PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The Seed-Fungus of Lolium temulentum, L., the Darnel

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Phil. Trans. R. Soc. Lond. B 1904 **196**, 1-27
doi: 10.1098/rstb.1904.0001

PHILOSOPHICAL TRANSACTIONS.

I.—*The Seed-Fungus of* Lolium temulentum, *L., the Darnel.*

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Communicated by Professor Marshall Ward, *F.R.S.*

Received June 6,—Read June 19, 1902.

[Plates 1–3.]

Introductory.

It has long been known* that the grains of the Darnel contain a poisonous body (Lolium), which can be extracted by ether. This substance has marked toxic action on rabbits and certain carnivorous animals, and is said to induce vomiting and other unpleasant symptoms in man, but to affect pigs, cattle, and geese but little, or not at all.†

In 1898‡ attention was drawn to the fact that a large percentage (80–100 per cent.) of the grains of this grass contain a definite fungus-mycelium, always situated in a definite layer of the seed, *i.e.*, in the remains of the nucellus, just outside the aleurone-layer of the endosperm.

Although certain details were made out regarding the nature and position of the hyphæ, their relations to the seed and seedling, and to the poisonous properties referred to, almost nothing was discovered regarding the systematic position of the fungus, the course of its life-history, or even how it obtains its entry into the *Lolium*.

In the following account of an investigation pursued in the Cambridge Botanical Laboratory during the past session, I have succeeded in carrying our knowledge of this remarkable fungus considerably further, and especially in rendering clear the principal points concerning its life in the plant, and the mode of infection of the embryo. Nevertheless, all attempts to grow the fungus outside its host-plant, or to induce it to form spores, have failed with me as with other investigators, and although some suggestive facts have been obtained which may help in future efforts to establish

* According to Guérin (5) since Roman times.

† Hackel in Engler and Prantl, 'Natürliche Pflanzenfamilien,' II. Th., 2 Abth., 1887, p. 76.

‡ By Vogl, Guérin, Hanausek, and Nestler, in four papers referred to below.

the systematic position of the fungus, we are still utterly baffled as to whether it is an Ustalagine, a Uredine, or an Ascomycete of the nature of ergot or other toxic species as yet unknown.

The evidence so far points to the present case being an interesting example of symbiosis.

Historical Summary.

Previous to 1898 the research work on *Lolium temulentum* was entirely of a chemical nature, dealing especially with the toxic constituents. In January, 1898, Vogl (2) announced the presence of a layer of fungus hyphæ in the crushed nucellar remains just outside the aleurone layer, and later on in the same year three other papers appeared on the subject.

In August, Guérin (5) published the result of his investigations, the object of which was to determine the cause of the toxicity of Darnel.* He described and figured a densely interwoven layer of hyphæ between the exterior wall of the aleurone layer and the hyaline layer of the grain. He found the hyphæ lining the entire grain, except along the groove on the inner side and in the vicinity of the embryo. No mention is made of the fungus in the early stages of the growing plant. He observed the fungus in the young ovary before the fertilisation of the egg-cell, as also the growth of the endosperm, which results in the compression of the layer of hyphæ found in the mature grain. *Lolium arvense*, With., and *L. linicolum*, Sond., were also observed to contain the fungus, and since these three species are those to which poisonous properties are assigned, Guérin suggested that their toxicity may be due to the fungus found in them. *Lolium italicum*, Braun, did not contain hyphæ at all, and of *L. perenne*, L., only a single example with the fungus was obtained. Samples of *L. temulentum* from South America, Asia, Africa, and Europe, were examined, and only three were found to be devoid of the hyphæ. The nature of the fungus could not be determined. Guérin points out the pronounced differences which distinguish it from *Endoconidium temulentum*, described by Prillieux and Delacroix (10). The germination of these grains of *Lolium temulentum* was found to be excellent, and the fungus was said to be never met with in the neighbourhood of the embryo. Finally, Guérin suggests that the presence of the hyphal layer in Darnel grains indicates a case of symbiosis rather than of true parasitism. The reagents used by Guérin were chloral hydrate or lactic acid for swelling, and "coton bleu" for staining.

The papers of Hanausek and Nestler appeared in September. Hanausek (3) described the position of the fungus essentially as did Guérin. He also found the hyphæ in the nucellus of the young ovules, where it produced knots ("Knäuel"), which, however, developed no further. He pointed out similarities with the Ustila-

* In a subsequent note (11), Guérin claims that his work wa at least contemporaneous with that of Vogl, although published later.

gineæ, and was of opinion that there is much evidence in support of the view that the fungus is an Ustilagine, which rarely forms spores. No hyphæ were found in *L. perenne*.

Nestler (4) agrees with the previous authors as to the position of the hyphæ in the grain. Darnel grains were allowed to germinate under various conditions after treatment with ether. On the eighth day some hyphæ were found in the growing point of the seedling stem and in the base of the young leaf rudiments. They were subsequently found also in the internodes, and eventually in the vegetative cone and in the youngest leaf rudiments, occupying the intercellular spaces. They were even traced through the axis of the inflorescence into the ovular rudiment, entering through the funicle, and were found in abundance in the nucellus. Nestler's description of the subsequent development agrees with that of Guérin. Nestler also placed pieces of the hyphæ from the nucellar layer of the mature grains in various nutrient solutions of different strengths; but the hyphæ would not grow. The examination of the grains during germination showed, after a few days, a formation of isolated hyphæ, with rounded cells at the end or in the middle of the hyphæ ("Sporenbildung"). Later on, little but imprints on the aleurone cells were to be found. When the culms were about 1 decim. high, the endosperm of the grain was found to contain numerous thin, long, segmented hyphæ with perpendicular branches. These occurred even in partially sterilised grains. Nestler adds that the identity of these with the nucellar hyphæ is not proved. As to the method of penetration, the author suggests that the fungus is in the vegetative cone from the beginning of germination, but he found it in the growing point of the embryo in only a single instance. He also argued that penetration could not have taken place from the exterior, as his careful manipulation must have prevented such an entrance. No definite systematic position is assigned to the fungus, but a similarity between the toxic physiological action of Darnel and of Woronin's (12) "Taumelroggen" is suggested as possibly indicating relationships. The great differences in appearance and behaviour of the fungi which cause "Taumelroggen" are, however, pointed out. Nestler made use of chloral hydrate and potassium hydrate as clearing reagents.

No fungus layer was found in the other species of *Lolium*. The species examined were *L. perenne*, L., *L. multiflorum*, Lam. (= *L. italicum*, A. Br. = *L. Boucheanum*, Kunth), *L. remotum*, Schrank (= *L. arvense*, Schrad. = *L. linicolum*, A. Br.), *L. festucaceum*, Link. (= *L. perenne*, L. × *Festuca elatior*, L.), and others.

A short paper by Micheletti appeared last year (1901), and deals almost entirely with the toxicity of Darnel (6).

Methods.

For the demonstration of the presence of the fungus in the growing point of the plant, the grains were placed in an ordinary germinating chamber, and the embryos or seedlings dissected out at different stages. Flemming's weak solution (7, p. 41)

and chromic acid, 1 per cent. and ½ per cent., were used as fixing fluids. The material was cut, in the usual way, in paraffin. Aniline-water-safranin (8, p. 185) and HAIDENHAIN'S hæmatoxylin proved most useful for staining. The latter is particularly helpful in the detection of the hyphæ by means of the nucleoli, which stain deeply, and the method proved quite successful, especially in the examination of the seedling. Analine-water-safranin is very useful in the study of the ovary. Chloral hydrate, potassium hydrate, and lactic acid are useful in certain cases in the examination of the grains, but they are not suitable for the study of the growing point; their use probably accounts for the failure of previous writers to discover the hyphæ in the earliest stages of germination. As the alcoholic dehydration and high temperature of the paraffin-bath make the starchy endosperm unfit for the preparation of paraffin sections, the grains were cut in an ether-freezing-microtome after fixing in the usual way.* Of sections so cut, some were examined without further treatment, and others were treated with the various reagents above mentioned.

Attempts at cultures of the nucellar hyphæ taken from the grain were made in various media, in hanging drops in the Marshall Ward cell (9, p. 131). The difficulties of obtaining mounts free from bacteria and from other fungi are considerable, and details of the cultures will be described later on.

Numerous grafts of embryos of various species of *Lolium* upon the endosperm of other species were also successfully obtained. In the early part of this investigation the small percentage of grains of *Lolium temulentum* which do not contain the fungus, as contrasted with the similarly small number of grains of *L. perenne* which do contain it, occasioned some difficulty, especially in view of the erratic occurrence and variation, as explained below. This difficulty was completely obviated later by examining sections from the stigmatic end of all grains used, so that the condition of each grain (as regards presence or absence of fungus) worked with was accurately known. In the grafting and other experiments of a similar nature, no method was devised which could ensure absolute freedom from various moulds and bacterial forms without at the same time injuring the embryo, but several were found helpful in checking the growth of these forms, and in some cases perfectly clean seeds were apparently obtained. Dry heat at 95–98° C. killed the embryos; immersion in ether for 15 minutes (the method of NESTLER) gave fairly satisfactory results in checking the growth of moulds, but failed to completely prevent contamination; the embryos in almost all cases survived the treatment. One per cent. corrosive sublimate applied to the dry grains for 10–15 minutes usually cleaned the grains, but the embryos which survived such severe treatment were comparatively few in number. A 7-minute immersion in the same solution, however, proved satisfactory, at least in checking, and often in completely preventing, foreign growths; the growth of the

* I am greatly indebted to Mr. A. W. HILL, Demonstrator in Botany, for the use of apparatus in this part of the work.

embryos was at most but slightly retarded. In all cases where antiseptic solutions were used the grains were subsequently thoroughly washed in boiled distilled water.

The Grain and its Fungus.

The cross-section of the average grain of *Lolium temulentum* shows a layer of densely woven fungus hyphæ just outside the aleurone layer in the crushed remnants of the nucellus, of which the outer cell-rows form the "hyaline" layer. This is the hyphal layer which has been described by NESTLER, GUÉRIN, HANAUSEK and others.

