Cordyceps ophioglossoides (Ehrh.).

BY

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With Plate XXVIII.

MOST of the species of *Cordyceps* are well known to be parasitic upon insects, particularly upon caterpillars. *C. ophioglossoides*, however, together with *C. capitata*, is parasitic upon various Tuberaceae. *C. ophioglossoides* occurs upon *Elaphomyces granulatus*, *muricatus*, and *variegatus*. Masee, in his 'Revision of the Genus *Cordyceps*¹', separates these fungus-inhabiting species from *Cordyceps*, and places them in a distinct genus, *Cordylia*, restricting *Cordyceps* to the insect-inhabiting species. The character, however, seems hardly to be of generic importance. An account of the relationships and distinctive characters of the genus is given in Masee's paper.

The stroma is much like that of the other species of *Cordyceps* (Pl. XXVIII, Fig. 1). It is upright and club-shaped. It is composed of a stalk about 3 inches long and $\frac{1}{8}$ inch thick, and of a head from 1-1 $\frac{1}{2}$ inches long, thicker in the middle and tapering to a somewhat obtuse end. The stalk has a smooth surface and is firm and fleshy in consistency; the head is covered with numerous small papillae, at the tips of which the perithecia open.

¹ Ann. Bot., 1895.

The material with which this research has been carried on was given me by Prof. H. Marshall Ward, who collected it in the autumn of 1900 in Scotland. The tubers upon which it was parasitic were those of *Elaphomyces variegatus*. The greater part was fixed in ordinary methylated spirit, some part in Kieser's solution. The chief stains used were Delafield's and Heidenhain's haematoxylins. I also tried Flemming's and Hermann's safranin-gentian-violet double stains, but the material was not fixed well enough to permit the use of these with advantage.

STRUCTURE AND DEVELOPMENT OF ASCI AND SPORES.

Unfortunately all of the material at my disposal was mature; none of it showed any young stages in the development of the stroma or perithecium.

The cavity of the mature perithecium is roughly egg-shaped (Fig. 2); it passes above into a very narrow neck, and this opens at the top of small, flattish papilla.

The general mass of tissue of the fertile part of the stroma is very loose: it would appear to have been pulled apart by the growth, during the development of the perithecia. The outermost layers, however, are composed of closely woven hyphae, which run for the most part parallel to the short axis of the stroma and transversely to the long axis.

The wall of the perithecium itself is also composed of closely woven parallel-running hyphae. This gives the perithecium the appearance of being an invagination of the surface layers of the fertile stroma.

The hymenium occupies the basal part of the perithecium-cavity: the hyphae of which it is composed are closely packed and run parallel to the long axis of the perithecium.

In a mature perithecium the cavity is occupied by a large number of very long cylindrical asci in all stages of development. This continuous development of asci alone rendered it possible for me to obtain different stages in the formation of the spores. When mature, the asci extend up to the neck of

the perithecium; young ones are continuously being pushed up between the bases of the older ones.

The youngest ascus I have observed is shown in Fig. 3, which represents the upper two-thirds of the ascus. It contains already about half a dozen nuclei, irregularly distributed. The protoplasm is finely granular, and stains darkly with haematoxylin; a cap is present even at this stage. I have obtained all stages intermediate between this and the mature ascus.

The nuclei divide repeatedly, and the resulting nuclei distribute themselves quite irregularly in the protoplasm of the ascus. Successive stages in this process of division are shown in Figs. 5 *a*, *b*, *c*, 6, 7, and 8. Figs. 5 *a*, *b*, and *c* show some of the nuclei in the process of division. I was, of course, unable to observe any karyokinetic figures. The protoplasm throughout these divisions remains finely granular, and continues to stain darkly with haematoxylin. During this period of nuclear division the ascus increases slightly in breadth and considerably in length.

At the end of this period we have then the long, narrow ascus, tapering towards the base, and with a well-marked cap (Fig. 12) stretching the greater part of the length of the perithecium. It is somewhat twisted, so that it is rare to get any considerable length of an ascus in one section, even though the section be median to the perithecium; moreover, the large number of asci in a perithecium renders it almost impossible to piece together the whole of any single ascus from a series of sections. The protoplasm is dense, and shows no traces of divisions. Scattered irregularly about in the protoplasm are immense numbers of small, roundish nuclei (Figs. 7 and 8).

After divisions have ceased, the nuclei become arranged in rows longitudinal to the long axis of the ascus. Owing to the large numbers of nuclei and the narrowness of the ascus, this does not involve any great change of position for the nuclei, as may be seen from Fig. 8. Eight longitudinal rows are formed, of which three are usually seen in section.

