# On the Structure and Life-History of Entyloma ranunculi (Bonorden)

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VIII. On the Structure and Life-History of Entyloma Ranunculi (Bonorden).

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[Plates 10-13.]

THE attention of mycologists has been long directed to the study of the Ustilagineæ, not only on account of their morphological peculiarities, but even more especially because the economic questions arising from their relations to our crops, &c., have assumed such proportions as to force this group of parasites far into the foreground. Interesting and important as are the parasitic habits of the Ustilagineæ, however, and much as they have been investigated, it has to be admitted that we know as yet very little about them. Two or three of the most common forms, it is true, have been so often studied by different observers that they may be regarded as worked out sufficiently to allow of our regarding them as types; but it needs no very extensive acquaintance with the group to satisfy ourselves that the best known forms are not the simplest, and that much still remains to be accomplished in this large group. is not only that the Ustilagineæ are so minute, but they are so peculiarly modified, and so specialised as parasites, that the most careful observation is necessary in making out the numerous points in their structure; in addition, observers still differ considerably as to the interpretation of some of the facts of structure which are established.

Taking the most recent systems \* of classification, we may regard the Ustilagineæ as comprising the following genera, Ustilago, Tilletia, Sorosporium, Urocystis, Schizonella, and Entyloma, and so far shall be in accordance with all the modern authorities; when we come to such genera as Geminella, Sphacelotheca, Doassansia (Cornu), and Graphiola (Fischer) and some others, the matter becomes more complicated, and special investigations are still needed to determine the limits of the genera and group. Entyloma, however, is a well-established genus,† and now includes some fifteen or

- \* E.g., DE BARY, 'Morphol. d. Pilze,' p. 186, and Winter, 'Rabenhorst's Kryptogamen-Flora,' p. 80.
- † DE BARY, 'Botan. Zeitung,' 1874, p. 101. Schroeter, in Cohn's 'Beiträge Biol. Pflanz.,' vol. 2, pp. 368 and 439.

sixteen species. They are parasitic in the mesophyll of the leaves of various plants, and are characterised by producing rounded resting-spores as intercalary swellings on the very fine, septate, intercellular mycelium; these spores germinate like those of *Tilletia*. In *Tilletia*, however, the resting-spores form dense powdery aggregates, which is not the case with the more isolated spores of *Entyloma*.

Of the various species of this genus referred to above, three are described as occurring in the leaves of species of Ranunculus. One of these—Entyloma ranunculi—appears to be extremely common in some places, and has a wide distribution; its resting-spores have often been described, and it is regarded by all the authorities as a well-marked species. It has, however, so far as I know, not been specially investigated in detail, and, in addition to the other facts contained in the following memoir, it is of interest to have observed the germination of the conidia for the first time; the infection experiments are also new, and consequently important, and they establish beyond doubt the relations of the conidia to the Entyloma.\*

I now proceed to the description of my own observations, the completion of which has been rendered possible by an outbreak of the disease which the fungus induces on a large patch of densely crowded plants of Ranunculus Ficaria during the spring and early summer of this year; this patch of diseased plants was so favourably situated, and the fungus spread so rapidly and in such enormous numbers on it, that I was enabled to observe and record some facts of considerable interest respecting the origin and spread of the epidemic due to the action of the parasite. Moreover, material was to hand in abundance and in excellent condition, and the circumstances were so fortunate that it was possible to note day by day the symptoms of the disease, and the progress, climax, and decline of the epidemic. I mention this expressly because it is not sufficiently recognised how necessary is the study of the diseases of plants in the field—clinically, as it were—as well as in the laboratory.

The patch of Ranunculus referred to above extended some distance along the course of a conveniently situated damp ditch: during February and March thousands of young fresh green leaves sprang up, and in April the ground was densely carpeted with them; the leaves were so closely packed that the ditch appeared full of them. The ditch itself runs nearly due north to south, and is only damp as a rule; it becomes filled with water during heavy or continuous rain, however, and the water

\* With respect to these conidia, it appears that they have been described at various times as the spores of other species of Fungi, and that Winter first suggested their connection with Entyloma. Then Schroeter observed conidia in connection with Entyloma serotinum. (Schroeter, loc. cit., pp. 369 and 438.) Winter ('Kryptogamen-Flora,' p. 113) says:—"Sporidien bildung auf der lebenden Nährpflanze," which conveys the impression that the resting-spores germinate in the leaf and bear "sporidia" on their promycelia. The bodies here referred to are true conidia, however, as is clear from my observations, and are developed independently of the resting-spores. Moreover, this is the first time the germination of the conidia of an Entyloma has been followed and described (cf. De Bary, 'Morphol. d. Pilze,' p. 194), thus placing their connection with the Entyloma beyond doubt, and explaining their nature as true conidia.—May 5, 1887.

runs off rather slowly to the south. A fairly dense growth of hazel and other trees overshadows all, and in the summer but little sunlight reaches the plants in the ditch after noon: the morning sun nevertheless reaches the plants under and through the trees during the earlier months named. One consequence of all this is that the leaves of the *Ranunculus* were very succulent, bright, and long-stalked, and, as already mentioned, appeared to fill up the hollow of the ditch.