Previous writers have found but a very small per cent. of the grains of *L. temulentum* devoid of the hyphæ. HANAUSEK states that he examined many hundreds of grains and found none without the fungus! The number devoid of the fungus found by GUÉRIN was very small, and NESTLER also reports only a few. I have found the proportion between those grains with and those without the fungus exceedingly variable. In one package of grains grown at the University Botanical Gardens in Cambridge, 15 per cent. (12 out of 76) were devoid of the fungus, and in a package received from Upsala, out of ten grains examined eight did not contain hyphæ. Another lot of thirty-five from the University Botanical Gardens at Cambridge gave thirty-three with and two without hyphæ. The latter probably approximates the usual proportion. The error which might arise from an admixture of other species is practically nil, on account of the marked characters of the grains of Darnel.*

After examining about twenty grains, I discovered that certain microscopic differences were sufficient to enable me, even without the aid of a lens, to pick out most of those grains devoid of the fungus, subsequent microscopic examination in almost every case confirming the selections. The grains without the fungus often appear incompletely developed. They are usually more slender in both lateral aspects, and less swollen in the centre, and are usually devoid of the yellow to dark-brown or grey colour of those which contain the fungus. In many cases they are more or less yellowish-green in colour; but sometimes of a dark straw-yellow hue. Nevertheless examples are to be met with which are microscopically indistinguishable from those which contain the fungus. The latter are usually swollen considerably in the central region, and are yellowish-brown or grey in colour, seldom green. These general differences are based upon the examination of material of 1901, grown in the Botanical Gardens at Cambridge. The colour is perhaps the most useful diagnostic character. Apparently grains kept several years tend to lose the colour difference. I have specimens of *L. temulentum* var. *arvense* which are so dark in colour that at first sight one would pronounce them to be ergoted; they are, however, perfectly healthy grains containing a broad layer of hyphæ.

For the examination of the fungus in the grain and embryos, grains of Darnel were placed in ordinary germinating chambers for 18 to 24 hours. In such grains no

* See MARSHALL WARD, 'Grasses,' p. 168, and authorities quoted.

actual growth of the embryo has as yet taken place, since very rarely indeed are mitotic figures found. The considerable increase in the size of the embryo is of course due simply to the absorption of moisture. These embryos were then dissected out and cut by the paraffin method, while the endosperm was cut with an ether-freezing-microtome. Longitudinal and cross-sections of the grain show the hyphal layer in the nucellar remnants, distributed almost co-extensively with the aleurone layer (figs. 3–9).

According to all previous writers, the hyphæ are not found near the embryo nor along the groove on the inner side of the grain; but although this is true for the upper part of the groove, at the base of the latter, where the end of the aleurone layer (fig. 4*a*) comes into contact with the outer surface of the scutellum, the hyphæ are again found (figs. 4 and 5), often as a fairly broad layer, which appears to have entirely escaped the observation of previous investigators.

The hyphæ at this point penetrate round the end of the aleurone layer, and hence come into direct contact with the embryo, and it is from these hyphæ that entrance into the embryo has been effected. In this *infection layer*, as I will term it, the hyphæ run, chiefly longitudinally, for some distance above and below the end of the aleurone layer (figs. 22–24), and since the layer is often broad, a more or less circular area of hyphæ can be seen on the outer surface of the scutellum at its base (fig. 4*b*). The absence of hyphæ along the groove of the inner surface is explained in the development of the ovule. The funicular region of the young ovule, along which the groove subsequently arises, contains at first hyphæ, as elsewhere, though they are less abundant than in the rest of the nucellus; but in the elongation of the ovule no growth of hyphæ seems to take place in this region, leaving the groove devoid of the fungus in the mature grain.

In all cases observed the infection has taken place from the *infection layer* described above. The aleurone layer in this region is often bent back along the scutellum towards the tip of the latter, thus forming a double layer (fig. 22). This is also explained in the development of the grain. The hyphæ gain entrance to the embryo before the latter has attained its ultimate intra-seminal size, and firmly fasten the ends of the aleurone layer to the embryo; so that the continued growth of the embryo results in a dragging and doubling of the aleurone layer, sliding being prevented by the hyphæ. In some cases, however, this bending back of the aleurone layer does not occur, and the hyphæ are then found on both sides of the end cells of the layer (figs. 23 and 24). The hyphæ may frequently be seen growing through between the aleurone cells in this region (figs. 22 and 23); such a penetration is not usually found in any other part of the aleurone layer. Under certain conditions, to be referred to subsequently, however, it does occur, and is then usually very frequent (figs. 37–39).

On the outer side of the grain (fig. 1) the aleurone layer reaches to the ligule of the scutellum, and the hyphal layer on this side does not reach the end of the aleurone*

* Guérin's figure (*loc. cit.*, p. 235), showing the aleurone extending downward on the outer surface to a point opposite the growing cone is, according to my observations, incorrect.

(figs. 3, 5 and 8); it may, however, extend to within a dozen cells from the end. It is not impossible perhaps that infection may, in exceptional cases, take place from this side of the scutellum; but, if so, it occurs very seldom. I have seen no evidence either in the mature grain or in the developing ovary to indicate that such an infection is ever accomplished. In all cases which I have examined, the infecting hyphæ have been found in the area described above (figs. 4 and 16).

Along a median longitudinal line of the outer surface a shallow groove marks the line of a considerably deeper groove, found in the young ovary, and along this groove the hyphal layer is often narrower than in the neighbouring regions. Moreover, the layer of hyphæ varies considerably in size in different grains as well as in different regions of the same grain. A fair average of the thickness is perhaps 10 to 15μ, but in some grains it acquires a thickness of 45μ. It is thickest in the lateral prominences and narrowest along the shallow groove of the outer surface, as described above. Along the inner groove, where the layer is entirely wanting, it may cease abruptly or taper off gradually. Beyond the end of the aleurone layer the embryo comes into direct contact at all points with a thin covering of the fused pericarp, seed-coats, and nucellus (figs. 5, 8 and 9*b*).

Previous writers have not recorded any penetration of the aleurone layer by the hyphæ. In addition to the penetration near the *infection layer*, described above, the hyphæ may, under favourable conditions, penetrate at any point (figs. 37–39). In material from Ghent (which did not appear quite normal or very vigorous, but of which the germination proved to be equally good with that of apparently normal grains), in almost every grain examined, hyphæ from the hyaline layer were found in great abundance, which had forced their way through the aleurone layer into the starch endosperm. These hyphæ are always intercellular and, as far as I have seen, penetrate where the walls are thickest, *i.e.*, at the junction of three or more cells. As many as three or four hyphæ often traverse the same wall (fig. 37). In the starch endosperm these hyphæ continue their intercellular growth (fig. 38) and may penetrate even to the centre of the grain. In the intercellular spaces at the corners of the large starch cells the hyphæ usually form small knotted masses by branching and by convolutions (fig. 38*a*), and the hyphæ themselves may become considerably swollen. At apparently any point in the wall these hyphæ may send out numerous coral-like branches (fig. 39), but no extensive growths of this kind were found. Invaginations of the wall into cell-lumen of the starch cells are frequent, but I have seen no undoubted example of intracellular hyphæ. As to contents, size and septation these hyphæ were otherwise similar to those found in the layer exterior to the aleurone. Their significance will be discussed later. In material from Hamburg, marked *L. multiflorum* (but which was probably *L. linicolum*), one case of penetrations similar to above was also found, a third case of similar occurrence was discovered in an undoubted specimen of *L. temulentum* grown in Cambridge.

The Fungus in the Embryo.

The relation between the hyphæ in the grain and those in the embryo is best seen in a median longitudinal section of the latter with the aleurone layer of the grain still attached (fig. 16). In sections overstained with hæmatoxylin the hyphæ can be seen entering the scutellum between the epidermal cells from the *infection area* described above (figs. 4 and 22–24); I have found as many as six hyphal penetrations in a single embryo; sometimes two occur contiguous to a single cell (fig. 25). The entire subsequent course of the hyphæ, as far as I have seen, is always intercellular. From the point of entrance they converge more or less toward the angle in the bend of the vascular bundle of the scutellum as it turns downward in the stem (fig. 15 and *k*, fig. 16), traversing a tissue composed of elongated thin-walled cells. From this region the hyphæ grow past the vascular bundle of the scutellum, and also close to that of the first leaf, in the neighbourhood of which they can usually be very clearly seen. They then penetrate the growing point of the embryo, approaching often to within two cells of the tip (figs. 11–17).

In view of the well-developed condition of the hyphæ in the growing point of the embryo, it must be accepted that the hyphæ have gained entrance previous to the mature condition of the grain, and the study of the embryology confirms this view. Perhaps the best method of demonstrating the presence of the hyphæ in the growing point is by means of aniline-water-safranin; fortunate staining shows the hyphæ in longitudinal view and also the cut-off ends (figs. 11 and 12). HAIDENHAIN'S hæmatoxylin stains the protoplasm of both host-cells and hyphæ, leaving the walls unstained; by careful washing out, the nucleoli of both host-cells and fungus-hyphæ can be brought into view, darkly stained upon a light grey background of cytoplasm (fig. 10). By means of these nucleoli the hyphæ can often easily be recognised and their course traced. Hyphæ near the surface, especially in overstained sections, are sometimes stained an opaque dark blue (figs. 13–15). In the more or less conical region, the apex of which is the bend of the vascular bundle of the scutellum (fig. 16, *k*), and indeed wherever they are found in the scutellum, the hyphæ stain very readily with hæmatoxylin, but often irregularly as though the protoplasm had aggregated into dense masses, suggestive of initial stages of degeneration. A similar appearance is frequently found in the hyphæ of the nucellus during the later stages of germination of the grain.

The hyphæ in the growing point are found in a conical region corresponding with the growing cone. Toward the apex most of them lie longitudinally in the cone (figs. 11 and 14), but toward the base there is a denser network in which the hyphæ often appear on all sides of many of the cells (fig. 12); in this region the cut-off ends of the hyphæ are very abundant. Owing to the density of the protoplasm of the host-cells, and the difficulty in obtaining unobstructed views of the fungus in the growing point, the details in this region can be only imperfectly made out. In a

24-hour stage the hyphæ are 2–3μ in diameter, branch frequently, have conspicuous nucleoli, and septa are very rare, if present at all. I have seen no distinct septa in an embryo of this age. This may be merely an indication that the growing tips of the hyphæ are as yet unsegmented. The hyphæ never enter a cell lumen, but are always intercellular, and are so closely covered by the substance of the cell walls in which they are imbedded, that cross-sections appear like very small interpolated cells (fig. 12), and can be seen only in carefully-stained preparations.