Following this rearrangement, longitudinal lines of division

appear in the protoplasm of the ascus (Fig. 9), dividing this up into eight filiform ascospores; these are thus multinucleate from the beginning. The ascospores soon become rounded off and acquire a wall, not, however, till further divisions have occurred. In sections transverse to the ascus, seven outer ones are seen surrounding a single inner spore (Fig. 14 *a*).

Immediately following, indeed almost contemporaneous with, the longitudinal divisions, transverse lines appear, dividing each ascospore into a large number of cylindrical, uninucleate sporidia (Fig. 10). Stages such as that shown in Fig. 9 are very rare. The divisions seem to occur simultaneously throughout the ascus.

The sporidia, once formed, become separated, and each acquires a wall; they still, however, remain eight-ranked (Figs. 11, 14 *b*). The ascus becomes considerably broader and rather longer during this process (compare Figs. 14 *a* and *c*), and its wall becomes very fine and almost invisible. The sporidia become rounded at the ends; when ripe they are cylindrical-ellipsoidal in shape, about $4\ \mu$ long by about $2\ \mu$ broad. During the ripening the protoplasm becomes clear and free from granules, and stains much less darkly; the nuclei become somewhat larger, the contrast between young and ripe asci is thus very marked. During this time also the walls of the sporidia become changed chemically, and are probably rendered harder. They now resemble the caps of the asci: this was very clearly shown in sections of material fixed in Kieser, which were stained with Hermann's safranin-gentian violet; the walls of the ripe sporidia and the caps of the asci were coloured dark violet, while the walls of the younger asci and spores were pink (the nuclei and protoplasm were not stained). Fig. 13 shows part of an ascus with ripe sporidia: these are probably contorted in fixing.

ANATOMY OF STERILE PART OF STROMA.

The sterile part of the stroma shows nothing of special interest. The central mass consists of closely interwoven, septate, narrow hyphae, with thin walls, running quite irregu-

larly. The outer layers are composed of larger hyphae, with thicker walls and less cell-contents than the central ones: these outer hyphae are interwoven as are the central ones, but they are more regular; their general course is parallel to the long axis of the stroma (Figs. 15 and 16 *a*). There appear to be no special conducting or 'laticiferous' tubes present.

CONNEXION BETWEEN CORDYCEPS AND ELAPHOMYCES.

When sections were made of the lower part of the *Cordyceps* stroma, they were found sheathed with a layer of large, rather thick-walled hyphae belonging to *Elaphomyces*. Lower still, masses of these hyphae appeared as islands in the central mass of the *Cordyceps* stroma. These hyphae were considerably larger even than those of the outer layers of the *Cordyceps* (compare Figs. 16 *a* and *b*): they were much more loosely arranged, and their walls always stained more readily than those of the *Cordyceps* hyphae, so that there was little difficulty in distinguishing between the two sets.

The relation that the *Cordyceps* thus bears to the *Elaphomyces* tuber has a broad resemblance to that borne by the young sporophyte of an Archegoniate plant to the gametophyte, which is essentially a parasitic one. The basal part, then, of the *Cordyceps* stroma may be compared to the foot of the *Anthoceros* or Fern embryo, or to the absorbent cotyledon of a grass or palm in the endosperm. In all my material, which only showed mature *Cordyceps* stromata, very little of the original *Elaphomyces* tuber remained: it has been exhausted, and the tissue replaced by the advancing 'foot' of the *Cordyceps*.

If the *Elaphomyces* hyphae be more closely examined, they are found to be interwoven with smaller, thin-walled hyphae of *Cordyceps*. It seemed possible that the parasitic hyphae developed haustoria, by means of which the nutriment was absorbed from the host. I spent accordingly a considerable time in examining a large number of sections, taken in all directions and from several specimens, in order to determine, if possible, the presence or absence of haustoria.

I was, however, unable to distinguish anything of the nature of a definite haustorium. Though, of course, in such a case negative evidence is of little value, still it is hardly probable that if haustoria were present in any number, I should never have observed them.

Unions, however, do exist between the two sets of hyphae, and these are fairly frequent. Where they occur, the two adjoining walls fuse and become considerably thinner; the thickness of the partition is certainly not more than that of the walls of the *Cordyceps* hyphae themselves (Figs. 17, 18, 19, 20). In some cases, moreover, the *Cordyceps* hypha projects into the cavity of the other (Figs. 21, 22, 23), in which case the union has the appearance of a rudimentary haustorium.

Whether or not these hyphal fusions are sufficient for the nutrition of the *Cordyceps* I cannot say: they suggest again the comparison with a Phanerogamic embryo parasitic upon the endosperm, or the sporophyte of a fern upon the gametophyte. Possibly an examination of the *Elaphomyces* tuber in the earlier stages of the attack, and while the *Cordyceps* stroma is still actively growing, would give more definite results.

THE MYCORHIZA OF *ELAPHOMYCES VARIEGATUS*.