Towards the middle of April the bright green glossy leaves of the *Ranunculus* plants in a certain part of the above patch were noticed to be slightly spotted with white flecks, which increased in size and number day after day until before the middle of May a long tract—several yards—of the thickly growing leaves were infected and thus spotted.

Before describing the phenomena more closely, and simply speaking of the white flecks as the chief obvious symptom of the diseased condition, I may call attention to one or two points which seem significant. Ranunculus Ficaria is an extremely common plant all over the neighbourhood of Englefield Green, and nevertheless I have failed to find the white spots on leaves in many places near. Nor is this all; hundreds of plants on the eastern side of the garden (the ditch runs along the western side) have been examined, and no traces of the spots found, and even in the ditch referred to none of the plants in the northern two-thirds of its length were spotted as described. The disease—the epidemic, I will say—commenced on a few plants in April, and spread southwards for several yards during April and May. I account for this as follows:—The easily spread spores (conidia) of the fungus causing the disease were transferred by wind, and especially by water flowing southwards in the ditch during the rains occurring at various periods in April: the wind, as I had occasion to notice, was chiefly from the north and east at these times, hence the immunity of the plants in the northern parts of the ditch and on the other side of Even the fact of a south-west or west wind occasionally does not the garden. contradict the conclusion when all the circumstances are known, for a high bank and hedge lie to the westward of the ditch, and the hazel trees mentioned above would screen other parts of the garden.

An extraordinarily severe outbreak of the white spots occurred over the patch during the period May 6th to 12th, and it was then I noticed particularly how the epidemic spread to the south, and not to the north; the period referred to was remarkable for very warm "steamy" mornings and very bright noons. A storm broke over Englefield Green on Saturday, April 24th, and the ditch was flooded and overflowing for several hours, all the *Ranunculus* plants being bent downwards towards the south when the water had passed over and through them: that the flooding in question distributed the spores which caused the sudden and extensive outbreak on May 6th to 12th will hardly be questioned after what follows, for I shall show that it requires from a fortnight to three weeks to develope a white disease-spot in the leaf from a spore germinated on its epidermis.

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I now pass on to a more detailed description of the white "disease-spots" on the leaves (Plate 10, fig. 1). On its first appearance the spot is pale and greenish, and not sharply marked off from the surrounding tissue, and it requires close watching to be sure when it first becomes visible to the unaided eye; shortly afterwards, the central parts of the enlarging fleck are pure white, resembling powdered chalk, especially when the air is warm and still, and the conidia to be referred to have accumulated in large quantities. As the white speck ages and enlarges centrifugally, it turns more ashcoloured or yellowish in the centre, and finally becomes brownish, or even dark brown, and the patch of tissue is dead.

These spots appear on both sides of the leaves, and are alike, except that the white stands out more sharply in contrast with the darker glossy green of the upper side of the leaf. On a warm, still morning, it is possible to collect relatively large quantities of the white chalky powder (conidia) from the more active flecks, and it will be seen how important this must be in the reproduction and spread of the fungus causing the infection.

The white flecks are confined to the mesophyll of the leaf, and can be seen sharply bounded by the vascular bundles of the venation—for instance, in the fork whence two chief veins diverge—whereas they fade imperceptibly into the green of the mesophyll. The leaf is not thickened at the infected spots, but it is very noticeable, as the spot increases in age, that it becomes thinner and dries up or rots; in the former case cracking and tearing away from the healthy tissue, and, in the latter, falling down as putrid shreds (fig. 1). The difference depends on weather. In both cases the bits may soon disappear, and the leaf look as if a piece had been nibbled out.

It will thus be seen that the diseased condition is confined to a given area; the spots are local centres, and do not spread indefinitely over the leaf. I have counted 57 on a leaf less than  $1\frac{1}{2}$  inches broad and long, and many more can co-exist on that In some cases spots run together as they age. As I shall show later, each spot spreads from one centre only, i.e., from a stoma through which a germinal tube from a spore has passed; the stomata are on both sides of the leaf.