A small percentage of embryos, corresponding in general to that of fungus-free grains, are found to be devoid of hyphæ. In those sections of the embryo to which the aleurone layer is still attached, the absence of the hyphæ from the embryo is seen to be correlated with their absence outside the aleurone layer. In all cases examined, where hyphæ were present in the embryo, they could also be found in the nucellus. As to the possibility of their presence in the embryo and absence from the grain, all evidence is at present negative. Again, where hyphæ are found in the grain they are also present in the embryo. I have met with only one doubtful case where this did not appear to be true; the staining in this case may have been faulty, but, as far as could be seen, it was similar to other successful attempts. It is impossible at present to determine definitely whether these embryos, which appear devoid of the fungus, are so owing to the occasional failure of the hyphæ in ordinarily infected plants to gain admission to all the grains, or whether they indicate a fungus-free race of the Darnel, hitherto confounded with that containing the fungus. In two instances, in packets of "seeds" I have obtained spikelets or parts of spikelets with more than one grain still attached, in which one grain was devoid of hyphæ, and in both cases all the other grains of the spikelet (in one case three, in the other five) were likewise free from the fungus. This, together with the variable proportion of grains with and without the fungus, indicate in all probability that all or none of the grains of a given plant are infected.* Experiments are now being made which it is hoped will clear up this and several other related points. In order to check the evidence of the microtome sections as to the presence of hyphæ in the growing point of the embryo, and in order to try to obtain information as to the function of the general nucellar layer of hyphæ in the grain, the following experiments were undertaken:—

Thirty-five grains each of *Lolium temulentum* and *L. perenne* were freed from their paleæ and immersed in 1 per cent. corrosive sublimate for 7 minutes.†. They were then quickly shaken in several changes of boiled distilled water, and placed in sterile germinating chambers, which were also moistened with boiled distilled water. After

* Guérin (*loc. cit.*, p. 235) states that of more than forty samples examined, only three were devoid of the fungus, and in two of these there was no exception. He adds: "Faisons observer de plus que, pour une localité donnée, lorsqu'un grain est parasité tous le sont." According to my observations, the last statement would be correct if restricted to single plants, but certainly will not hold for localities.

† Preliminary experiments indicated that this treatment was not noticeably injurious to the embryos, but retarded or altogether prevented disturbing growths of moulds, &c.

24 hours in this chamber, they were removed, and with sterilised instruments the embryos from the *L. temulentum* grains were grafted on to the *L. perenne* endosperms, and *vice versâ*. Each embryo of *L. temulentum* was examined under a hand-lens with a magnification of 10 diameters, and all traces of the pericarp, nucellus, and endosperm were removed.* The cleaned embryos were then placed in suitable cavities prepared in the endosperm of *L. perenne*, and the grains then replaced in sterilised chambers. Of the thirty-five grafts of *L. temulentum* on *L. perenne*, all but one germinated successfully, growing to a height of from 1–3 centims. in the germinating chamber, and remaining almost absolutely free from moulds. They were then removed to soil, where most of them continued their growth. In the grafts of *L. perenne* on *L. temulentum* no precaution was taken to remove the pericarp, since *L. perenne* does not usually contain a hyphal layer. Thirty-four grafts were made, and of these, eighteen germinated. All of the grafted grains were kept in the germinating chamber 7–8 days. The resulting seedlings were in both cases uniformly smaller and more slender than the ordinary seedlings, but otherwise normal. On the 16th day five of the seedlings from *L. temulentum* on *L. perenne* were killed in 1 per cent. chromic acid, and sections of the growing point were cut. Three of these contained the fungus, and the other two did not. The distinguishing macroscopic differences between grains which contain the fungus and those devoid of it were discovered subsequent to the beginning of this experiment. To ignorance of these differences, and to the erratic occurrence of grains without the fungus in groups, as described above, is probably due the large percentage given of seedlings devoid of the fungus. The remaining seedlings have been kept to determine the influence, if any, on the formation of fruit. The presence of the fungus in those seedlings which were examined confirms, however, the results of the microtome sections. It is just possible, perhaps, that infection from external sources might have occurred during the grafting, but, in view of the life-history described below, and in view of the precautions in the manipulation, it is exceedingly improbable.

The growing points of two of the seedlings of *L. perenne* on *L. temulentum* were also examined, and both contained hyphæ. I have since found that, although not commonly, yet occasionally, normal grains of *L. perenne* contain a hyphal layer similar to that of *L. temulentum*.†

The occurrence of the fungus in both seedlings above mentioned is either a remarkable coincidence, or else (as seems far more probable) hyphæ from the *infection layer* of the *L. temulentum* grains were able to gain entrance to the embryo of *L. perenne*.

* I now know that, in spite of all possible precautions, hyphæ from the infection layer would still cling to the scutellum. They can be seen in sections of almost every dissected embryo.

† Guérin (*loc. cit.*, p. 235) also reports one such occurrence.

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The Grain and Fungus during Germination.

In order to study the development of the fungus in the germinating grass seed, and subsequently in the plant, grains were germinated in an ordinary moist chamber, and each day up to, and at longer intervals after, the 17th day, material was fixed and cut in the usual way. The hyphal layer in the grain undergoes no noticeable change for some time, except perhaps a swelling due to the absorption of water. I have seen no evidence of growth of these hyphæ; on the contrary, all the indications point to the conclusion that they have been crowded out, as it were, and lie inert in the nucellar tissue. From a 24-hour grain, fragments of the hyphæ can easily be dissected out (figs. 34 and 35). Examined in distilled water, they are usually much contorted and bent, and from the ease with which they fragment appear to be quite brittle. They measure 3μ or more in diameter, and are slightly larger than those in the tissue of the growing plant. They branch frequently, the branches usually being at right angles, and about equal in diameter to the hyphæ from which they originate, and the septa are numerous and easily seen. The protoplasmic contents are usually homogeneous and finely granular, and in several days' time often become vacuolate; they greedily absorb hæmatoxylin, but the nuclei are either absent or hidden.

The hyphæ undergo no noticeable change until the fifth or sixth day, when the protoplasmic contents begin to contract and aggregate into coarse granules, which often assume a rounded form; the latter seem to mark early stages in the breaking down of the hyphal protoplasm (figs. 30, 31, and 33). By the eighth day much of the protoplasm, as well as the wall, has disappeared, and left only grooves, which mark the former position of the hyphæ in the nucellus (fig. 32). The rate of disintegration varies very considerably in different grains. Whether this disintegration is due to bacterial action or to that of enzymes, produced either in the hyphæ themselves or by the adjacent aleurone layer, cannot at present be determined. It may be difficult to see how bacteria could gain entrance through the pericarp, and unless some process analogous to enzymatic action in the mature aleurone occurs, it is difficult to say which seems most probable. The hyphæ completely disappear in the later stages of germination.

In the vast majority of grains examined I have never found a hypha penetrating either into the lumina of the aleurone cells or through this layer into the starch endosperm, except in the region of the *infection area* already described. With the exception described above, I have also never found, in ordinary grains, hyphæ in the starch endosperm. In a late stage of germination the glutinous products of the starch-cells often produced (in teased preparations of the endosperm) tenacious thread-like strings, remarkably similar to delicate wefts of fungus hyphæ, but their nature is easily detected on applying suitable tests. It is also very probable that the saprophytic fungi which frequent the outer surface of the grain, and which at about the

eighth day can be found in abundance in the tissues of the palea and pericarp, might eventually gain entrance to the starchy endosperm. NESTLER (*loc. cit.*, p. 213) has described hyphæ permeating the starchy endosperm when the culm is 1 decim. high, even in plants derived from grains treated in ether for 15 minutes or singed in the Bunsen flame. The ether method, according to my experience, although it retards, does not completely destroy the external saprophytes, and dry heat at 95–98° C. for 10 minutes is insufficient to destroy the bacteria. In view of these facts, and also of the failure of my culture experiments with separated hyphæ, as well as those of NESTLER, it seems very probable that the hyphæ in the starch endosperm described by NESTLER are not identical with the fungus in the nucellus; the latter has disappeared usually before the culm is 1 decim. high.

The following culture experiments are of interest in connection with the possibilities and development of the nucellar hyphæ of the grain.

In addition to the recorded attempts given below, numerous attempts have been made to get cultures of these hyphæ separated from the grain. The method pursued was the following:—Grains, with or without previous treatment, were placed in clean germinating chambers. At successive stages these were removed and, with a razor, properly sterilised, were cut in two parts in a median lateral longitudinal plane. The half containing the groove of the grain was rejected, and mounts made from the other half, after removing the starch and laying bare the aleurone and nucellus. With sterilised needles small amounts of the hyphal layer, often entirely free from starch, were obtained. Care was taken to prevent the needles from piercing the pericarp of the grain, to avoid contamination from species of *Cladosporium*, *Dematium*, and *Alternaria*, which are very commonly found on the pericarp. Bacteria are very difficult to exclude, but careful manipulation will give pure mounts of the hyphæ. The hyphæ were quickly placed in hanging drops in the Ward cell (9), and observed frequently. The media used were beerwort gelatin, 2 per cent., horse extract gelatin, 2 per cent., and distilled water. A large number of mounts were observed, in which bacteria and mould spores had developed to some extent, but had not completely overrun the drop in the course of a week. In these there was little or no possibility of the hyphæ having been killed in the manipulation previous to immersion in the medium, and it is probable that, if they were at all capable of growth in these media, they would at least have shown indications in a week.

Some mounts in beerwort gelatin were also placed in an incubator at 25° C. In all these attempts isolated fragments of hyphæ (figs. 34 and 35), and also clumps of hyphæ still attached to the aleurone cells, were used. To obtain pure mounts for a longer time-test, the following experiments were performed. Two per cent. beerwort gelatin was the medium used in all, and they were kept at ordinary room temperature.

1. Dry grains, from which the paleæ had been removed, were placed at a dry heat of 95° to 98° C. for 10 minutes, then put into sterilised germinating chambers,

which were moistened with boiled distilled water. The embryos were all killed, and no pure cultures were obtained; eventually bacteria always prevailed.

2. Grains cleared by carefully removing the paleæ with sterilised needles were placed in ether for 15 minutes (method used by NESTLER), and then shaken with boiled distilled water several times and put in sterilised chambers. All the grains germinated, and in one or two cases I was fortunate enough to dissect out one or two clean pieces. A pure mount of hyphæ from a 3-day grain showed no growth in 10 days, and was then discarded. A pure mount from a 6-day grain was kept 18 days, and no growth of the hyphæ took place.
3. Grains cleaned as before were immersed in 1 per cent. corrosive sublimate 10 to 15 minutes, then shaken well in several changes of boiled distilled water and placed in a sterilised chamber. Of these, almost 25 per cent. germinated, but the germination was retarded. A pure mount from a 3-day grain was kept 10 days; another, from a 6-day grain, was kept 18 days; and a third, from a 13-day grain, was kept 13 days. In no case did growth of the hyphæ take place.
4. From an untreated grain in an unsterilised chamber a fortunate pure mount was obtained, and kept 56 days, and no growth resulted.

