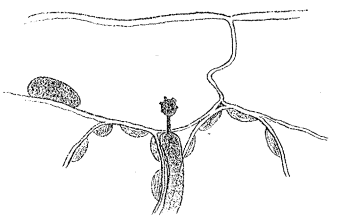

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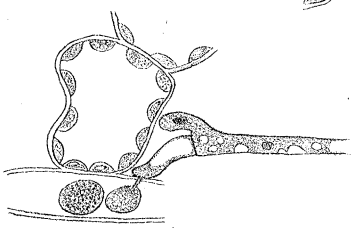

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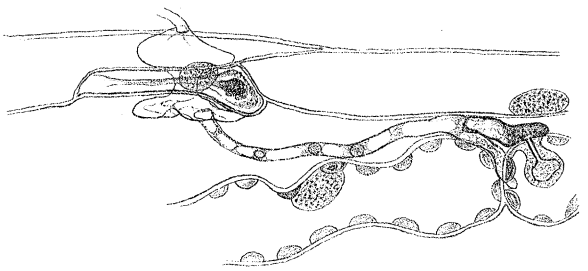

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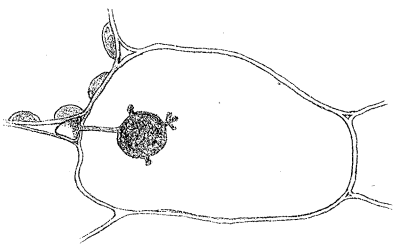

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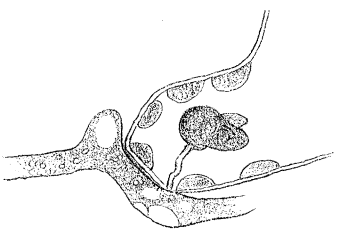

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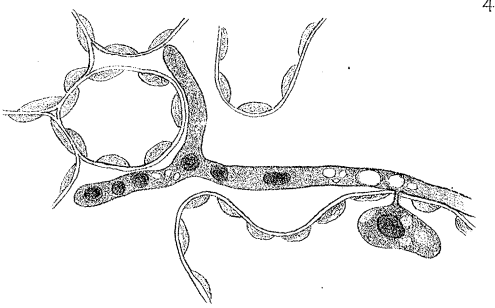

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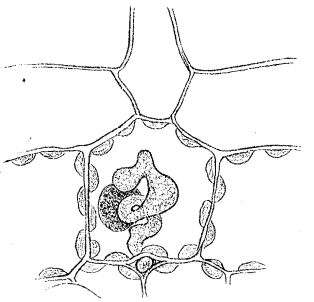

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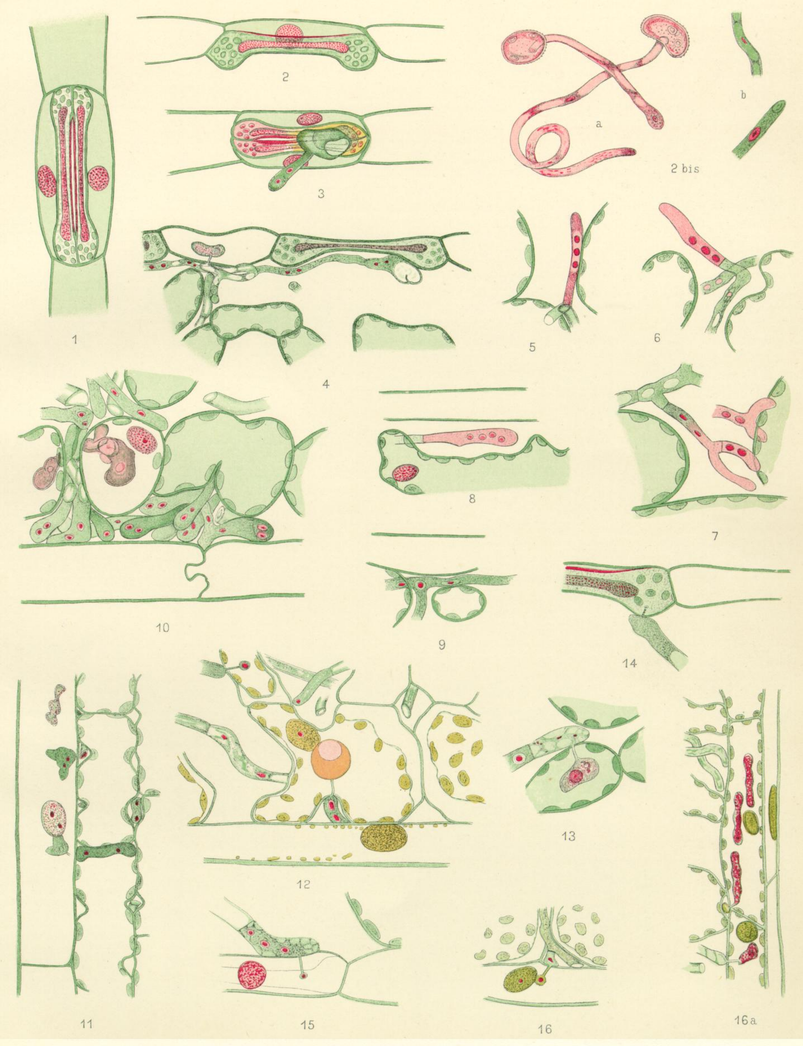