A curious, though by no means isolated, phenomenon is presented in the case of old leaves, which have shown the spots at a late stage of their life, and then turned yellow before the spots reach their matured condition: this is the existence of a vivid green ring around the spot, and is, without doubt, due to the mycelium of the fungus keeping the cells active after their neighbours are dead. I have noticed the same fact in the case of other parasitic fungi.

Sections through a young white spot show that a very delicate, copiously branched, mycelium exists between the cells of the mesophyll, both in the palissade and spongy tissue (figs. 7-14). Closer examination shows that the mycelium is segmented at rather long intervals, but the septa are very difficult to observe without reagents, owing to their thinness and that of the outer walls, and to the dense, finely granular

protoplasm in the hyphæ. If the sections are made through somewhat more advanced spots, the following additional peculiarities are noticeable (fig. 11). The mycelium has increased, and now sends branches into the lacunæ beneath the stomata. These branches fill up the interspace, and at length project through the orifices of the stomata in dense tufts or pencils (figs. 2, 6, and 11). The hyphæ are also seen to be mingled with numerous small spherical bodies—the resting-spores of the fungus. Subsequently the number of these spores increases enormously, until, in old spots, every nook and cranny between the cells is packed with them. Meanwhile, the pencils of hyphæ projecting to the exterior have produced innumerable colourless conidia from their free ends (figs. 3, 4, 5, 12, 13). It is these tufts and conidia which give the white powdery appearance to the spots.

Having thus given a general account of the fungus, I may proceed to describe further details as to the intercellular mycelium. It is not difficult to observe that at the margins of the spots (in the mesophyll tissue) the tips of the hyphæ are extending radially in all directions, branching as they do so, and forming septa behind the apices. Where the hyphæ pass along the wall of a cell, they frequently form flattened short branchlets or tufts, closely appressed to the outside of the wall of the cell (fig. 7), i.e., on the side bounding the lacunæ. These flattened tufts of branchlets are strikingly suggestive of haustoria, though they do not obviously pierce the wall. The hyphæ appear never to be intracellular. In some cases, with the aid of reagents, I have convinced myself that the attachment of these haustorium-like branchlets to the cell-wall is very close, and cannot help suspecting that either fine threads of protoplasm pass out to them from the sac of protoplasm inside, or that they send such fine threads through the cellulose; it has so far been impossible to place this beyond doubt, however. Although the hyphæ do not penetrate into the cavity of the cell, they can pass in the primary cell-wall (the middle lamella), and so force their way between two contiguous cells. Good sections show this distinctly, though, owing to the delicacy of the hyphæ, they are not easy to obtain. Moreover, as will appear clearer shortly, the tips of the hyphæ can make their way to the exterior between contiguous cells of the epidermis (figs. 11 and 14).

Following those hyphæ the tips of which protrude through the stomata, their ends are found to give rise to delicate colourless conidia by abstriction. Taking a given hypha, it grows out into the damp air or water, and its tip swells up slightly into an ovoid body which may lengthen considerably or not before it is separated off as a very delicate colourless conidium, with an extremely thin cell-wall and finely granular and vacuolated protoplasmic contents, in which minute brilliant oil-like drops are suspended.

In some cases, apparently in drier warm weather, the protruding hyphæ are relatively short, and the conidia ovoid or slightly reniform (fig. 3); in other cases, apparently in wet weather, and certainly in water (fig. 2), the hyphæ may protrude twice as far before the conidia are abstricted, and the latter are then longer, more

curved, and relatively thinner (fig. 5). Under such circumstances a conidium may be seen to germinate before it falls off from the hypha; or the hyphæ may go on growing longer and longer for many hours, to end at last, however, by forming long conidia at the extremity, the intermediate part dying off (fig. 2, a). Such abnormally long conidial segments are easily obtained by allowing the tufts to grow out from the stomata of cut-off pieces of epidermis suspended in water; the tufts thus produced are curiously suggestive of the so-called Ramularia, Cercospora, &c., of authors. The tufts of conidia are like Glæsporium Ficariæ (Berk).

The normal conidia are club-shaped or long ovoid bodies, slightly curved, and more pointed at the attached end. They were to be obtained in any quantity, on the leaves in the ditch referred to above, in May, and I was able to obtain pure sowings of them with ease, by removing them lightly with a fine camel-hair pencil, and thus not only to observe all stages of germination, but also to infect clean plants with certainty.