In Experiment 3 there is considerable possibility that the hyphæ were injured by the corrosive sublimate, but they are better protected than the embryo, on account of the somewhat gelatinous condition of the nucellar remnants in which they are imbedded. In appearance the hyphæ from these cultures are indistinguishable from those of untreated grains. In Experiment 2 there is at least very great probability that the hyphæ have remained unharmed, for germination of the embryo proceeds as in untreated grains. In Experiment 4, and in the very numerous less fortunate attempts above mentioned, there is no reason for supposing the fungus to have been in any way injured. Growth was not observed in a single instance. NESTLER (*loc. cit.*, p. 212) has also failed to cultivate these hyphæ.

The conclusion to be drawn from these results is that the hyphal layer (not, however, including the *infection layer*) in the nucellus of the matured grain is ordinarily incapable of further germination, or else that the fungus is so closely adapted to a parasitic life that its mycelium cannot be cultivated in artificial media, as is well known to be the case with rusts and smuts. Of course, both of these conditions may at once be true. The fact that the general hyphal layer in the grain is of no further use in the infection of the embryo, the common failure of the hyphæ to penetrate into the endosperm through the aleurone layer, the comparatively rapid disintegration of the hyphæ under natural conditions (figs. 28–32), the irregular staining reactions (fig. 33), the contortion and compression of the layer (fig. 28), and, finally, the behaviour of the hyphæ in the above-described culture experiments, all

appear to indicate that the nucellar hyphæ of the mature grains are incapable of further development. At the same time, it is very probable from the behaviour of the fungus that a very close adaptation exists between the latter and its host. That the formation of the thick layer of hyphæ in the nucellus functions for the purpose of penetration of the endosperm, is probable from the success which sometimes attends the attempt. There is apparently a struggle going on between the fungus-hyphæ and the endosperm, particularly the aleurone cells, the walls of which are in this case very serviceably thick, and external conditions are probably of importance in deciding the result. In some cases the hyphæ succeed in gaining entrance, but in the vast majority of cases the endosperm excludes them from any further success in attacking the contents.

The Fungus in the Growing Plant.

When the embryo of the grain resumes growth on germination, the hyphæ (already in its tissues, as we have seen) keep pace with this growth, and can be detected in the growing point throughout the life of the plant. NESTLER'S inability to see the hyphæ earlier than the eighth day was probably due to an incomplete method. As the stem elongates, the hyphæ can be seen for some distance below the growing point, and are especially easily demonstrated in the central thin-walled parenchyma. The development is best illustrated in a 17-day seedling (fig. 17). In a median longitudinal section the hyphæ can be traced first in the growing point, just as in the embryo, then stretching downward in the stem to the first node above the scutellum, and sometimes they may even be followed into the epicotyl. I have found but few cases of the latter, however, and it is probable that the hyphæ at that distance from the growing point have undergone disorganisation. It may also be noted here that the hyphæ which in the embryo can be found between the growing point and the *infection-area*, also apparently undergo early disorganisation; I have never seen them later than the sixth day of germination. In addition to their occurrence in the above described area in the 17-day seedling, the hyphæ extend into the growing points of the lateral buds (figs. 17b^2 and b^3, fig. 18b^1), and very pronounced evaginations of this kind are found at the base of each leaf, where the hyphæ form a vigorous network of characteristic appearance, extending through most of the cross-section of the leaf base (fig. 17d).* They are, however, very scarce or wanting in the vicinity of the vascular bundle. The hyphæ of these networks are easily seen, and furnish excellent material for detailed study (figs. 19, 20, 21). There are no hyphæ in the base of the first leaf-sheath (fig. 17l^1), and hyphæ never enter the lateral root rudiments, although they are often found very close to them.

As to the explanation and the function of these patches of hyphæ in the leaf-bases,

* NESTLER saw these hyphæ in the leaf-rudiments, but does not mention them in the later development.

I have little direct evidence to offer. It seems possible, however, that they have to do with the infection of the lateral buds. The latter are usually infected directly by hyphæ, from the centre of the stem; but it is possible that in case of failure of the terminal hyphæ, during the elongation of the stem, to accomplish the infection, of the lateral buds, the hyphæ in the leaf-base (which seems to remain vigorous for some time) may eventually effect an entrance into the bud. In this case, it may be that the above named hyphæ afford a safety precaution in case of first failure. In one case I traced hyphæ from the base of the second leaf downward to the growing point in the axil of the first sheath. Whether this would be the normal process, or whether the growth would be to the bud in the axil of the same leaf, cannot be determined. If this is the function of these hyphæ in the leaf-base, distance would probably be an important determining factor of the course in growth.

It is also conceivable that the tendency to form patches of hyphæ in the leaf-base is associated with the habit of entering the carpels previous to the infection of the ovules. As described later, the hyphæ can be found in the bases of all morphological equivalents of leaves, *i.e.*, paleæ, glumes and stamens.

Again, it might be considered that the hyphæ are led—probably by chemotactic attraction—into the young leaf rudiments as into a growing point, but the absence of the fungus from all roots, and particularly from lateral roots, points to the fact that adaptation is very highly developed.

In general the hyphæ are not found in the vascular bundles, and seldom even in close proximity to them, but are abundant in the thin-walled parenchyma. They are more easily seen here than in the embryo on account of the less dense protoplasmic contents and the frequent shrinking of the walls of the host-cells. They are usually, however, just as closely compressed by the surrounding walls as they are in the embryo.

The hyphæ, as seen in the leaf-bases (figs, 19–21), have a finely granular protoplasm in which a high power shows numerous large vascuoles, which are, however, absent from some regions (fig. 20). The nuclei are ellipsoidal or spheroidal, with a well-marked spherical nucleolus, which stains very readily with hæmatoxylin, and also, but less easily, with aniline-water-safranin. The nuclei are often found at the corners of the host-cells, where the hyphæ are frequently swollen. Septa are very seldom seen and probably rare (fig. 21). As in the embryo, so in these later developments, the course of the hyphæ remains intercellular, and I have seen no haustorial branches of any kind.

Inflorescence and Ovary.

Some weeks before the spicate inflorescence emerges from the enveloping leaf-sheaths its initial inception exists as a short cylindrical mass of merismatic tissue, embossed with the rudiments of the floral parts, glumes and paleæ (fig. 40). Sections show that almost the entire mass is permeated by the fungus-hyphæ, so that it seems very

improbable that a single ovule need escape infection. In the later development each ovule is seen to be infected by way of the funicle, and the hyphæ, as before stated, extend into the bases of all glumes, paleæ and filaments in a manner similar to that met with in the case of the vegetative leaves. The hyphæ in the internodes are very conspicuous.

In the hope of finding spore-formation in the pollen sac, a number of stamens were carefully examined, but the hyphæ were never found to extend far into the filaments, and never invade the anthers.

The rudiments of the ovules, however, contain hyphæ from the earliest stages. In a very young stage, the spheroidal ovule is attached to the placental margin of the carpel by its flattened funicular side, and in this condition (fig. 41*v*) I find the hyphæ uniformly distributed throughout the ovule except in the external layers of cells. With the formation of the embryo-sac accompanying the change of the ovule to an ovoid form, the distribution of the hyphæ becomes remarkably characteristic. This is very well seen when the embryo-sac is in the 8-cell stage,* as indicated by the dotted line in fig 43. The hyphæ are then found throughout the upper end of the ovule, but they are completely wanting on the outer side in the embryo-sac end—stopping at about the antipodal end of the sac; on the inner or axial side, however, a tongue of hyphæ extends from the funicular region almost to the micropyle (see dotted area figs. 41–45). A patch of hyphæ can always be detected outside the embryo-sac opposite the egg nucleus (fig. 48*h*). In the upper part of the ovule a differentiation of the nucellar tissue is already evident, into an outer small-celled layer of compact cells, 4–6-cells deep (fig. 47), and a central area of large thin-walled cells with thin watery protoplasm (fig. 47 *c.ent*). The hyphæ have already formed a dense network throughout the outer small-celled region, and extend usually to within two cells of the exterior. They may extend along the radial walls of the sub-epidermal layer of cells to the epidermis, but are seldom found along the inner periclinal wall of the epidermis, and never extend into the radial walls of the latter. In the central thin-walled tissue the hyphæ are very conspicuous, though not so abundant as outside this. They are in every way similar to those in the leaf-bases, except that septation is apt to be more evident and abundant (fig. 50).

The next noticeable change occurs during the elongation of the ovule, in the period preceding and accompanying pollination and fertilisation (figs. 44 and 45). As the ovule elongates, the growth of the hyphæ in different regions varies. Along the broad funicular side, practically no growth of the fungus takes place, so that very soon the hyphæ in this region are reduced to very few. The tongue which extends toward the micropyle, however, sustains a vigorous growth, and the mycelium here, having become detached from the rest of the hyphæ by the elongation of the ovule and cessation of the growth of the funicular hyphæ, now forms an isolated patch lining the inner lower side of the rapidly growing embryo-sac. This constitutes the

* By this phrase I mean when the embryo-sac contains the complete egg-apparatus, antipodal cells, &c.

young *infection layer* of the grain. The embryo-sac as it increases finds little resistance in the thin-walled cells of the central region, and these break down readily. The hyphæ between these cells are not injured, but are shoved along and gradually accumulate into a special layer as the embryo-sac elongates and swells (figs. 45 and 49). The peripheral denser network of hyphæ has, by further growth, become still more dense.

The further development is best seen when the embryo has attained a length of about 3/10 of a millim. In such an embryo the lateral growing point is fairly well differentiated from the terminal scutellum, but the rudiments of the leaves have not yet appeared; walls have now been formed between the nuclei of the embryo-sac, and the endosperm has invaded the nucellus in all directions to within a short distance of the dense peripheral layer, in which the growth of the hyphæ has also continued. The *infection layer* remains localised in its growth, and does not increase greatly in length. Only a few isolated hyphæ can now be found in the funicular region. The embryo has absorbed all but a few remnants of the cells in the micropylar end of the endosperm, and on both inner and outer surfaces has come into direct contact with the nucellus (fig. 46). On the inner surface the latter touches the *infection layer*, and numerous hyphæ can now be seen *entering the embryo directly and penetrating to various depths in its substance* toward its growing point; some can be traced continuously from the *infection layer* to within two cells of the tip of the cone (*cf.* figs. 51, 11 and 14). I have found no infection take place previous to the formation of the growing point; it is probably, therefore, the special chemotactic influence of the vegetative cone which brings about the entrance of the hyphæ, as soon as the absorption of endosperm has brought the embryo into contact with the nucellus.