Elaphomyces has long been known as one of those Fungi which form a mycorrhiza with the roots of Conifers. Reess¹ has described the mycorrhiza on the Pine roots.

Among my material was a rootlet of *Pinus*, bearing a coral-like mass of secondary and tertiary rootlets forming the mycorrhiza; this was still in connexion with a part of the rind of an *Elaphomyces* tuber, so that no doubt could be entertained as to the identity of the mycorrhiza Fungus. One specimen which is now in the Botanical Museum at Cambridge shows the whole connexion between the *Cordyceps ophioglossoides*, *Elaphomyces variegatus*, and the mycorrhiza on the Pine root; it is probably almost unique.

As my observations differ somewhat from the account given

¹ Bibl. Bot., 1887.

by Reess, I thought it advisable to give a short account of the mycorhiza-structure, so far as could be made out from the material at my disposal.

Reess describes the mycorhiza as being ectotrophic and says, at all events for the young stages, that the Fungus extends into the root only a distance equal to the thickness of the fungus-sheath outside. He describes these internal hyphae as being intercellular, and as giving rise to peculiar mulberry-like ingrowths into the cell-cavity of the cortical cells. He is in doubt as to whether these structures are haustoria, or merely hypertrophies of the cell-wall caused by the action of the intercellular hyphae; he seems on the whole to favour the former view, at any rate in some cases, while in others the growths appear to show no lumen and give the reactions of ordinary parenchyma-membranes, while their connexion with the intercellular hyphae is doubtful. These structures are described as being conspicuous and of frequent occurrence.

My observations show that *Elaphomyces*, as a mycorhiza Fungus, is rather variable in its structure.

The structure most frequently found shows in the young stage the ordinary fungus-sheath on the outside of the root, and this is continuous with an intercellular network (Fig. 25) between the cells of the root-cortex. The network in surface view has the appearance of a reticulate thickening of the cell-walls. This structure is that described by Frank¹, for the mycorhiza on the roots of Cupuliferae, but in his case only the outermost layer of cells of the root was concerned. In my sections the whole of the root-cortex was seen to be penetrated in this way by the Fungus (Fig. 24).

One very characteristic feature of this mycorhiza is the action of the Fungus on the cortical cell-contents. These are transformed into (apparently) slimy, deeply staining masses, which contain fat (Figs. 25, 27, 28, 29). This change takes place right through the cortex of the root. Sometimes a large, more or less disorganized nucleus is present in the mass.

¹ Bot. Zeit., 1885.

In none of my preparations showing this intercellular network was there any sign of intracellular hyphae or of haustoria. I may say now that in no case have I ever seen anything of the peculiar mulberry-like ingrowths, figured and described by Reess.

The next older stage of this form of the mycorrhiza is shown in Fig. 26. On the outside is the pseudoparenchymatous fungus-sheath, which has invaded in parts the root-tissue—the outer layer of cells of the root is quite broken up, leaving only bits of cell-wall surrounded by the fungal network. Following this fungus-sheath we get the root-cortex: this no longer shows the intercellular network of internal hyphae; the cells are separated by large empty spaces; they are filled with the slimy, dark-staining masses mentioned above. In nearly every case the walls of these cortical cells are beginning to break down. In the centre of the section we get the feebly developed stele, which appears quite healthy and unaffected by the Fungus.

The oldest stages have a very peculiar appearance (Figs. 27, 28). As before on the outside we get the fungus-sheath quite healthy and unchanged. In the centre of the section, also healthy and unchanged, is the stele. But between the fungus-sheath and the stele we have simply an almost homogeneous mass of slimy, disorganized cell-contents, in which we are still able sometimes to trace the outlines of the original cortical cells. Scattered in this we get broken bits of cell-walls and some few nuclei.

Stahl¹ has recently treated afresh the whole question of mycorrhiza; he reviews all previous work and himself supports Frank's view that in all cases the mycorrhiza is a symbiotic union between the Fungus and the host root. He regards the Fungus as the dominant partner, which has forced itself on the host by cutting off the food-supply, and so compelling the higher plant either to compete with it by means of an extensive root and leaf-system, or to submit to an alliance.

Certainly in the union between *Elaphomyces* and the Pine

¹ Prings. Jahrb., 1900.

root, the Fungus would seem to be dominant. My observations do not show whether or not the Pine gains any compensating advantage for the loss which it undoubtedly sustains through the presence of the *Elaphomyces* hyphae. Certainly the roots have the appearance of suffering from the attack of a true parasitic Fungus, and it is difficult to imagine such a cortex as shown by them being of any service in the transport of nutrient material to the stele.

I must now briefly describe other appearances presented in the Pine roots, by apparently the same Fungus.