Sown in water, in hanging drops kept over damp cells, the conidia germinate readily under favourable conditions. I have noticed that in many cases a sowing of two or three conidia in a drop remained unaltered for several days, the conidia finally dying off, or one or two germinating at last; whereas, in drops containing some dozens of the conidia, the germination sometimes followed more rapidly and certainly. At first I put the phenomenon down as probably due to temperature; further experience leads me to doubt the accuracy of that conclusion. Another point I am convinced of: conidia sown in a drop of water on a leaf of the living plant germinate more readily than those in a similar drop on glass. Nevertheless, it has been sufficiently easy to get the conidia to germinate in rain water, and I have seen hundreds, and perhaps thousands, of them in all stages of germination.

To describe a concrete case. The conidium (a, fig. 23) was sown in the morning about 8 o'clock, and remained almost unaltered for 24 hours; next day, at 2 P.M. i.e., 30 hours after sowing—it had commenced to germinate (b), throwing out a delicate tube at either end; at about 10 P.M. (the same night) the stage c was reached, the germinal tube at the one end had grown to a short length only, and then its end had swollen up into a secondary conidium, taking the protoplasm from the rest. It will be noticed that the thin tail-like germinal tube at the other end of the conidium became empty, and that three septa appeared—one cutting off this empty tail-like tube, another dividing the main body of the conidium, and the third cutting off the successful germinal tube (as we may term it) from the now empty conidium. mention these facts because it will be seen that these septa—usually three in number --constantly recur, and the tail-like unsuccessful appendage seems to be always formed and emptied as described. At 9 A.M. on the third day—i.e., 49 hours after sowingthe secondary conidium (fig. 23, d) had commenced to put forth a short lateral hypha, which by 2 P.M. (e) had grown out as a thin, feebly-coiled, and very delicate hypha, while a second similar hypha was forming above. These thin hyphæ grew a little longer, and then stopped (f).

In fig. 24 are shown other conidia germinating in the same way, under the same conditions; the slight variations in detail do not affect the general conformity to the above type. It frequently happens that two conidia "copulate" after they have formed the secondary conidia, or during the development of the latter (fig. 25). This often occurs when the conidia are numerous, but by no means always; the after-effects of such copulations, if they exist, do not manifest themselves clearly in the further fate of the secondary conidia; they appear to behave exactly as if no copulation had occurred.

There is a small point of some interest to be noticed here. It has already been stated that the cellulose wall of the conidium is exceedingly delicate; it results thence that when the conidium is deprived of its protoplasmic contents the remaining empty shell is barely visible, and easily overlooked, and the same is true of empty portions of hyphæ. It often happens, therefore, that such specimens as those in fig. 24 (n and r) are not at first sight quite intelligible, until more careful search results in the discovery that the empty conidia, &c., are still attached; in other cases, however, the remains of the conidia become destroyed (e.g., by bacteria, &c.), and the delicate hyphæ containing the protoplasm persist alone. I have excellent reasons for believing that such hyphæ are not necessarily dead, and that the presence of certain fine hyphæ on the leaves is to be explained as above.

First, however, it will be advisable to see what occurs when the conidia are germinating in a drop of water on the leaf of the living *Ranunculus*, and where the increased supply of oxygen may be one of several causes for the fact stated above—that the conidia germinate more rapidly.

In figs. 26 and 27 are shown several specimens of germinating conidia, which had been sown in drops of dew on the living leaf, the plant being kept in a cool green-house under a glass bell-jar. It is at once noticeable that several of the conidia have proceeded at once to the development of the germinal hyphæ without the preliminary formation of the secondary conidia; the germinal tubes are thicker and stronger than is the case with sowings in pure water on glass. Here and there a case occurs (fig. 27) where the secondary conidium is interpolated as it were, but this at once proceeds to develope the germinal mycelium. Of course, the specimens figured are such as have not sent their hyphæ through a stoma; very many of them would do so about the second or third day after sowing, as shown in figs. 29 and 31.

It is now necessary to return to the mycelium in the intercellular spaces of the mesophyll of the leaf. It is observed in all cases where the white spots are fairly advanced, and wherever the conidia are developed in abundance on the exterior, that numerous spherical resting-spores exist among the closely weaved branching hyphæ. Thin sections and careful examination show further that these resting-spores are formed in the course of the hyphæ themselves as local dilations, or, more rarely perhaps, at the ends of branches which may be very short (figs. 9, 10, 17). It is possible to macerate or tease out specimens showing all the chief stages of develop-