The transition from this condition to that of the mature grain is fairly simple. The embryo continues its growth and further invades the endosperm, while the infecting hyphæ continue to penetrate it and to fill up the growing point. The endosperm differentiates into an external aleurone layer and a starch-bearing central region, and by swelling out further crowds the nucellus, with its contained hyphæ, into a mere crushed lamella in the above-described position in the grain. The outer part of the hyaline layer of the mature grain is devoid of hyphæ (fig. 28), and is derived from the external (usually two) layers of cells of the nucellus, as described above. Along the funicle, where the inside groove forms, the hyphæ have entirely disappeared, and on the opposite (outer) side they have not grown towards the micropyle, but have simply kept pace with the growth of the ovule, so that they do not extend past the scutellum. Finally, the *infection layer* still persists, as already described, at the base of the scutellum (fig. 5).

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Other Species and Varieties of Lolium.

GUÉRIN had already pointed out that a hyphal layer is to be found in *Lolium linicolum* and also in *L. arvense*, and he found one specimen of *L. perenne* with the fungus. My search among the various species confirms his results and adds a larger percentage of infected specimens of *L. perenne*, and examples of *L. italicum* as well; besides some other doubtful species. Samples of grains have been collected from numerous places throughout Europe, and of these many are obviously wrongly determined, whence it will be necessary to grow the plants for an exact diagnosis.

The following tables give results regarding grains of which the determination appears to be correct. Of *Lolium temulentum* a very large additional number of grains have, moreover, been examined of which no record is given :—

Lolium temulentum, L.

No. of plants examined.	No. devoid of fungus.	No. with fungus.	Source of sample.
10	1	9	Bonn.
11	3	8	Ghent.
10	8	2	Upsala.
5	1	4	Marseilles.
10	4	6	Vallombrosa.
8	1	7	Lemburg.
8	3	5	Munich.
8	0	8	Lyons.
76	12	64	Cambridge.
35	2	33	„
181	35	146	

L. temulentum, L. var. *arvense*, With.

No. of plants examined.	No. devoid of fungus.	No. with fungus.	Source of sample
5	1	4	St. Petersburg.

Lolium perenne, L.

No. of plants examined.	No. devoid of fungus.	No. with fungus.	Source of sample.
10	9	1	Cambridge.
1	1	0	,,
19	15	4	,,
30	25	5	

Lolium italicum, Braun.

10	10	0	Cambridge.
10	9	1	,,
9	9	0	Ghent.
10	9*	1	Italy.
20	20	0	Cambridge.
59	57	2	

Lolium linicolum, Br.

1	0	1	Paris.
8	0	8	Greifswald.
8	0	8	Lyons.
8	0	8	Cambridge.
25	0	25	

In the following list of grains of *L. multiflorum* the samples are probably, but not certainly, correctly determined :—

L. multiflorum, Lam.

No. of grains examined.	No. devoid of fungus.	No. with fungus.	Source.
3	3*	0	Hamburg.
2	2	0	Paris.
2	2	0	Kew.
1	1	0	Utrecht.
8	8	0	

* One of these contained the young sphacelial stage of an ergot.

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The following is a list of doubtful forms: the names under which they were received are given each case :—

Species.	No. of grains examined.	No. devoid of fungus.	No. with fungus.	Source.	Remarks.
L. strictum .	8	8	0	Lyons. . . .	Very similar to *L. temulentum.*
„ .	1	0	1	Paris	Similar to *L. linicolum.*
L. rigidum .	8	8	0	Hamburg . .	Similar to *L. perenne* and *L. italicum.*
L. scabrum .	10	10	0	Malta	Similar to *L. linicolum.*
L. multiflorum	10	2	8	Lyons. . . .	„ „
„	10	1	9	St. Petersburg .	„ „

As a very large number and very varied selection of samples is necessary for generalisations, it will be readily seen that the above tables are insufficient, but they serve to point out the fact that the fungus of *L. temulentum* (or a closely related form) has a wider distribution than has heretofore been known.

General Conclusions.

The life cycle of the fungus of *Lolium temulentum* is probably unique. From the absence of spore-formation, and from the facts above described, we must conclude that this fungus can live for a considerable time without forming spores, passing from plant to embryo through succeeding generations. It is, of course, not inconceivable that such hyphæ should go on indefinitely without an intervention of spore-formation, though the phenomenon is so remarkable that it is impossible to resist seeking for the spores. Many mycorhizal fungi, as far as is known, form no spores, and several domesticated flowering plants might also be cited as analogous examples of sterility. The spore-forms of the *Lolium* fungus may, however, still exist in a form which has hitherto escaped detection.

Then, again, we find developed in *Lolium* a unique and very successful method of infection, which the fungus has apparently substituted for the usual method of spore infection. It is conceivable that such a method might arise during unsuccessful attempts of the fungus to form spores or a sclerotium in the ovary, and the subsequent infection of the embryo might easily arise on account of the chemotactic influence of the growing point on the baffled and subjugated, but still vigorous, hyphæ. After the successful establishment of this method of infection, it is conceivable that a symbiotic relationship advantageous to both fungus and host might arise, which would probably result in the cessation of attempts by the fungus to form spores or sclerotium; the continued sterility would then be explained by the success of the new infection method. That it is very successful there can be no doubt, since probably over 95 per cent. of *Lolium temulentum* grains contain the fungus.

The response of the hyphæ to the attractive influence of the growing point is indeed remarkable. They often pass very near the rudiments of lateral roots, but never enter them, and they never penetrate any considerable distance into a leaf. The distribution of hyphæ in the young ovary, as shown above, is another striking illustration of this close adaptation.

As to the relation between host and fungus in the grain generally, we have already seen that GUÉRIN considers it as a true symbiosis. As regards the usual life cycle, this is probably true, but the large hyphal layer of the grain, and the occasional penetrations of the endosperm, suggest vestigial indications that the action of the fungus is, or has been, at times injurious to the endosperm of the plant. Otherwise the fungus seems ordinarily to exert an almost stimulating influence on the host. Those grains which contain the fungus are, as described above, apparently better developed and larger. The germination of the infected grains is, moreover, excellent, as all who have worked with Darnel agree. No sufficient number of grains free from the fungus have been experimented with to allow of generalisations on this point, but, so far as I have seen, the power of germination of uninfected grains is at least as great. It may also be pointed out that the grains of *L. temulentum* are larger than those of other species of *Lolium*, and according to my experiments have a higher germination efficiency than those of *L. perenne* and *L. italicum*, which are usually devoid of the fungus. With the possible exception, therefore, of the (as yet unknown) cases in which spore or sclerotial formation may occur, the fungus is not injurious to the host, and it is even possibly of advantage.

It was natural to connect the phenomena here concerned with the remarkable hypothesis of so-called mycoplasm.

The recent suggestion of ERIKSSON (14), that the fungus of *Lolium temulentum* may exist in the grain-embryo in a mycoplasmic form, is based upon the incomplete researches of NESTLER. As I have already pointed out (13), such a supposition is entirely unnecessary, since the hyphæ can be very clearly seen in the grain-embryo, and throughout the life cycle of the grass. Infection occurs by direct ingrowth of true hyphæ, and no question of intracellular admixture of fungus and host-protoplasm can be entertained. Moreover, the fungus here concerned has few or none of the characters of a Uredine.

If no spore or sclerotial development ever takes place, it is difficult to see how plants developed from fungus-free grains (and such occur) of *L. temulentum* can become infected. In this case one might consider the present *L. temulentum* as consisting of two races, one with a symbiont, and the other fungus-free. The macroscopic differences between the grains, as above described, accentuate this view. The susceptibility to infection is probably not lost, however, unless the results of my graft experiments are due to mere coincidence; the long-continued difference might conceivably lead to specific distinction. It must remain for future research to test this point; as already said, we may yet meet with some kind of spore-formation under special conditions, or

possibly some sort of sclerotium may be found to occur when circumstances favour the fungus. Meanwhile, however, it seems preferable to keep speculation within the bounds set by the observed facts.

Nature of the Fungus.

The nature of the fungus has been the subject of some speculation, but little direct and positive evidence has been brought forward in proof of the various views.

The stated similarity of the physiological action of Darnel to that of "Taumelgetreide" of Woronin has led some observers to refer both to similar causes. Nestler suggests that one of the numerous hyphomycetous saprophytes which, according to Woronin, cause "Taumelroggen," may be identical with the fungus of Darnel. There is really no foundation for such a supposition other than the similarity of physiological action. The life-histories, and the effects of the fungi on the grains, are so very diverse that there can be little doubt that they are different forms. The fungi of "Taumelgetreide" are saprophytic Hyphomycetes and Pyrenomycetes, which gain an entrance by the early death of the immature grain during very damp weather, and which shrivel the more or less dark-coated grains. There is not the slightest similarity here with the effect of the fungus on Darnel.

Poisonous rye was also examined by Prillieux and Delacroix (10), and attributed by them to a new fungus, which they described as *Endoconidium temulentum*. Guérin, who has examined material of *Endoconidium* sent by Prillieux and Delacroix, states that it is not at all identical with the fungus of Darnel.

Hanausek affirms that there is evidence that the fungus is a Ustilagine. He does not, however, produce any evidence, except the formation of knots (Knäuel) of hyphæ in the ovary, and his figure of "Knäuel" formation is anything but convincing in this respect. He also cites the presence of various Ustilagineæ in *L. temulentum* as evidence. I have found no such knotting of hyphæ to indicate the commencement of Ustilagine spore-formation. There are, however, several points not observed by Hanausek which might possibly give colour to his conjecture. The infrequency of septa, and the detailed appearance of the hyphæ in the tissues of the young plant, are somewhat suggestive of an Ustilagine, but the abundant septation of the hyphæ in the ovary, and the invariable intercellular course of the hyphæ, are certainly different from the usual conditions in Ustilagineæ (15).

The real similarities to Ustilagineæ are in the mode of invasion of the young plant, the continued on-growth of the hyphæ at the growing point, and their dying off behind, and especially in the mode of invasion of the inflorescence and entrance into the ovaries.