PLATE 4.

All figures on Plate 4 are from preparations stained with Diamant-fuchsin and Licht-grün.

Fig. 1. A stoma of *Bromus secalinus*, L., seen from above. The nuclei of the companion and guard-cells red, the chlorophyll corpuscles of the latter and the protoplasm green. Ridges of guard-cells red.

Fig. 2. The same in vertical longitudinal section. The ridge bounding the aperture red; nuclei as before.

Fig. 2. *bis*. Two Uredo-spores (*a*) of *Puccinia glumarum* after 20 hours germination, hardened and stained *in situ*; one showing the nucleus in the body of the germ-tube, the other, having swollen at the tip in preparation to form the appressorium, has its nucleus in the swelling. (*b*) Pieces of similar tubes found in sections.

Fig. 3. A stoma seen from the inside at the moment of entry of a germ-tube. The internal vesicle has put forth an infecting tube containing two nuclei and has been cut by the razor. The external vesicle (*appressorium*) can be seen on the other (exterior) side of the stoma slightly out of focus, both stained green. The chlorophyll corpuscles of the guard-cells (stained red) are paler at the point of attack.

Fig. 4. Stoma in longitudinal vertical section, with the inner vesicle and infection tube: the latter has passed to the left and branched beyond its first septum, and one of the branches has put forth a nucleated haustorium into the epidermal cell. Nuclei and vacuoles occur in the hyphæ. From a six-day culture.

Fig. 5. End of a hypha showing septum and three nuclei. The latter and the younger segment containing them, red; the older parts of the hypha, green.

Fig. 6. Similar preparation showing a vacuole and red nuclei in the older portions.

Fig. 7. Similar preparation showing two nuclei in each terminal segment.

Fig. 8. Similar preparation showing four nuclei in the terminal segment.

Fig. 9. Similar preparation of older hypha, showing nuclei (green) and nucleoli (red).

Fig. 10. Portion of vertical section of leaf, showing two parenchyma cells, and parts of three others and of two epidermal cells. The two left-hand cells have "corpuscules" in them; these are really the heads of haustoria of which the connecting necks are not visible. A cell nucleus lies near the large branched and twisted haustorium. Numerous hyphal ends, clavate and each with two nuclei, are pressing close to the epidermal cells preparatory to the formation of spores. From an eight-day culture.

Fig. 11. Similiar preparation showing the nucleus and three "corpuscules" (haustoria) in an epidermal cell, and nucleated hyphæ in the neighbourhood. The haustorium in contact with the cell nucleus shows traces of the neck, pointing to the contiguous hyphal end. From a six-day culture.

Fig. 12. Portion of a section of an older leaf, on which pustules had already appeared. In addition to the old and swollen haustorium in contact with the cell-nucleus in the centre, note the young haustorium with its conspicuous red nucleus in the cell to the left above. The cell-contents are partially collapsed and the chlorophyll-corpuscles, &c., stain faintly.

Fig. 13. Terminal segment of a vigorous hypha, showing a nucleus beneath the septum, vacuoles and nuclear substance, and a distinct neck connecting it through the cell-wall with the large nucleated haustorium in the cell. From a six-day culture.

Fig. 14. Hyphal end which has just pierced the wall of a guard-cell of a stoma, and formed the earliest stage of a haustorium.