When sections were taken of the main rootlet they showed that no external fungus-sheath was present. The root, however, was not free from the Fungus; in many of the cortical cells the cell-cavity was filled with a pseudoparenchymatous network of hyphae (Figs. 29, 30). The outer cortical cells appeared to be first attacked, but the Fungus afterwards extends throughout the cortex, and in many cases the network was to be seen even in the cells of the endodermis. The walls of the cortical cells are fairly thick, some of these cells, as also some of the parenchymatous cells of the stele are filled with dark-staining substance (Fig. 29), similar to that shown by the cells of the cortex in the other roots as mentioned above. There is certainly a considerable difference between these hyphae and the intercellular network of the smaller rootlets, but there seemed no trace of the presence of another Fungus, and I myself am convinced that both sets of hyphae belong to *Elaphomyces variegatus*.

Another appearance presented by this mycorrhiza was only seen by me in one series of sections. It here showed one of the main rootlets of the mycorrhiza, and arising from this a smaller one. Both were covered with a fungus-sheath which was continuous over the point of origin of the secondary rootlet. The smaller rootlet showed the younger stage of the intercellular network. The larger rootlet on the contrary showed nothing of this, the walls of the cortical parenchyma were thickened and quite normal (Fig. 31). But in many of these cortical cells were seen bundles of fungal hyphae

or single hyphae (Fig. 31). These do not agree in any way with the description and figures given by Reess of his haustoria.

If these three forms of mycorrhiza are really due to the action of one Fungus, and this certainly appears most probable, it would seem to show that *Elaphomyces variegatus* is capable of varying its method of attack, according to the strength of the root attacked; when this is small, with thin-walled cortical parenchyma, we may suppose the intercellular network is sufficient for the destruction of the cortex. On the other hand we must presume that the older roots had not been attacked until the cortical cells had thickened their walls, in which case the formation of the intercellular network was not possible or not sufficient, and so the Fungus resorted to the intracellular method of attack. I offer this merely as a suggestion; it may be, of course, that three distinct Fungi were present.

In conclusion I have to thank Prof. Marshall Ward for his kindness, not only in giving me the material with which to work, but also for his assistance while the work was in progress. I have also to thank Mr. R. H. Biffen for much advice and assistance.

EXPLANATION OF FIGURES IN PLATE XXVIII.

Illustrating Mr. Lewton-Brain's paper on *Cordyceps ophioglossoides*.

- Fig. 1. General drawing of *Cordyceps ophioglossoides*, with two mature stromata.
Fig. 2. Mature perithecium in median section; *hy.*, hymenium; *as.*, asci in different stages of development; *o.*, opening of neck of perithecium.
Fig. 3. Part of young ascus in longitudinal section, with very few nuclei (*n.*).
Figs. 4, 5 *a, b, c*, 6, 7, 8. As Fig. 3 showing progressive stages in division of nuclei, before divisions occur in the protoplasm.
Fig. 9. As before, showing longitudinal line of division in the protoplasm.
Fig. 10. As Fig. 9, showing both longitudinal and transverse divisions.
Fig. 11. Part of ascus with nearly mature sporidia.
Fig. 12. Cap of ascus.
Fig. 13. Part of ascus showing three rows of ripe spores.
Fig. 14. Transverse sections of asci of various ages.
Fig. 15. Transverse section of sterile portion of stroma; *ex.*, outer hyphal layer; *in.*, central hyphae.
Fig. 16 *a* and *b*. Camera lucida drawings with same magnification (about 1000), (*a*) of external hyphae of *Cordyceps*, (*b*) of *Elaphomyces variegatus*.
Figs. 17–23. Various unions between large hyphae of *Elaphomyces* and the smaller parasitic hyphae of *Cordyceps ophioglossoides*.
Fig. 24. Part of tangential section of cortex of Pine root, showing intercellular network of hyphae (*int.*).
Fig. 25. Transverse section of root in same condition as in Fig. 24; *sh.*, fungus-sheath; *int.*, intercellular network; *c.*, cortical cells; *st.*, stele. $\times 225$.
Fig. 26. Part of cortex of Pine root, showing more advanced stage than Fig. 25. $\times 225$.
Figs. 27, 28. Sections showing still more advanced stage; *cor.*, remains of cortex. Fig. 27 $\times 225$.
Fig. 29. Part of rootlet in transverse section, without external fungus-sheath; *x.*, intracellular network of hyphae; *c.*, cortical cells; *par.*, parenchyma of stele.
Fig. 30. Part of cortex of similar root.
Fig. 31. Part of cortex of another rootlet, showing bunches of intracellular hyphae (*h.*).

Lewton-Brain, L. 1901. "Cordyceps ophioglossoides (Ehrh.)." *Annals of botany* 15, 521–531. <https://doi.org/10.1093/oxfordjournals.aob.a088832>.

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