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The resting-spore, at first simply a thin-walled dilation of the hypha, becomes separated by a septum, and its wall thickens gradually, bright granules and fat-like drops accumulating in the granular protoplasm. When quite mature, the protoplasmic contents assume a yellowish cast, but the thickened wall is only slightly yellow or colourless, perfectly smooth, and devoid of markings or mucilage, simply showing a slight tendency to stratification. It is not an uncommon event to meet with specimens like fig. 9, where one or two little branchlets arise close to the young spore and even appear closely applied to it (fig. 17); whether these are to be looked upon as representing degraded pollinodial branches, or whether they are merely of the nature of the haustorium-like branchlets referred to before, I cannot decide; it is quite certain that no fertilising tube is formed—the resting-spore arises purely asexually. The ripe spore exhibits a paler translucent spot in the centre, shining through the fatty and finely granular contents; it is perfectly easy to convince one's self of the attachment of the spore to the hypha in macerated specimens (figs. 10, 14, 19).

It is an obvious question: On what does the proof that the resting-spores and the conidia belong to the same fungus rest? The reply is simple and conclusive, though it has been by no means easy to obtain it. Putting aside the universal occurrence of the resting-spores in the white spots (fig. 1) as soon as they are well developed, and passing over the suggestive similarities to the "sporidia" of other Ustilagineæ, shown in the germination of the conidia (fig. 25), there are two series of observations which, together with these, place the connection between the spores beyond doubt.

In the first place the examination of very large numbers of careful sections has resulted in the obtaining of preparations like figs. 12-14, in which, although the anatomical continuity between a conidium and a resting-spore is not absolute, there can be no doubt as to the existence of that continuity. In fig. 14, the clearest case, the branch of the mycelium, passing up to the exterior, would end in a conidiophore, and it is attached to a branch bearing a resting-spore; and an examination of the other figure leads to the same conclusion: the difficulty of laying bare the hypha along its whole course is, of course, immense. The conclusion of the proof of continuity, however, is fully established by the production of the resting-spores from the conidia sown on the leaf.

I have already mentioned that when the white spots are at their best they are covered with the numerous conidia as with an impalpable chalky powder; the restingspores do not come to the outside, and thus it is perfectly feasible, and even very easy, to obtain pure sowings of the conidia by lightly passing a camel-hair pencil over the spots on an undamaged leaf. I have paid a great deal of attention to this matter of pure sowings and pure culture, and most of the clean sowings on glass (e.g., fig. 23) were obtained in this way: a clean, new camel-hair pencil was drawn lightly over a vigorous white spot, and a leaf infected in the manner described below, and then a glass slide was touched with the pencil, which still retained conidia; the purity of the last sowing was evidence of the purity of the infective sowing.

The method of infection is simple. The camel-hair pencil (or a clean needle), charged with conidia, is lightly placed for a moment in a drop of dew, or of distilled water, on a leaf of Ranunculus Ficaria. The sowing is then kept moist, either by means of a bell-jar placed over the plant, or by means of a damp-cell kept over the drop containing the sowing.

Precautions were taken to obtain the experimental plants from a distance, and from localities where no white spots were found on the leaves; moreover, I kept uninfected plants from the same neighbourhood in the same closed greenhouse, during the progress of the observations, as control plants. In all but one or two cases the infections succeeded perfectly, and in most cases the infective capacity (if I may so term it) of the conidia was most strikingly displayed.

It was perfectly easy to obtain such preparations as those figured at figs. 29 and 31 by stripping off the epidermis at the spot where the sowing had been made a few (12-24) hours previously; and similar preparations were obtainable any rainy day from the wet leaves in the ditch referred to above, especially when the leaf was first laid on water for a few hours. As the figures show, the conidia germinate normally, and at once proceed to push their germinal hyphæ through the wide orifices of the stomata; very often the germinal hypha makes coils, and it is usually at least sinuous. The unsuccessful tail-like hypha is developed at the opposite end of the conidium, as before; but the formation of the interpolated conidium is very rare—the germinal tube at once enters the stoma nearest it. Cf. figs. 23-27 and figs. 29 and 31.

When examining recently infected leaves, or young leaves from the damp ditch which were exposed to all the conditions necessary for infection, I often observed delicate little stretches of hyphæ lying on the cuticle, and looking like bits of a filamentous Schizomycete: two such bits are shown in fig. 29. Moreover, it was a by no means uncommon occurrence to see similar filaments on the inside of a stoma closely applied to the walls of the guard-cells, and evidently making their way inwards. It seems not improbable that these isolated filaments are really pieces of the germinal tubes, which have been formed at some distance from the stomata, and have become detached by the decay of the exhausted portions of hyphæ or spores behind them. I have already shown that the protoplasm passes along into the ends of the delicate germinal tubes, leaving the empty and exhausted portions behind to die off (cf. fig. 24), and it is certainly not impossible that by this means these filaments can creep forward, so to speak, to distances greater than the tube full of protoplasm can reach—in fact, we may say the germinal tube creeps along by building its own ladder behind it.