Fig. 15. Slightly more advanced stage in the development of a similar haustorium in the companion cell of a stoma. The hyphal end shows three nuclei, a fourth is visible in the young haustorium.

Fig. 16. Still more advanced haustorium in an epidermal cell, and in contact with its nucleus.

Fig. 16*a*. Part of a longitudinal section of a leaf of *Bromus mollis* attacked by the Uredo of *Puccinia glumarum*. One cell shows a nucleus and a haustorium of the pyriform type; another contains three elongated (sausage-shaped) haustoria—one of which is connected by its neck with a hypha—and the cell nucleus.

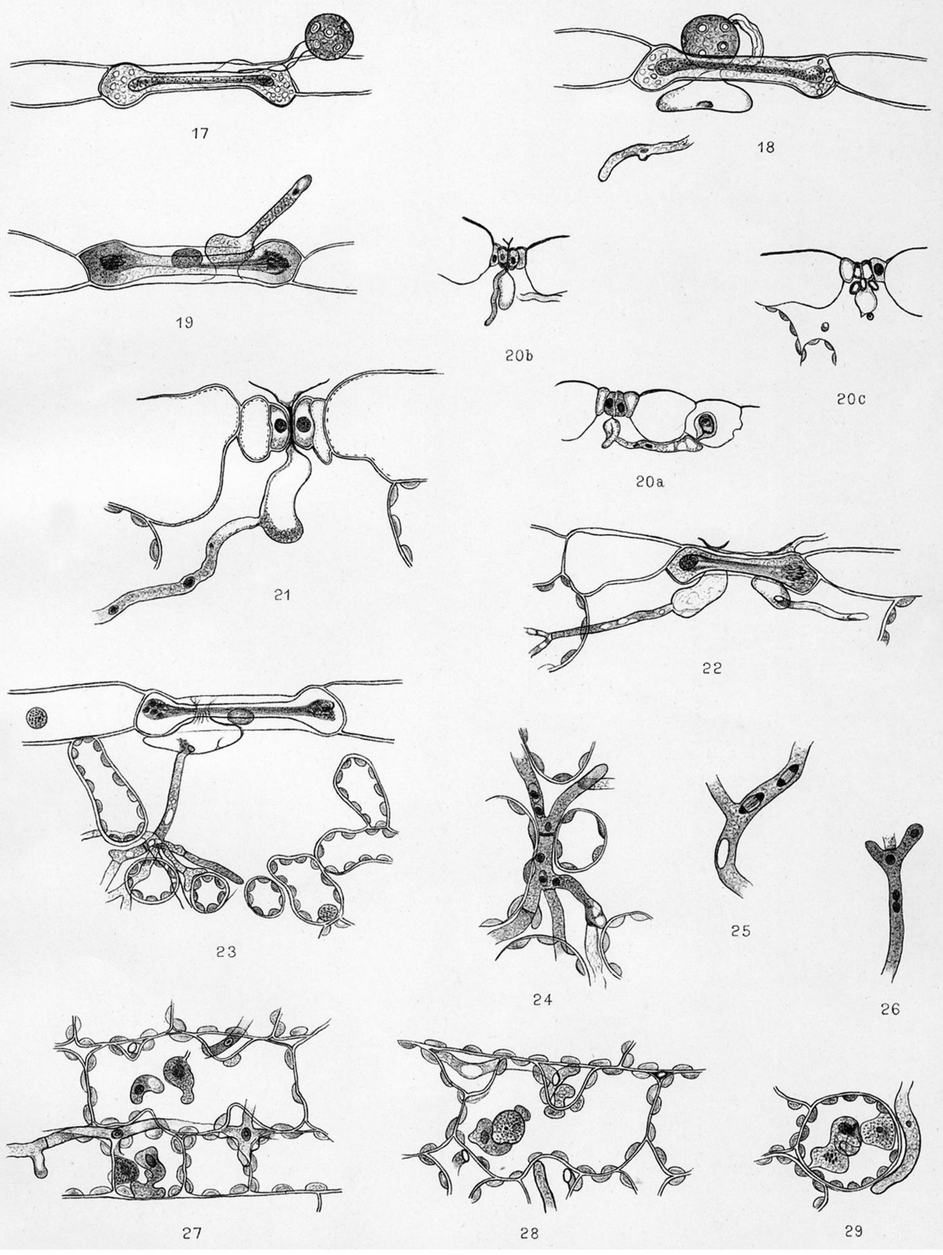

PLATE 5.