The probabilities of relationship with the ergot of *L. temulentum* are very interesting. The frequency of occurrence of ergots of *Lolium* in England* is

* According to R. H. Biffen.

strangely coincident with that of the fungus in the grain, *e.g.*, most abundant in *L. temulentum*, less so in *L. perenne*, and exceedingly rare in *L. italicum*. Moreover, the abundant septation of the hyphæ in the grain is also suggestive. Again, the occasional penetration of the endosperm lends a little colour to the supposition that the present conditions have arisen through a parasitic sclerotium-forming stage. On the other hand, it must be pointed out that the physiological action of ergot and Darnel are dissimilar, and that the life-history of the Darnel fungus is different in detail from that of any known ergot; and, finally, in the examination of ergoted material of *Lolium*,* what were apparently young sphacelial stages showed no hyphal layer outside of the aleurone, and the hyphæ, which permeated all tissues of the pericarp, nucellus, and endosperm, differed in character considerably from those of normal Darnel.

When pollination of young Darnel flowers is prohibited, the ovary remains small, and the entire nucellus, with the exception of the cushion of tissue bearing the *infection layer*, becomes transformed into a small dense mass of hyphæ, somewhat suggestive of sclerotial formations. Enough evidence is not yet to hand for generalisation from these experiments. Numerous other experiments have also been started to induce spore or sclerotial formation, but up to the present without success.

The abundance and importance of apparently sterile fungus-hyphæ in the life-histories of many orchids, and the rapidly increasing number of discoveries of mycorhizal forms, draw special attention to the strong probability of symbiosis in Darnel. We have at present no conclusive evidence as to whether a plant from a fungus-free grain ever produces infected grains, or, on the other hand, whether a plant from an infected grain ever produces fungus-free grains. Until this and other related points can be cleared up, the possibilities of spore-formation and infection can only be conjectured.

I wish, in conclusion, to express my great obligation and sincere thanks to Professor Marshall Ward, at whose suggestion this work was undertaken, and whose kindness in placing at my disposal all possible laboratory facilities and material, together with his very many helpful suggestions, have been invaluable in this investigation.

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Explanation of Plates.

All figures, except 1, 2, 34 and 35, have been drawn with the aid of the camera lucida.

PLATE 1.

Fig. 1 (× 6). A normal grain of *Lolium temulentum*, outer surface, *i.e.*, the convex or embryo side of the grain.

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Figs. 6–9 (× 22). Cross-sections at levels indicated in fig. 5; letters as in fig. 5

and v = vascular bundle of inner pale; s = scutellum; d = coleorhiza, r = root; l = leaf-sheath; c = area of hyphæ at base of the growing point; e = starch endosperm.

Fig. 10 (× 425). Detail of the growing point of the embryo, showing nucleated cells with delicate walls, stained with HAIDENHAIN'S hæmatoxylin. n = nucleoli of intercellular fungus hyphæ; h = heavily stained hyphæ at surface; a = outline of growing point.

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PLATE 3.

Figs. 37–39 (× 755). Cross-section of grains with hyphæ penetrating the cell walls of the aleurone layer and invading the starch endosperm (swollen with potassium hydrate and stained with iodine), fig. 37 = through the aleurone; fig. 38 = in the starch region, and 39 shows the peculiar

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Figs 42 = tangential, and 43 = median longitudinal sections from the same ovary. 43 contains an embryo-sac with eight cells; in 45 the embryo is still an undifferentiated ovoid body, and the walls of the endosperm cells have not all been formed; in 46 only the densely compacted hyphæ can be seen; the embryo is here about 300μ in length and has rudiments of the scutellum and growing point.

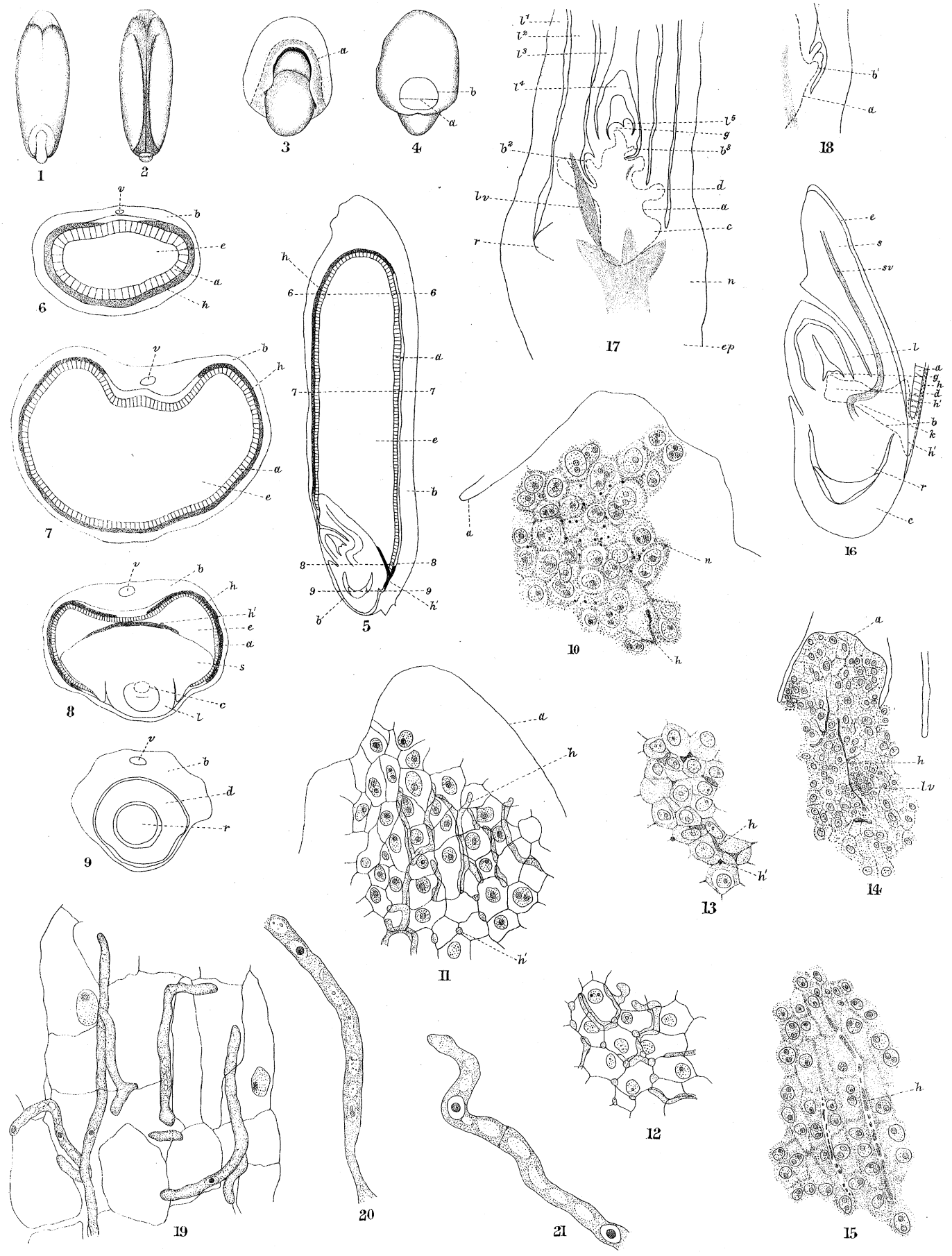
Fig. 47 (× 755). Detail of cross-section through the ovary of fig. 43; *peri* = inner wall of the carpel; *i. int.* = inner integument; *o. int.* = outer integument; *ep* = epidermis of the nucellus; *h* = hyphæ in denser external region; *h′* = hypha in cross-section; *cent.* = central large-celled region.

Fig. 48 (× 755). Longitudinal section of the egg cell end of the embryo sac with the nucellus on the inner (= axis) side of the ovule, from stage of fig. 43; *h* = hypha (future infection layer); *e. s.* = wall of embryo sac; *ov* = egg cell nucleus.

Fig. 49 (× 755). Longitudinal section of an ovule, the upper end of fig. 45. Shows central region with the embryo sac crowding the hyphæ into an accumulating layer; *a* = wall of the embryo sac.

Fig. 50 (× 755). Hyphæ from section of fig. 45. Nuclei and septation abundant. Occasionally a hypha is much swollen.

Fig. 51 (× 272). Detail of the embryo region of fig. 46. Shows hypha which has just reached the growing point, and its origin in the infection area; *g. pt.* = growing point; *sc.* = scutellum; *endo* = starch endosperm; *al* = the aleurone, which is commencing to differentiate; *i. int.* = inner integument; *nuc* = nucellus; *h* = hypha of *infection layer*.



M.P. Parker lith.

Parker & West imp.

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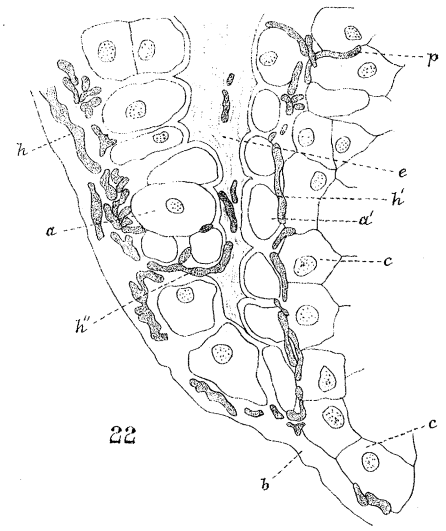

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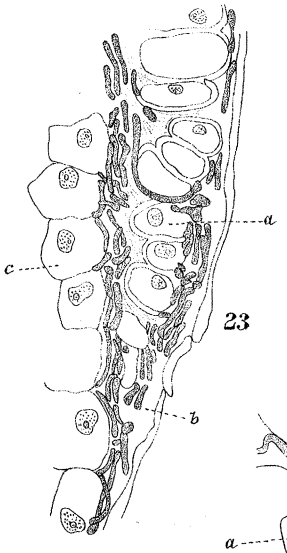

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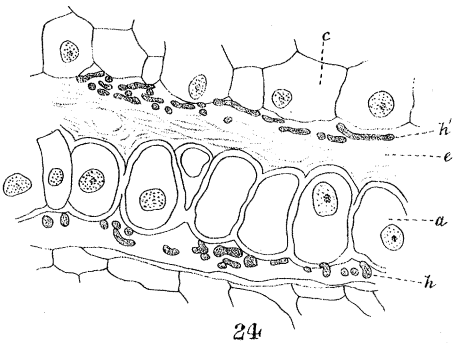

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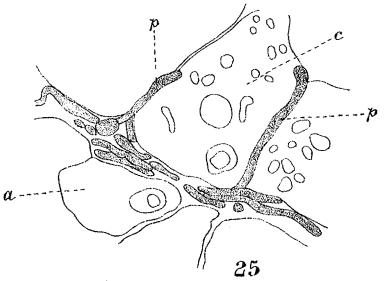

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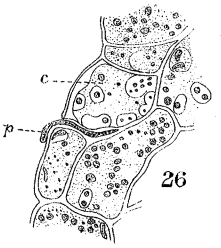