Be this as it may, the leaves are easily infected by means of the conidia, and in nearly every case a pallid greenish-white spot was found on the infected leaf in from 13 to 19 days from the sowing: moreover, the spot was always confined to the area on which the sowing was made.

The following list of infections will serve to illustrate this. In each case three

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leaves were infected—one leaf on each plant—and controlled as described. The date of sowing the conidia on the leaf is given in the second column, and the date on which the pallid spot was clearly visible in the third column: it should be noted that some difficulty occurs in deciding exactly when the pallid spot is visible, a difficulty which depends partly on the observer and partly on the hue of the leaf. The yellow spot of a Uredinous fungus is much easier to detect than these pallid greenish-white

spots in their younger stages.

Plant.	Date of sowing conidia.	Date on which the spot appeared.	Number of days occupied in developing spot.
	3.5	т ч	days.
$\mathbf{A}$	May 13	June 1	19
B	May 13	May 31	18
C	May 13 , . , .	June 1	19
D	May 16	June 2	17
E	May 16	Failed	
F	May 16	Failed	
G	May 24 ,	$\mathrm{June}\ 7  .  .  .$	14
$\mathbf{H}$	May 24	June 7	14
I	May 24	Failed	
K	June 2	June 21	19
$\mathbf{L}$	June 2 , .	June $19 \dots$	17
M	June 2	June 20	18
		,	

Hence the periods given in the Table are only approximate—indeed, they could not be otherwise so long as we are ignorant of the exact period occupied by the conidium in germinating, and by the fungus in making its progress through the tissues.

And now arises the question—can we throw any light on the problem as to the relative ease with which a parasitic mycelium invades its host?

So far as it goes, the following evidence seems of some value. I found that those plants of *Ranunculus Ficaria* which grew in the shaded damp ditch were infected more easily than plants growing in open drier situations. The differences between these two kinds of plants, so far as the leaves are concerned, are chiefly as follows:—

The more shaded plants have much larger leaves with much longer petioles: the laminæ are undoubtedly softer in texture, and brighter green in colour, the smaller tougher leaves of the plants in the open being of a dark and glossy green, especially above. These differences correspond to differences in minute structure: the shaded leaves have shorter, looser, palissade cells, and more intercellular spaces between them and in the large loose spongy parenchyma. Moreover, the stomata on the upper surface appear to be more numerous: on the lower surface the stomata seem to be larger, but I cannot say they are more numerous. The stomata have wider openings in the damp shaded plants, and the cell walls of all the parts are thinner and more watery; of course it may be assumed that there is more aqueous vapour in the intercellular spaces. Taking all these facts into consideration, I see no difficulty in explaining the differences in the times occupied in infections; and they also throw

light on the vexed question which has arisen around the unfortunate word "predisposition."

The plants in the ditch were certainly more apt to be diseased than others in the open, because the disease, once established, could spread like an epidemic under the conditions existing. I have other facts in other connections which bear out this, and I hope some time to be able to devote special and continuous attention to this question.

I do not propose to enlarge upon the subject of the resting-spores. After some months in the dormant condition they put forth promycelial tubes, from which sporidia are developed which seem to behave like the conidia described; the type of germination is like that of *Tilletia*.

#### DESCRIPTION OF THE FIGURES.

### PLATE 10.

- Fig. 1. A leaf of Ranunculus Ficaria with the white disease-spots containing the parasite. Two of the white spots are turning ashen grey in the centre, and a still older spot at the margin had turned brown, and the rotted tissues then fell away. The chalky appearance of the younger spots is due to the conidia. There are spots on both sides of the leaf.
- Fig. 2. A stoma with the hyphæ of the *Entyloma* protruding—from a leaf laid 12 hours in water. To the right is one of the hyphæ about to form a conidium at the apex. The hyphæ are here very long, since they grow into the water. (Zeiss, E.)
- Fig. 3. Similar pencil of hyphæ protruding from a stoma, and bearing conidia. The preparation is taken fresh from a leaf growing in not very damp air, hence the shorter hyphæ and conidia. (Zeiss, E.)
- Fig. 4. Similar preparation—one of the conidiophores slightly branched. (Zeiss, E.)
- Fig. 5. Similar preparation from leaf in damp ditch, and taken in wet weather, showing the elongated form of the hyphæ and conidia. (Zeiss, J.)
- Fig. 6. Stoma with protruding conidiophores from the margin of a young spore. The fungus is still young. (ZEISS, E.)
- Fig. 7. A cell from the spongy mesophyll of *Ranunculus Ficaria*, with copiously branched hyphæ of *Entyloma ranunculi* closely applied to its walls. (Zeiss, E.)
- Fig. 8. Cells surrounding an intercellular space, with the mycelium of the *Entyloma* on and between the cells. (Zeiss, D.)