The preparations from which the figures in this and the next plate were taken were stained in various ways: principally by Hæmatoxylin, by Saffranin and Lichtgrün, or by the triple stain. Magnification as before.

Fig. 17. Stoma in vertical longitudinal section, with a germinating Uredo-spore, the germ-tube of which can be traced to the orifice. From a two-days culture.

Fig. 18. Similar preparation, in which the germ-tube can be traced to the vesicular swelling inside the stomatal cavity. The latter has given off an infecting hypha, part of which is cut away. Two-days culture.

Fig. 19. Similar preparation showing traces of the appressorium, and the whole of the internal vesicle and the nucleated young infecting tube. Four-days culture.

Fig. 20. Three successive transverse sections through a stoma, showing the remains of the appressorium and its connection through the stomatal aperture with the internal vesicle, which has given off at least two infecting tubes. In *a* the principal infecting tube, containing two nuclei, is putting forth its first haustorium into the epidermal cell next but one to the stoma. (Zeiss D. Hom. Oc. 12.) From a five-day culture stained with saffranin.

Fig. 21. Similar preparation showing the connection between appressorium and inner vesicle through the stoma, and the greater part of the infecting tube containing two nuclei. From a five-days culture.

Fig. 22. Vertical longitudinal section of a stoma, through which two germ-tubes have passed and formed internal vesicles and infecting tubes. Four-day culture.

Fig. 23. Similar section, showing the vesicle and infecting tube; the latter branching into a spreading system of hyphæ which are beginning to run between the parenchyma cells of the leaf. Five-day culture.

Fig. 24. Portion of section showing the branched, septate, vacuolated and nucleated hyphæ rapidly spreading in all directions from an infection system such as that shown in last figure. Five-day culture.

Fig. 25. Portion of a branched hypha of such a system, showing vacuole and two nuclei in process of division. Five-day culture.

Fig. 26. Similar preparation with one nucleus just divided.

Fig. 27. Cells of the leaf-parenchyma of which one shows two "corpuscules" (haustoria), one with portion of its neck visible. In a lower cell the nucleus, and a branched haustorium. All three haustoria are nucleated, and nucleated hyphæ abound in the near neighbourhood. Six-day culture: Diam.-fuchsin and L. grün.

Fig. 28. Similar preparation showing the "corpuscule" (haustorium), closely investing the cell nucleus. Six-day culture.

Fig. 29. Similar preparation showing much branched "corpuscule," which exhibits traces of its haustorial neck, and nuclei. It is in contact with the cell nucleus.

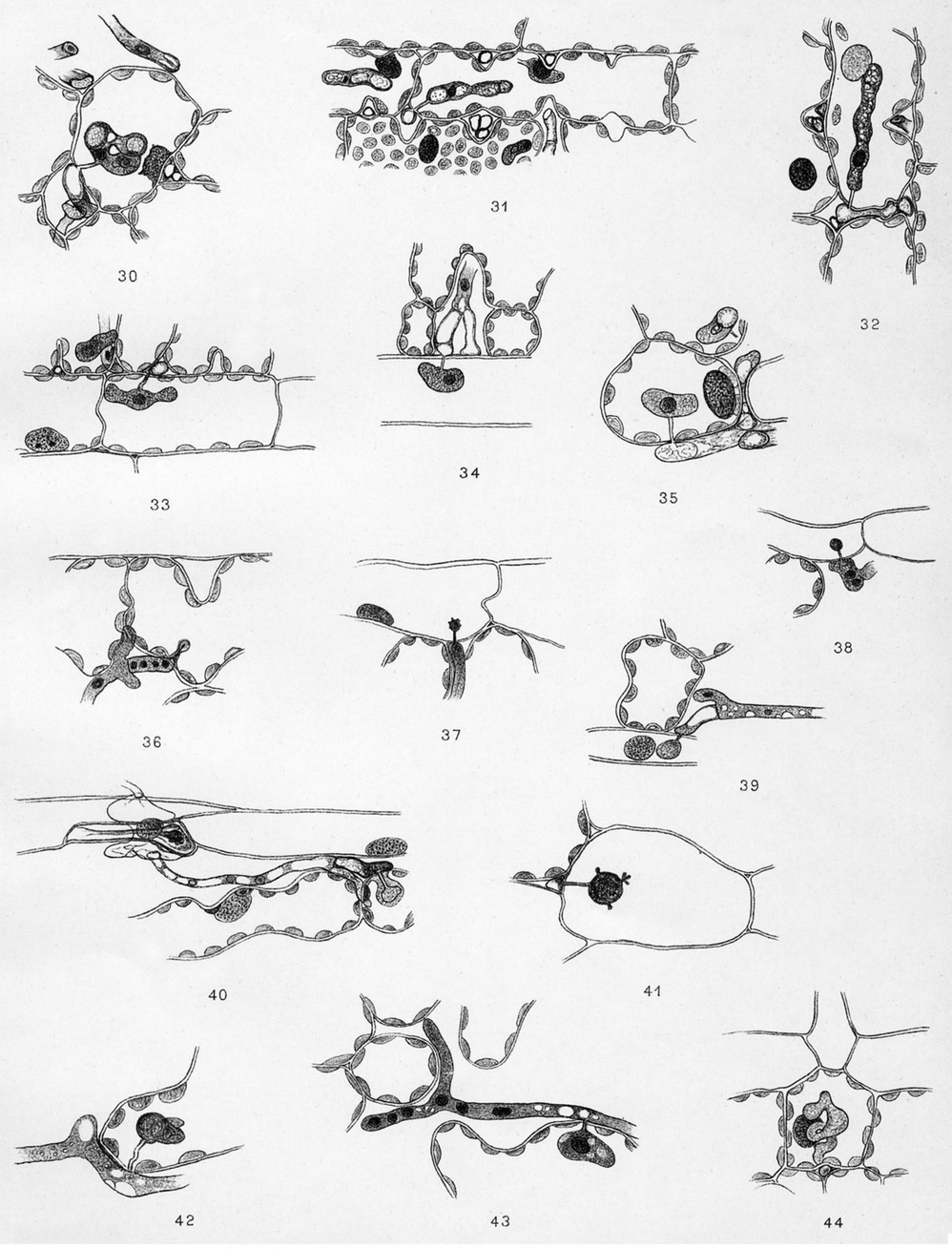

PLATE 6.

Fig. 30. Similar preparation, but betraying clearly the haustorium nature of the "corpuscule," which is branched, vacuolated, and contains two nuclei, and is attached by a distinct neck passing through the cell-wall to the hyphæ which has given origin to it. The cell nucleus is to the left. Six-day culture; Diam.-fuchsin and L. grün.

Fig. 31. Section of parenchyma near a vascular bundle. In the lower and left-hand upper cells are "corpuscules" and cell-nuclei; in the upper median cell the haustorium-character of the former is clearly betrayed by the connecting neck, of which part only is seen in that next the cell-nucleus, but the whole is visible in the long vacuolated and nucleated haustorium in the left-hand lower corner of the cell. Hyphæ abound in the section. From an old disease-pustule.

Fig. 32. Similar preparation showing the long sausage-shaped, nucleated and vacuolated haustorium in contact with a pale and nearly exhausted cell-nucleus. Old pustule.

Fig. 33. Cells with "corpuscule" and with a complete haustorium of the "hammer-head" type in a cell sheathing the vascular bundle. From a six-day culture.

Fig. 34. Similar preparation from a five-day culture showing haustorium in an epidermal cell.

Fig. 35. Similar preparation from older leaf with haustorium in a parenchyma cell.

Fig. 36. Early stage in the formation of a haustorium, from the terminal segment of a hypha; note the four nuclei in the segment, and the gelatinous halo round the haustorium. Four-day culture.

Fig. 37. Slightly older haustorium with papillæ on it—probably remnants of the gelatinous halo. Five-day culture.

Fig. 38. Similar preparation of a slightly more advanced haustorium. Five-day culture.

Fig. 39. Somewhat older haustorium, in contact with the nucleus of an epidermal cell.

Fig. 40. Germ-tube and appressorium, which, passing through the stoma, has developed the internal vesicle and infecting hypha, and the latter is in process of forming the first haustorium enveloped in its gelatinous halo. Five-day culture.

Fig. 41. Older haustorium with prominences, and neck joined to hypha seen in transverse section.

Fig. 42. Still older haustorium, showing lobed type.

Fig. 43. Portion of hyphæ showing nuclei and vacuoles, and a typical nucleated and vacuolated haustorium. Five-day culture.

Fig. 44. Much coiled haustorium in contact with nucleus in a cell beneath a hair.