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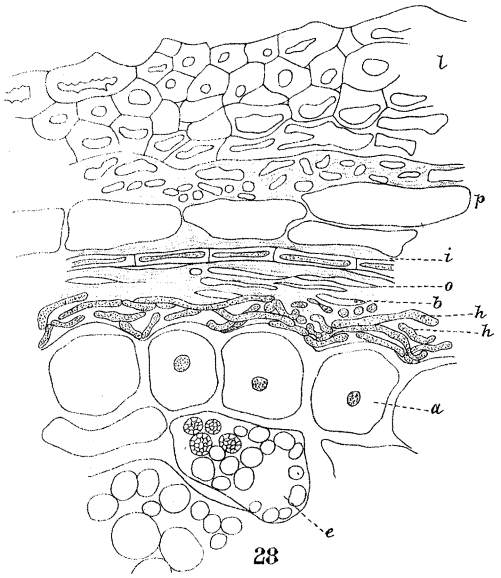

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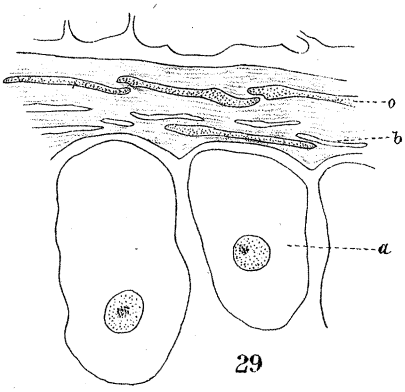

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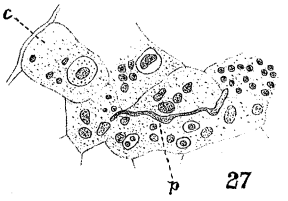

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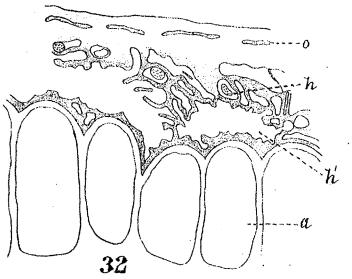

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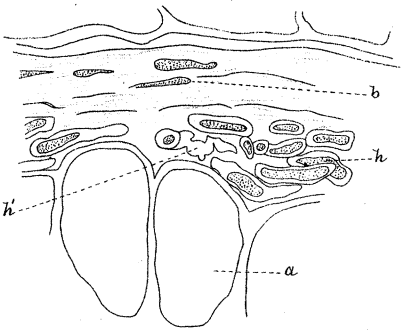

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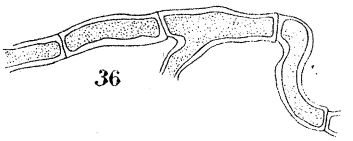

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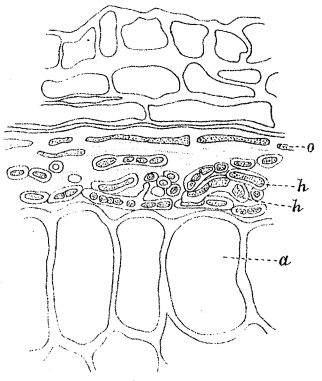

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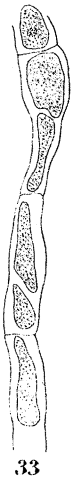

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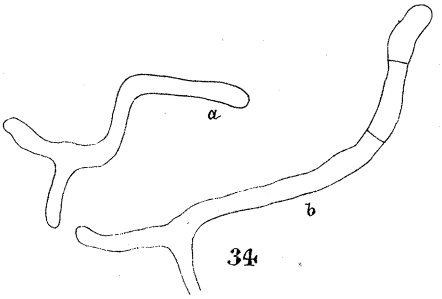

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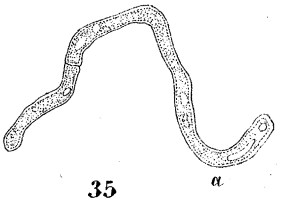

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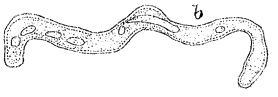

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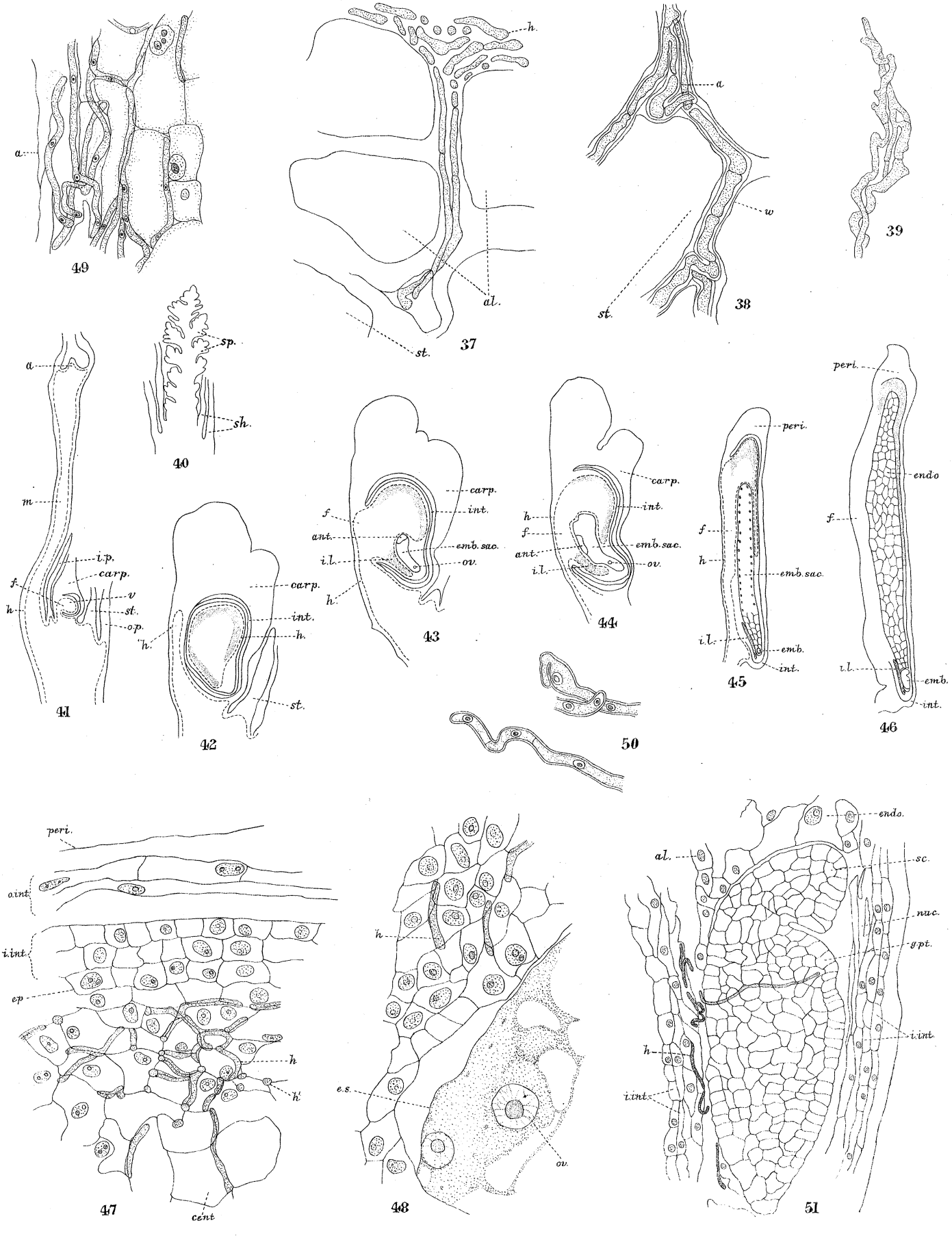

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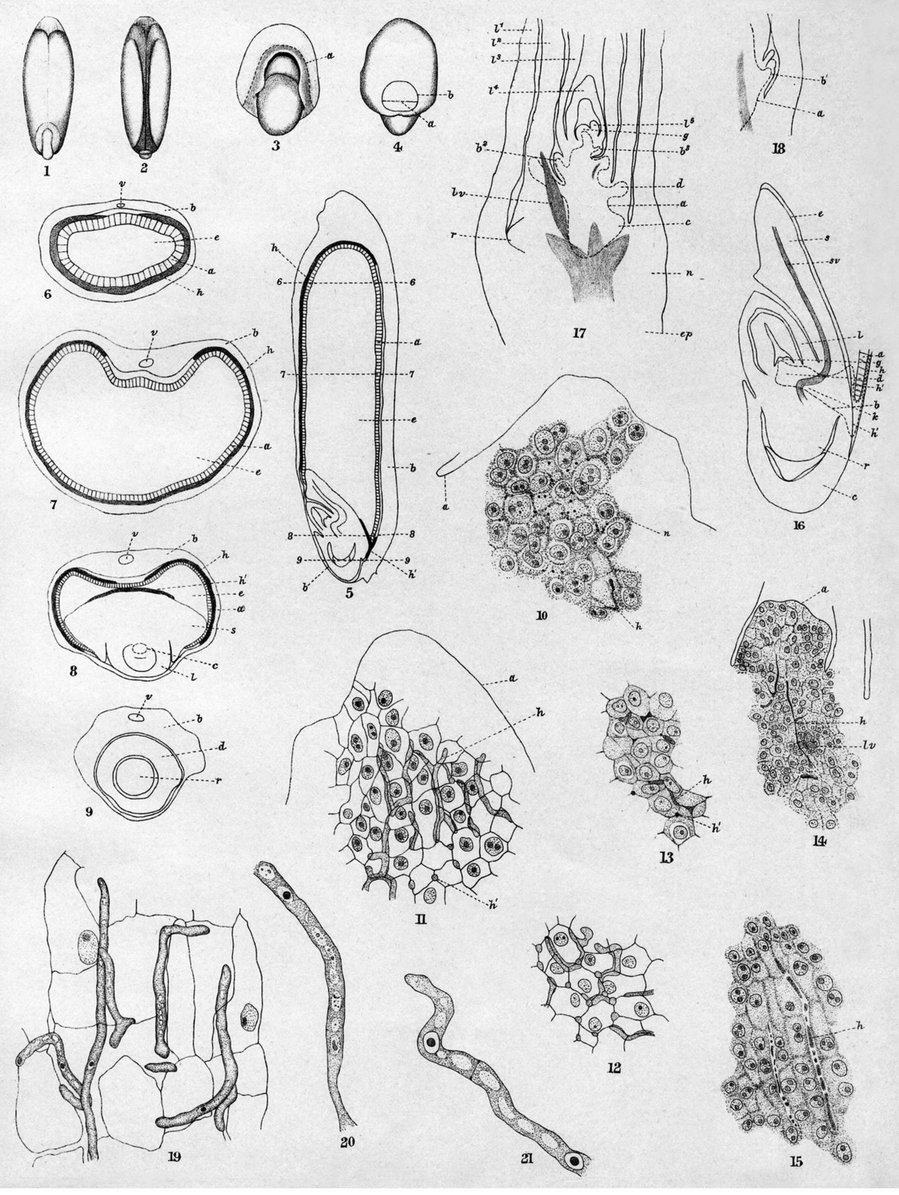