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Fig. 9. Portion of epidermis stripped off and examined from inside, showing branched mycelium of the *Entyloma* running between the underlying mesophyll cells (these are omitted for simplicity). Nuclei and plastidia are seen in the outlined epidermis cells. Resting-spores in various stages of development are being formed by the mycelium; most of these are intercalary, one is at the end of a short branchlet. Haustorium-like protuberances are frequently developed and some branches anastomose. (Zeiss, J.)

## PLATE 11.

- Fig. 10. Similar preparation, the mycelium much branched. (Zeiss, J.)
- Fig. 11. Transverse section of a leaf of *R. Ficaria*, through a well-developed and active disease spot. The intercellular spaces are blocked up with mycelium and resting-spores; in the lacunæ below the orifices of the stomata the mycelium puts forth dense pencils of conidiophores. In the figure the stoma on the upper surface is cut longitudinally, that on the lower surface transversely and nearer one end. Conidiophores are also seen forcing their way between the epidermis cells of the upper side. The mycelium is all intercellular; wherever it appears otherwise, close examination shows that it is applied to the exterior of the thin walls. (Zeiss, D.)
- Fig. 12. Portion of extremely thin section through a disease spot, macerated and teased out. The two contiguous cells of the epidermis between which the hyphæ forced their way to the exterior have become separated. Two resting-spores among the hyphæ below. (Zeiss, J.)
- Fig. 13. Similarly teased preparation. The hyphæ are older and show the septa more clearly. (Zeiss, J.)
- Fig. 14. Portion of very thin section as before, hardened and examined in glycerine. It shows very clearly the passage out of the conidiophore in the middle lamella of two contiguous epidermis cells. The vertical branch to the right appears to have passed out similarly, but was cut; it is attached below to a branch bearing a resting-spore. (Zeiss, J.)
- Fig. 15. Lacuna between palissade cells from similar preparation; it is filled with resting-spores. (Zeiss, J.)

## PLATE 12.

- Figs. 16-18. Mycelium with resting-spores teased out and isolated from macerated specimens. (Zeiss, D.)
- Figs. 19 and 20. Resting-spores in various stages approaching maturity. (Zeiss, J.)

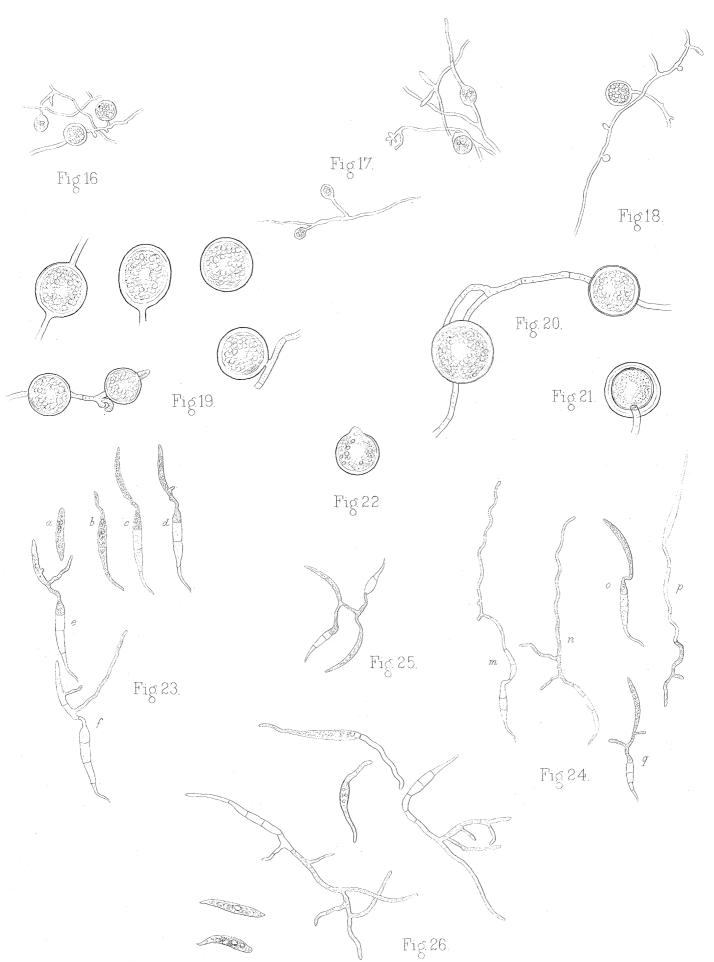
- Fig. 21. Mature resting-spore. The contents are more finely granular and translucent; a portion of its mycelium is still attached. (Zeiss, J.)
- Fig. 22. A resting-spore commencing to germinate. (Zeiss, E.)
- Fig. 23. Germination of the conidium of *Entyloma ranunculi*. After lying twenty-four hours in water it swelled up slightly and presented the appearance drawn at a; six hours later, i.e. at two p.m., it had commenced to germinate (b); c, the same conidium at ten p.m.; d, at nine A.M. next day; the secondary conidium is now commencing to germinate. At two p.m. the stage e was reached, and soon afterwards (f) the growth had ceased, the two little germinal tubes having taken all the protoplasm. (Zeiss, E.)
- Fig. 24. Conidia germinating in water on glass as before. The empty conidia and other parts decay and disappear, and the still living portions are thus completely isolated. (Zeiss, E.)
- Fig. 25. Two germinating conidia which have copulated. This is a very common event where they lie close together. (Zeiss, E.)
- Fig. 26. Conidia germinating in drops of water on the leaf. The secondary conidia are not formed, as a rule, and the germinating hyphæ are stronger and branch more. (Zeiss, E.)

## PLATE 13.

- Fig. 27. Similar preparation. (Zeiss, E.)
- Fig. 28. Conidia attached to branched conidiophore (below), which had been allowed to grow out in water; and a germinating conidium; both more highly magnified. (Zeiss, J.)
- Fig. 29. Portion of epidermis of *R. Ficaria* with conidia of *E. ranunculi* germinating on it. Germinal hyphæ are entering the orifices of the stomata; two pieces of germinal hyphæ (?) are lying on the epidermis cells. Inside the latter are nuclei and plastidia. (Zeiss, E.)
- Fig. 30. Portion of mycelium allowed to grow out into water; the protoplasm aggregates in certain cells and branches, and the septa are very distinct in the empty branches. (Zeiss, E.)
- Fig. 31. Stoma with two germinal hyphæ entering its orifice; seen from within. The spores are visible through the epidermis. (Zeiss, E.)
- Fig. 32. Stoma seen from within. On its walls are thin branched hyphæ of unknown origin, but which may possibly be isolated pieces of germinal tube. (Zeiss, E.)

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- Fig. 1. A leaf of Ranunculus Ficaria with the white disease-spots containing the parasite. Two of the white spots are turning ashen grey in the centre, and a still older spot at the margin had turned brown, and the rotted tissues then fell away. The chalky appearance of the younger spots is due to the conidia. There are spots on both sides of the leaf.
- Fig. 2. A stoma with the hyphæ of the *Entyloma* protruding—from a leaf laid 12 hours in water. To the right is one of the hyphæ about to form a conidium at the apex. The hyphæ are here very long, since they grow into the water. (Zeiss, E.)
- Fig. 3. Similar pencil of hyphæ protruding from a stoma, and bearing conidia. The Downloaded from retb.royalsocietypublishing.org preparation is taken fresh from a leaf growing in not very damp air, hence the shorter hyphæ and conidia. (Zeiss, E.)
  - Fig. 4. Similar preparation—one of the conidiophores slightly branched. (Zeiss, E.)
  - Fig. 5. Similar preparation from leaf in damp ditch, and taken in wet weather, showing the elongated form of the hyphæ and conidia. (Zeiss, J.)
  - Fig. 6. Stoma with protruding conidiophores from the margin of a young spore. The fungus is still young. (Zeiss, E.)
  - Fig. 7. A cell from the spongy mesophyll of Ranunculus Ficaria, with copiously branched hyphæ of Entyloma ranunculi closely applied to its walls. (Zeiss, E.)
  - Fig. 8. Cells surrounding an intercellular space, with the mycelium of the Entyloma on and between the cells. (Zeiss, D.)
  - Fig. 9. Portion of epidermis stripped off and examined from inside, showing branched mycelium of the *Entyloma* running between the underlying mesophyll cells (these are omitted for simplicity). Nuclei and plastidia are seen in the outlined epidermis cells. Resting-spores in various stages of development are being formed by the mycelium; most of these are intercalary, one is at the end of a short branchlet. Haustorium-like protuberances are frequently developed and some branches anastomose. (Zeiss, J.)

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