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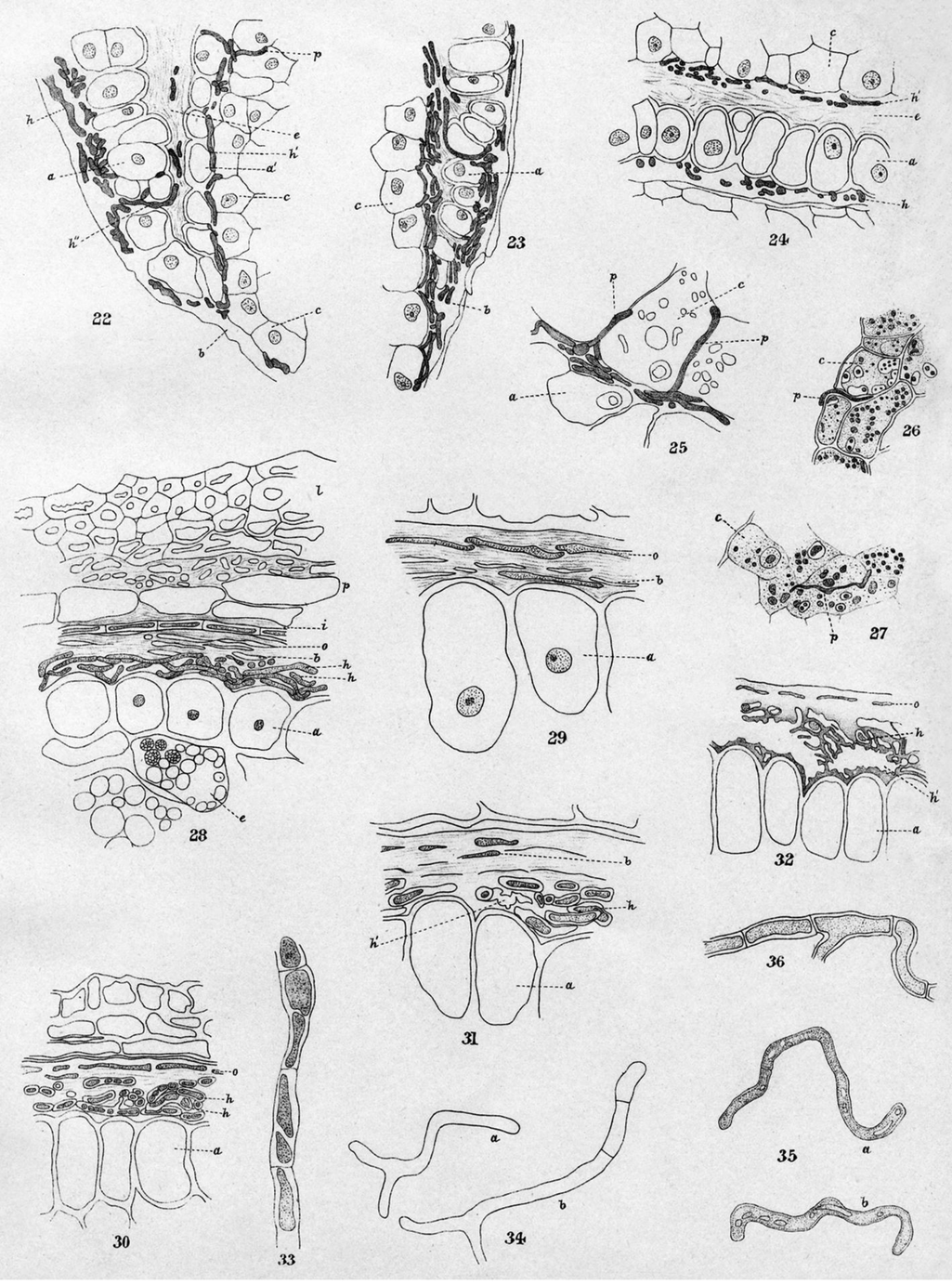

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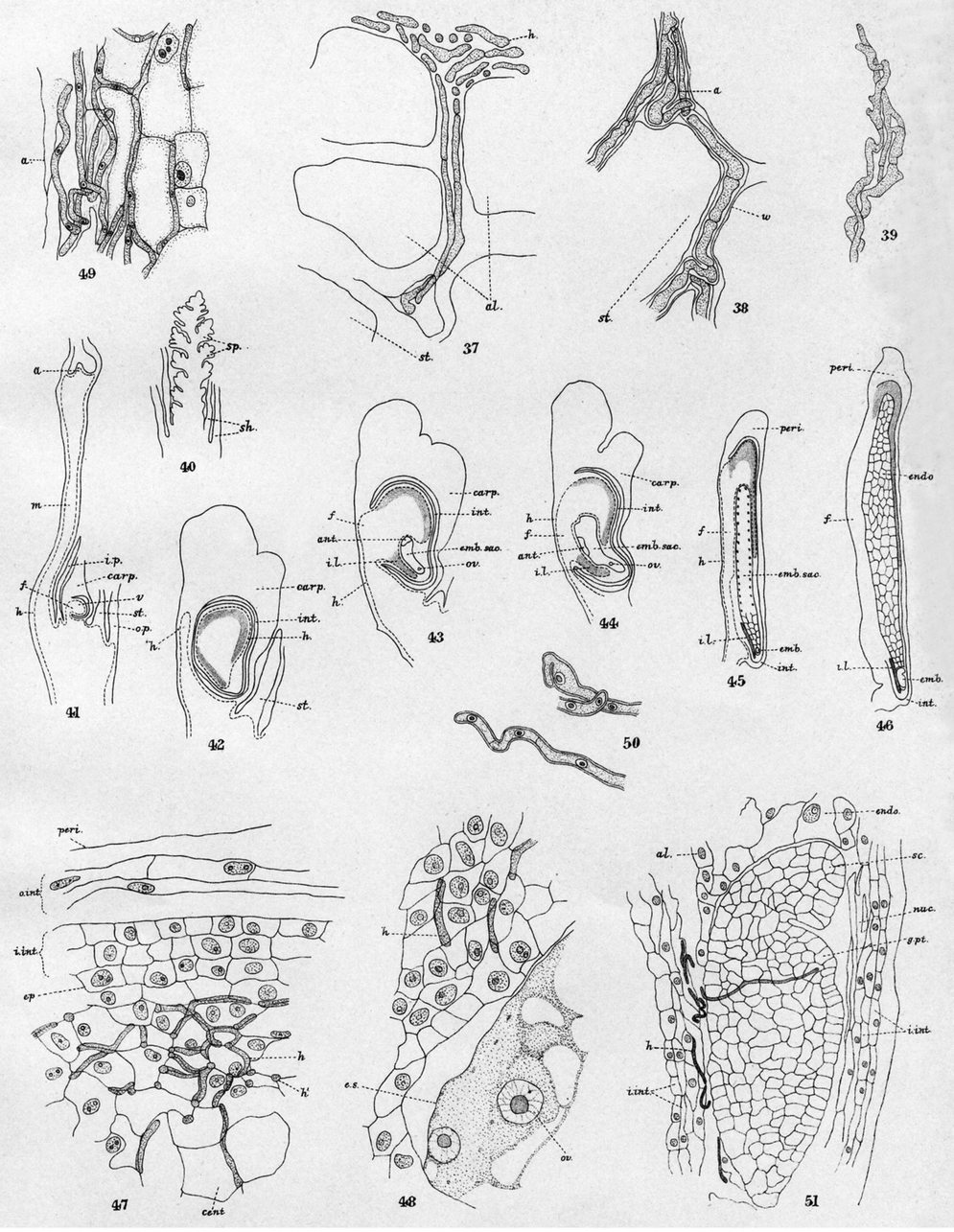

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Figs. 42–46 (42–44 = ×45; 45 and 46 = ×12). Illustrate the development of the ovule and the grain. Letters as in 41. The areas of denser aggregations of hyphæ are shaded; *int* = integuments; *ant* = group of antipodal cells; *i*, *l* = infection layer; *ov* = egg cell; *emb* = embryo; *emb. sac* = embryo sac; *endo* = endosperm; *f* = funicle.

Figs 42 = tangential, and 43 = median longitudinal sections from the same ovary. 43 contains an embryo-sac with eight cells; in 45 the embryo is still an undifferentiated ovoid body, and the walls of the endosperm cells have not all been formed; in 46 only the densely compacted hyphæ can be seen; the embryo is here about 300μ in length and has rudiments of the scutellum and growing point.

Fig. 47 (× 755). Detail of cross-section through the ovary of fig. 43; *peri* = inner wall of the carpel; *i. int.* = inner integument; *o. int.* = outer integument; *ep* = epidermis of the nucellus; *h* = hyphæ in denser external region; *h'* = hypha in cross-section; *cent.* = central large-celled region.

Fig. 48 (× 755). Longitudinal section of the egg cell end of the embryo sac with the nucellus on the inner (= axis) side of the ovule, from stage of fig. 43; *h* = hypha (future infection layer); *e. s.* = wall of embryo sac; *ov* = egg cell nucleus.

Fig. 49 (× 755). Longitudinal section of an ovule, the upper end of fig. 45. Shows central region with the embryo sac crowding the hyphæ into an accumulating layer; *a* = wall of the embryo sac.

Fig. 50 (× 755). Hyphæ from section of fig. 45. Nuclei and septation abundant. Occasionally a hypha is much swollen.

Fig. 51 (× 272). Detail of the embryo region of fig. 46. Shows hypha which has just reached the growing point, and its origin in the infection area; *g. pt.* = growing point; *sc.* = scutellum; *endo* = starch endosperm; *al* = the aleurone, which is commencing to differentiate; *i. int.* = inner integument; *nuc* = nucellus; *h* = hypha of *infection layer*.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES