

of the wood cells by the fungal hyphae, thus causing wilting and death of all parts above the point affected.

(5) No differences in power of infection or in behaviour in culture were observed between strains of the fungus isolated from different sources.

I should like to take this opportunity of thanking Mr F. T. Brooks for the time he has spent in directing the work and for many suggestions and criticisms, and Professor Seward for laboratory accommodation.

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ORCHID MYCORRHIZA*.

With Plates II—VII.

By J. Ramsbottom.

INTRODUCTION.

One of the most interesting phenomena in biology is that generally known as *symbiosis*—the living together of two organisms in close association. It is usually considered that this intimate relationship is of benefit to both components. Many examples occur in the plant kingdom. The lichen is probably the best known of these, being a composite plant formed of a fungus and an alga in definite union. Other well-known examples are the bacteria (*Pseudomonas radicola*) living in the root nodules of Leguminosae, and the Ginger-beer plant†, of which the lumps are composed of a yeast (*Saccharomyces pyriformis*) and a bacterium (*Bacterium vermiforme*). An intimate union can also occur between plant and animal, as in the case of the marine worm *Convoluta*, in the body of which an alga is always present,

* Reprinted from Messrs Charlesworth and Co.'s Catalogue, 1922. The Editors desire to thank Messrs Charlesworth for permission to reprint this article and for the loan of the blocks.

† The Ginger-beer plant is, at the present time, being widely distributed over the country as "Californian Bees," "Macedonian (Salonika) Bees," "Mesopotamian Bees," "Palestine (Jerusalem) Bees," "Wine Bees," "Water Bees," "Balm of Gilead," etc.

and as in the larvae of certain aphids, coccids, etc., where yeasts occur as more or less definite structures.

MYCORRHIZA.

That the roots of many plants have the threads or mycelia of fungi associated with them has become very well known during the last eighty years. It is of interest to find that cells containing fungi were first figured in an orchid (though not very clearly) by Link* in 1840, who observed them in the young seedling (protocorm) of *Goodyera procera*. He did not hazard a guess as to their nature—his idea being that the cells were filled with colourless granular material which finally disappeared.

At the beginning of the forties of last century the naturalists of this country who were curious in botany were very interested as to whether *Monotropa Hypopitys* was parasitic on the roots of beech in a manner similar to *Lathraea*. In 1842 we have T. G. Rylands† writing “On the nature of the byssoid substance found investing the roots of *Monotropa Hypopitys*.” Rylands concludes that “the ‘byssoid substance’ is really fungoid, and performs no essential function in the economy of the *Monotropa*.” It is, however, to Reissek‡ (1847) that we owe our first real knowledge. He examined numerous plants and came to the conclusion that fungi were normally present within the cortical cells of the roots of various flowering plants, being best developed in the underground roots of orchids. In these he studied most of the native and several exotic genera. He found that in *Orchis Morio*, for example, the fungus was present in almost all the cortical cells, whereas in the tropical species the fungal masses were arranged singly at the periphery. The presence of fungi was most frequent in underground roots, less usual in superficial ones and very rare in aerial roots exposed to the light. Moreover Reissek attempted to extract the fungus from the roots. In those days of imperfect technique it is not so surprising that he failed as that he should have made the attempt. The fungus he obtained he named *Fusisporium endorhizum*: it is probably one of the common saprophytic species of *Fusarium* so abundant in soils.

Another type of association between fungus and root is also well known, particularly in forest trees. Here the fungus mycelium forms a sort of mantle round the root, in contrast to being within the cells of the cortex. Apparently Hartig first

* H. F. Link, *Icones selectae anatomico-botanicae*, II, p. 10, t. VII (1840).

† T. G. Rylands, On the nature of the byssoid substance found investing the roots of *Monotropa Hypopitys*. *Phytologist*, I, pp. 341–8 (1842).

‡ S. Reissek, Über Endophyten der Pflanzenzelle, eine gesetzmässige den Samenfäden oder beweglichen Spiralfasern analoge Erscheinung. *Naturwiss. Abhandl. von W. Haidinger*, I, pp. 31–46 (1847).

noted this type in 1840 in the extremities of the rootlets of *Pinus sylvestris* although he mistook the hyphae for branched intercellular canals surrounding the internal cells such as are known to exist in the corky layer of the root cortex in *Juniperus* and *Thuja*. Rootlets so infected are most frequently coralloid in appearance. Gasparini in 1856 noted that such rootlets in *Castanea* and *Corylus* were surrounded by fungal hyphae.

The term *mycorrhiza* was coined by Frank* in 1885 for the fungus-roots. Even at that date it was known that in some plants the fungus occurred in rhizomes as well as roots (e.g. *Neottia*), and since then many cases have been found for which the term is quite a misnomer (e.g. Liverworts). It is a convenient term, however, and it is better to accept it with an extended meaning rather than to restrict it to those cases for which it is etymologically sound. Frank gave special names to the two types mentioned above. He used the term *endotrophic mycorrhiza* for those forms in which the fungus occurred within the tissues of the host, and the term *ectotrophic mycorrhiza* where the fungus hyphae surrounded the rootlet as a sheath. These are convenient general terms, but it is well to remember that the two types are not absolutely distinct, as is seen, for example, in *Monotropa*, which had been well described by Kamienski in 1883. Mycorrhizas, mainly endotrophic, have been described, either as usual, or occasional, in various Liverworts, Mosses, Horsetails, Club Mosses, Adder's Tongues, Ferns, Conifers and Flowering Plants: and in Algae apart from Lichens we have cases of constant association of fungi and seaweeds, as, for example, in *Ascophyllum* and *Pelvetia*, which each have their attendant *Mycosphaerella*. The antiquity of such associations is seen in the fact that they occur in the fossil plants *Rhynia*, *Hornea* and *Asteroxylon* from the Lower (or Middle) Devonian—vascular cryptogams which from their simple structure and age are of the greatest theoretical importance. Weiss (1904) moreover recorded mycorrhiza in fossil roots from the Lower Coal Measures for which he proposed the name *Mycorrhizonium*, and Osborn (1909) found fungus mycelia in the inner cortex of *Amyelon radicans*, the root of *Cordaites*.

ORCHID ROOTS.

As we have seen, fungi have been recognised in the roots of orchids since 1847. A transverse section of an infected root taken just above the root-cap shows the fungus in the cortical cells (Fig. 1). The distribution is more or less constant in the

* A. B. Frank, Ueber die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze. Ber. d. deutsch. bot. Gesell. III, pp. 128-145 (1885). Lehrbuch der Botanik, Bd. 1 (1892), p. 264.

same orchid, but varies in different genera. It is only in the young root where root-hairs are present that the fungus is, as a rule, recognisable as such. The epidermal cells are not infected. The fungus usually enters the root through the root-hairs, but in some species it apparently is able to make use of any portion of the piliferous layer. The hyphae* pass through the external layers to a more or less definite zone, where they reach their maximum development, rapidly spreading and completely filling the cells. If an exodermis be present the hyphae pass through the thin-walled transfusion or passage cells. The first two or three cortical layers of the root are thus generally free from fungus except where the hyphae of infection pass through them: even in these there is no balling of the mycelium in the cells. In some genera (*Habenaria*) (Figs. 1 and 14), the fungal zone occupies roughly the third and fourth layer of cortical cells. In other genera (*Neottia* and *Epipogon*) the fungal zone of the root occupies three layers or so of cells separated from the endodermis by about half-a-dozen cell rows. In other cases practically the whole of the cortex is occupied (*Cymbidium* and *Odontoglossum*). The central stele is never infected, the mycelium not entering the endodermis. The fungus also never infects the cells of the growing point of the root. Infected roots do not always show the endophyte in all their length, neither is it invariably present in a continuous zone. Infection does not generally occur once for all, but the hyphae from the soil infect the roots in several places and if the fungal zone be of several cells thickness it is frequently seen as patches in transverse section. Nor, as a rule, are all the roots of an orchid infected. Aerial roots particularly are free from fungus, the only exceptions being where the roots are applied to the soil and are without chlorophyll. Such a case is shown in Fig. 15. Aerial roots can sometimes be found in such a position with the exposed portion green: in these circumstances if infection occur the fungus is restricted in distribution to the portion of the root without chlorophyll. In addition to cells containing chlorophyll those containing tannin, mucus, raphides and other crystals are never invaded by the fungus. Lateral roots are more frequently infected than main roots and in those genera with numerous roots (*Orchis*, *Ophrys*) according to Stahl only one out of three of the roots arising from the rhizome have fungus present in their cortex. Moreover, certain genera such as *Listera* and *Epipactis*, which have their chlorophyll particularly developed, seem to be irregularly infected, whereas plants poor in chlorophyll, e.g. *Limodorum* and *Corallorhiza*, are well fungussed. All

* Janse has shown that in *Lecanorchis javanica* the infecting hyphae are sometimes united into a mycelial ribbon.

orchids so far investigated possess mycorrhiza, with the single exception of the saprophytic *Wulfschlaegelia aphylla**. Large numbers both of native and exotic species have been studied—Wahrlich†, for example, examined over 500 of the latter cultivated at Moscow.

Since the earliest workers, e.g. Reissek, it has been known that in some cells at least the fungus becomes changed from its original thread-like structure into glary yellow amorphous masses. In fact, it was owing to this phenomenon that the fungal nature of the cell infections of these roots was not at first generally realised. Wahrlich paid special attention to the changes which took place, and most investigators of orchid roots since then have taken note of them. Magnus‡ working with *Neottia* in which the alterations are well marked gave a clear description of the metamorphosis. He distinguished two main types of infected cells and held that there were no transitional stages. In the one type which he calls "digesting cells" (Verdauungszellen) the fungus always degenerates; in the other type, the "host cells" (Pilzwirthszellen) the fungus remains alive in the cells which lodge it and is thus able to hibernate. Magnus states that *Neottia* shows a more or less definite arrangement of these two types of cells, the digesting cells forming an outer and an inner and the host cells the middle layer. Such a regular arrangement is not usual in orchids—even in *Neottia* it is doubtful—and host cells are absent in certain native genera, such as *Goodyera*, and in most tropical forms. Bernard and Burgeff have also studied the question of the fungus digestion—the former mainly in seedlings, the latter principally in the root of *Platanthera chlorantha*. Before a hypha enters a host cell the nucleus of the latter increases in size. This action at a distance is also seen in the fact that starch disappears from the cells. The nucleus in the neighbourhood of the hypha becomes hypertrophied, often becomes modified in form and has increased attraction for stains. Where mycelial influence is great the nucleus becomes amoeboid and sometimes disintegrates: this would seem to indicate a parasitic action on the part of the fungus. The digesting cells are clearly recognisable by the degenerating mass which more than half fills the cells. The increase in the size of the nucleus is also a character as it becomes about four times its original diameter, i.e. roughly sixty times the

* Further investigation is needed on this plant. MacDougal first recorded that *Cephalanthera oregana* was free from fungus, but later found a somewhat sparse and intermittent infection.

† W. K. Wahrlich, Beiträge zur Kenntnis der Orchideenwurzelpilze. Bot. Zeit. XLIV, pp. 481, 497 (1886).

‡ W. Magnus, Studien an der endotrophen Mycorrhiza von *Neottia Nidus-avis* L. Jahr. f. wissensch. Bot. xxxv, pp. 205–272 (1800).

volume. The nuclei become amoeboid and put out pseudopodia which serve to attack the hyphae. The hyphae only stain slightly: they increase in diameter up to about double and also in length. The development in some cases is so great that the cell is quite filled with the thick mycelial mass and the nucleus is crumpled by the hyphae. Enclosed by the pseudopodia the latter gradually lose their outline until frequently they cannot be distinguished from protoplasmic trabeculae. The victorious nucleus then assumes a round form and normal volume and reconstitutes its chromatin network. The endophyte is reduced to an amorphous yellowish clump with indistinct contour, and is absolutely devoid of life: it is surrounded by a cellulose membrane. It would seem that as the root ages the clumps finally disappear. After the formation of the clump starch often reappears in the cell. Burgeff states that the fungus in the host cells can re-attack digesting cells when similar stages are again gone through. We shall return to the question of digestion when we consider the seedling.

GERMINATION OF SEEDS.

The difficulty in germinating the seeds of orchids is one which has been known for a considerable number of years. In fact, it was not until 1804 that any orchid seedlings were described, when R. A. Salisbury figured those of *Orchis Morio* and *Limodorum verecundum*. Later, many botanists such as Link, Irmisch, Beer, etc., added to our information concerning the stages of development. Orchid growers evolved the method of sowing seeds on the soil containing the parent plant*, and it was in this manner, or some modification of it, that most of the hybrids known in horticulture were raised. The facts known, i.e., the difficulty in germinating seeds unless placed on orchid soil and the presence of fungi in the roots, led many to suspect that the fungus was concerned in some way with the success or failure of germination.

We have mentioned that when Reissek recognised the fungal nature of the cell inclusions, he attempted to isolate them. This attempt, long before the days of bacteriological technique, was bound to end in failure and the fungus he isolated was a species of *Fusarium*, a genus which has been time and again proclaimed as the consort of the orchid root. (The genus *Nectria* also has often been assumed to be the endophyte.) It is to Noël Bernard that we are indebted for our chief knowledge of the facts of orchid germination. This brilliant young French investigator began his studies on mycorrhiza in 1899, and they extended until his death in 1911. His first investigation was on the germina-

* I believe Dominy of Messrs Veitch and Sons introduced this practice.

tion of *Neottia*. In 1902 in his thesis "Étude sur la tubérisation" he mentions that orchid seeds can germinate only in the presence of the root fungus and that the seedling is infected from its earliest stages. Realising the importance of this fact he turned his attention to investigating it thoroughly and following the various ramifications of the subject. Bernard's great work, "L'évolution dans la symbiose. Les Orchidées et leurs Champignons commensaux," appeared in 1909. In the same year a comprehensive work by Burgeff was published entitled "Die Wurzelpilze der Orchideen." Both these investigators succeeded in isolating the fungus from the orchid root and growing it on nutrient media. Orchid seeds germinated without difficulty on having the appropriate fungus supplied to them. In describing the course of events full use has been made of the work of Bernard and Burgeff, this being supplemented by observations made by the late Mr J. Charlesworth and the writer.

JOSEPH CHARLESWORTH (1851-1920).

It will probably not be considered out of place here if I venture upon a few remarks concerning my friend the late Mr Joseph Charlesworth. In the year 1913 I was invited to Haywards Heath to see his results in raising seedlings by what he styled the "pure culture method." He had succeeded in eliminating many sources of error and had achieved remarkable and consistent results in raising *Odontoglossum* and its allies by sowing seeds on nutrient media in which the appropriate fungus was growing. The probability that the mycorrhizal fungus in some way affected the germination of orchid seeds had influenced him for many years and he had earned his great reputation as a hybridist by his success in raising hybrids by modifications of the methods in common use. In an account of a visit to his establishment in 1906 it was written "Here is a veritable seedling land, thousands and thousands of them," and in 1909 "The raising of *Odontoglossum* and allied genera has become a very important business, and there are thousands of seedlings in existence. Messrs Charlesworth are reducing it to a system." It was to one so successful by the older methods that Bernard's work made such a strong appeal, and he eventually decided to adopt the system. His culture flasks were sufficient testimony to the success of the laboratory method when placed upon a commercial scale. One was not prepared to find, however, that at the same time he had, after the age of sixty, become so imbued with the new spirit as to have purchased microscopes, microtomes, ovens, stains, books, etc., and become proficient in microscopic technique. (The photomicrographs illustrating this paper are all taken from his preparations.) Naturally

he did not restrict his newly acquired activities to studying orchids, but the main part of his laboratory work dealt with them, and he was especially interested in the seed from its first formation and in the relations between fungus and seed in germination. The whole of the slides were generously placed at my disposal. We, however, drew up a scheme of collaboration and mapped out a series of investigations, which unfortunately had to be discontinued owing directly and indirectly to the war. When, in 1920, we were both again free to resume the work he was a sick man and beyond application to research.

I should wish to repeat here for the benefit of those orchid lovers who knew so well one part of his accomplishments that the other part was equally good. The fact that he should commence laboratory work at such a late age is as surprising as is the success which he attained practically unaided. To a botanist trained in the schools many of his expressions appeared whimsical, but when he termed the small cells at the distal end of an *Odontoglossum* seed the "soul of the plant," it was as a result of finding that it was there eventually that both stem and root were laid down—and he had a happy knack of coining such expressions and, one may add, a certain persistency in using them. If his early days had been spent in acquiring a knowledge of academic botany rather than in connection with his father's wool business, there can be no doubt that the name of Joseph Charlesworth would have been writ large in the annals of British science. As the firm of Messrs Charlesworth are carrying on the traditions of their late chief, it may be possible at some future date to complete and put on record certain of the investigations; and it is hoped it may be possible to carry out the original plan in which his knowledge of orchid culture would have played an essential part.

ORCHID FUNGUS.

Bernard in his first attempts to isolate the fungus from orchid roots obtained a species of *Fusarium*. When, however, he succeeded in extracting the right fungus he established a criterion which enables one to settle without doubt whether the true fungus has been isolated, viz. that the endophyte is able to bring about the germination of the seed.

The fungus, when living within the cells of the plant, shows no characters which give a clue to its systematic position, but when it is grown on nutrient media it shows additional stages of development which are characteristic.

When extracted from the root and placed in a culture medium the fungus always appears to behave in the same way. The fungus spreads over the surface by the apical growth of its

septate filaments. Meanwhile lateral branches arise and anastomoses take place between the hyphae. Later, balls of hyphae appear here and there in the culture and on the sides of the tube or flask containing them, usually some distance from the ends of the hyphae. These balls are very similar to those which appear in the cells of the root, being formed by the rolling up of the ends of young growing filaments, and often becoming very compact. When seen in the host cells this method of growth suggests adaptation to the needs of the special environment, and its presence in cultures might lead to the supposition that the character is so impressed upon the fungus that it also shows it when living free. The character is, however, not rare in the group in which we must classify this fungus.

As the mycelium becomes older shorter filaments arise with very short and swollen segments, which are apparently rich in food reserve. (It was this appearance that caused Bernard to place the fungus in the genus *Oospora* when he first studied it.) These filaments ramify abundantly and in certain forms anastomose amongst themselves and give rise to yellow or brown sclerotia* (Figs. 4 and 5), small spherical bodies formed of intertwined and massed hyphae. These structures are capable of withstanding drought and other inclement conditions and are remarkably tenacious of life. Bernard has pointed out that these swollen filaments are very like those which occur in *Rhizoctonia violacea* Tul.† which is common on potatoes, lucerne and other crops, where it forms small, blackish, irregular sclerotia, and he considers that the orchid fungi fall into the same genus. He classed the fungi obtained from about twenty orchids as three species, *Rhizoctonia repens*, *R. mucoroides* and *R. lanuginosa*. The first, which was by far the most commonly isolated (*Laelia*, *Laelio-Cattleya*, *Spiranthes*, *Paphiopedilum*, *Cymbidium*, *Aerides*, *Bletilla*, *Coelogyne*), does not form sclerotia. *R. mucoroides* was found in *Phalaenopsis* and *Vanda*, and *R. lanuginosa* in *Odontoglossum* (Figs. 4 and 5). Burgeff, unaware of Bernard's latest results, proposed a new genus *Orcheomyces* for the reception of the orchid fungi. He fully describes fifteen species, naming them after the orchid from which he obtained them and mentions another fourteen by name: he divides them into five main groups.

A discussion of the different systematic interpretations given by Bernard and Burgeff would be out of place here and for convenience the more generally adopted name *Rhizoctonia* will

* Sclerotia are known in all groups of fungi, often reaching considerable dimensions, e.g. the size of a man's head in *Polyporus Mylittae* (the "black fellows' bread" of Australia).

† *Corticium vagum* B. and Br. var. *Solani* Burt.

be used. The diversity in the number of species is simply a case of the usual "lumping" and "splitting." Bernard found in his experiments that fungi obtained from different sources, but to which he gave the same specific names, varied somewhat in their behaviour, and it is quite probable that these physiological distinctions are related to slight morphological differences. Bernard later recognised certain of Burgeff's species as falling within his, e.g. *Orcheomyces Sambucinae*, *O. mascula*, *O. insignis* and *O. Luddigi* were regarded by him as *Rhizoctonia repens*—but he apparently took into account merely the gross characters of growth.

The endophytic fungus is able to ferment cellulose, which accounts for its ability to penetrate cell walls. Burgeff made a study of the physiological characters of the species he isolated. He found that they were able to absorb carbohydrates in the form of sugars, these being in all cases transformed by a diastase-invertase in some species, maltase in others. Having regard to the prevalent ideas as to the function of mycorrhizal fungi it is of particular interest to note that these forms are apparently unable to fix free nitrogen: the nitrogen of organic compounds, such as peptone, can be made use of as a source of nitrogen: ammonium compounds are better assimilated than nitrates. By growing cultures in the dark and in an atmosphere devoid of carbon-dioxide he established the fact that the carbon compounds of the soil can suffice as a source of carbon.

Bernard in his experiments found that the fungi if grown in culture gradually became inactive. Cultures two years old were quite unable to bring about germination. Burgeff, on the other hand, found that his cultures after twenty-six and twenty-eight months retained their power. In connection with this point a culture of a root fungus which had been regularly cultivated for at least eight years, though not used during that time for germination, was recently tried. A very feeble germination occurred in certain of the tubes. As the activity of the fungus when it was first isolated is not known, it is impossible to say whether there is any decrease in intensity, though this is probable. The gradual attenuation with final loss of activity noted by Bernard may be a consequence of "staling" through too infrequent renewal of cultures. He found that the intensity of an attenuated form can be increased by extracting it from a plant which it had been successful in germinating.

GERMINATION OF SEEDS—*continued*.

The seeds of orchids are very small, the embryo being frequently only just visible to the naked eye*. They possess a

* The embryo in Fig. 2 is approximately 200 μ , i.e. c. $\frac{1}{16}$ inch.

single integument which is in the form of a characteristic network (Fig. 2) which varies somewhat in shape and structure in the different genera. On sectioning the seed (Fig. 3), or on viewing when stained and mounted whole, there is seen to be no differentiation into cotyledon, stem, radicle, as is almost universal in flowering plants*. It appears to be most usual for the cells at the suspensor end of the seed to be somewhat larger than at the upper end (Fig. 3), though this is not always the case (*Cypripedium*). Sometimes the suspensor cells are permanent (*Cattleya*)—the suspensor is the stalk by which the developing seed is attached and nourished—at other times they disappear before the seed is matured (*Phalaenopsis*). Seeds taken from the capsule under sterile conditions and sown on ordinary substrata where no fungus is present do not as a rule develop. Generally they merely swell and become green (*Odontoglossum*) though sometimes even this does not happen (*Epidendrum*); in other cases they may form stomata and the rudiments of hairs (*Cattleya*). The only case so far known in which any considerable development can take place under these conditions is *Bletilla hyacinthina* where Bernard found that thin slender seedlings developed with distinct leaves. The food reserve of orchid seeds is most frequently oil, part of which becomes transformed into starch. The reserve food comes to its end just as the seed commences to become green. This is usually after three or four months, during which time very little, if any, nutriment can be obtained from the substratum, as absorbing hairs are lacking. If no fungus infection take place then, the seedling dies. It is somewhat surprising that after the production of chlorophyll death should occur rather than autonomous growth by aid of photosynthesis: the seedling appears to form chlorophyll as a sort of last despairing effort.

If, however, the appropriate fungus (i.e. the fungus from the root of the parent or some closely allied plant) be added now at the latest, an extraordinary change takes place. The fungus seems to give an impetus to development.

In the culture flasks it is only in prearranged experiment that infection takes place at such a late stage. The fungus enters the seed usually within a few days. The course of events may be made out from the photomicrographs, which are taken from different genera in order to show the general similarity in the phenomena. Entry takes place at the suspensor end of the seed between the suspensor cells themselves, if such be retained. The cell walls here are unmodified, though the general surface of the

* *Bletilla hyacinthina* shows a rudimentary cotyledon according to Bernard. Treub has indicated a cotyledon in *Sobralia macrantha*, and Pfitzer records a green embryo with a differentiated cotyledon in *Platyclinis glumacea*.

seed is slightly cuticularized. As we have seen, the cells at the suspensor end of the seed are generally larger, and it is into these that the fungus passes (Figs. 3, 6, 7, 8). The cells are invaded by degrees, the hyphae becoming twisted into a ball in each cell before passing on to the next. Almost immediately the smaller cells at the opposite end of the seed undergo division. It is here that the meristem of the stem is laid down. The meristematic cells in orchids are never entered by the fungus: the only cells capable of division which ever harbour the endophyte appear to be those of the seed where it first enters. Eventually the developing seedling takes on a swollen shape most frequently more or less turbinate (Figs. 9, 10).

Bernard uses the term "protocorm" for this swollen tubercle and regards it as of theoretical importance, as it simulates the protocorms of Lycopods and the colourless underground prothalli of Adder's Tongues, etc. It is of interest to remark that a similar structure, also associated with fungi, occurs in the primitive fossil plant *Hornea* from the Devonian. The fungus remains restricted to the larger cells and follows in the wake of their division. The epidermal layer is free from infection. Meanwhile the rapid division taking place in the smaller cells at the anterior end of the seed gives rise to the young stem apex and the first leaf (cotyledon). About the time this young leaf becomes visible to the naked eye the cell-division has become extended along the axis and the beginning of the central stele is seen (Fig. 10). In this manner the young root is formed and begins to absorb its way through the tissues of the protocorm (Fig. 11). Finally it passes out into the soil (Fig. 12). In no orchid studied in the present series (*Odontoglossum*, *Oncidium*, *Cattleya*, *Cymbidium*, *Vanda*, *Cypripedium*, etc.) does the developing root when passing through the tissues enter the fungal zone nor do the hyphae extend into the root. In fact there is often a suggestion of a delimiting membrane separating the two areas (cf. Fig. 12). Thus when the root enters the soil it is absolutely free from infection; in none of the usually cultivated orchids does the root receive fungus from the swollen protocorm. Infection takes place from the soil most frequently when the root is about a quarter of an inch in length, the hyphae entering by the root hairs a little behind the region of greatest growth. This throwing off of the fungus, as it were, is repeated in orchids with tubers which do not retain their roots: the tuber is not infected and the new roots receive their fungus from the soil. In fact, in orchids so far studied it is only in the saprophytic *Neottia* that constant infection obtains. Here infection progresses gradually from the widely infected protocorm into the body of the plant, gains the rhizome and infects the successive

roots. The region of infection is thus perfectly continuous throughout the plant from the tip of the protocorm to the base of the inflorescence: as Bernard remarks, according to the evidence the whole of the mycelium harboured by a *Neottia* has for its single origin the mycelial filament which first penetrates the embryo*.

The question arises as to whether root infection *per se* is obligate in orchids with abundant chlorophyll or whether it is a necessary evil. If the latter, one would expect the fungus to be lodged in the roots, though restricted in distribution. As stated above, all the cells entered seem to act as digestive cells in cultivated orchids. Is such digestion a device for protection or for nutrition?

What has been happening to the fungus during these stages? The course of events was first followed by Bernard. As we have seen, the fungus enters at the suspensor end of the seed by the cells of the suspensor near the point of attachment (*Odontoglossum*) or by the cells of the pole of the embryo where the suspensor is attached (*Vanda*). There appears to be an attraction, though feeble, towards the place of entry. The first filament entering the seed apparently excludes all others, though it may be of an attenuated form and unable itself to bring about germination. Bernard compared this with vaccination: the infection immunizes the seed. In successful germinations the fungus, after seed entry, follows the development of the cells forming mycelial balls in all the posterior portion of the seedling. According to Bernard, when the fungus reaches the cells bordering on the meristematic region digestion takes place. This is regarded as being analogous to phagocytosis such as occurs in animals where the white corpuscles of the blood attack, engulf and digest any invading micro-organisms: the cells in which the digestion takes place are the phagocytes.

In general these may be regarded as definite cells often recognisable, even before infection on account of their nucleus sometimes becoming lobed. The balling of the fungus in the cells is compared with agglutination, and the manner in which this occurs only in cells of the developing seedling which have achieved their growth is compared with cases of mortal infection where the balling is abandoned sooner or later and the fungus grows on in every direction and invades all the tissues indifferently.

* The association can be even more close under certain conditions. Flower scapes are frequently unable to pierce the humus covering them and the flowers and seeds develop underground, sometimes beneath the root-tufts which produce them. Mycelium apparently from the rhizome of the plant passes up the central cavity of the stem and infects the seeds in the subterranean fruits which are thus able to germinate.

Digestion eventually takes place in all the more deeply lying cells, while the external layers act as host cells. The fungus can pass out of the protocorm by way of the hairs present on its surface.

This application of the theory of phagocytosis is a most attractive one. Gallaud* first suggested the similarity of the function of the digestive cells and that of phagocytes, but it is to Bernard that we owe the working out in detail†. Much investigation on the germinating seed is still needed. Bernard's account of the distribution of the phagocytes is not satisfactory. As the photomicrographs (Figs. 10, 12, 13) show it is not unusual for all the infected cells of the protocorm to be able to digest the fungus eventually.

GERMINATION WITHOUT FUNGUS.

How far is it possible to replace the fungus by artificial conditions? Bernard concluded from a consideration of the way in which the endophyte can act at a distance, i.e. bring about changes in cells to which it has not access, that there is a general modification of the physico-chemical properties of the sap which can reach all the tissues. He tried the effect of solutions of salep and saccharose of increasing concentrations on seeds of *Bletilla*, *Cattleya* and *Laelia*. In *Bletilla* where, as we have seen, germination takes place with the formation of slender seedlings in the absence of fungi, in high concentrations most of the seedlings showed thickened protocorms and short internodes comparable with fungus infected individuals. The seeds of *Cattleya* and *Laelia* at low concentrations swell and become green. With higher concentrations development is always much slower and more irregular than with fungi, but one can obtain seedlings of quite normal appearance. As the concentrations increase the development is increasingly better, but more irregular: but there is an upper limit beyond which there is no germination.

Thus it appears that augmentation of the culture medium can, in certain cases, supply the place of fungus action. In fact Bernard states that in the condition of his experiments it was more certain and easier to germinate certain seeds by the action of concentrated solutions than to have recourse to fungal infection. Germination was slow, but very regular, the protocorms had a normal appearance and the seedlings when fairly developed could be transplanted. Experiments showed that *Rhizoctonia* was able to increase the concentration of the solutions in which

* F. Gallaud, Études sur les mycorrhizes endotrophes. Rev. Gén. Bot. xvii, pp. 5 et passim (1905).

† Bernard (1911) also showed that the bulbs of *Loroglossum* contain a diffusible substance which has a fungicidal effect on *Rhizoctonia*.

it grew and Bernard considered it probable that it acts similarly in orchid tissues and increases the degree of concentration of the sap. This problem of autonomous germination recalls to mind that of parthenogenesis—the development of an ovum without the intervention of a spermatozoon. The egg possesses all the substances necessary for activation: the spermatozoon is an inciting cause of these reactions within the egg system on which development depends. Parthenogenesis occurs naturally in certain groups, but it has been brought about experimentally in numerous cases where fertilization normally obtains*, †. Apparently the first successful attempt was made by Tichomirow in 1886, who stimulated the unfertilized ova of the silk moth to development by rubbing them between two pieces of cloth. Various methods have since been used such as treatment with fatty acids, certain salts such as barium chloride, lipid solvents such as chloroform, hypertonic and hypotonic solutions, etc.‡.

Another significant similarity is that artificially activated eggs always show a marked slowness in their rate of development, even with the best methods, as compared with the fertilized eggs. This suggests, according to Lillie, some factor that has not yet been successfully imitated in any artificial way. Is it possible that in both cases accessory food factors (vitamines) may play a part? In considering the case of seeds it might be pointed out that there are many instances of peculiar germination known in other phyla. Pinoy§ showed that spores of Myxomycetes such as *Chondrioderma difforme* do not germinate unless bacteria are present. Ferguson|| discovered that the only way in which she could germinate the spores of the common mushroom effectively was by having a little mycelium of the fungus present in the cultures, and Servettaz¶ found that a species of *Oospora* activated the growth of the moss *Phascum*

* F. R. Lillie, Problems of Fertilization. Univ. of Chicago Science Series (1919).

† Y. Delage and M. Goldsmith, La parthénogénèse naturelle et expérimentale. Paris (1903).

‡ The only case in which parthenogenesis has been induced in the entire vertebrate phylum is in the frog, where Bataillon in 1910, after years of vain attempts, finally succeeded by the exceedingly simple method of pricking the eggs with a fine needle. It is necessary that blood or tissue extract should be carried into the egg by the needle. This method has been abundantly confirmed and tadpoles so obtained have been reared to maturity by Loeb and Bancroft.

§ E. Pinoy, Rôle de bactéries dans le développement de certains Myxomycètes. Ann. Inst. Pasteur, xxi, p. 632 (1907).

|| M. C. Ferguson, A preliminary study of the germination of the spores of *Agaricus campestris* and other Basidiomycetous fungi. U.S. Dept. Agric. Bureau of Plant Industry, Bull. No. 16 (1902).

¶ C. Servettaz, Recherches expérimentales sur le développement et la nutrition des mousses en milieux stérilisés. Ann. Sci. Nat. 9 sér. xvii, pp. 111–224 (1913).

cuspidatum to a remarkable degree, though the favourable action was of short duration in the conditions of his experiments.

GASTRODIA.

An unusual and interesting type of mycorrhiza occurs in *Gastrodia elata**, a non-chlorophyllous orchid widely spread throughout Japan, where it occurs mostly in woods under *Quercus serrata* and *Q. glandulifera*. The full-grown flowering tuber is oblong and slightly curved, attaining almost without exception a length of 10–17 cm. This tuberous rhizome is the whole vegetative part of the plant and consists essentially of parenchymatous cells. Multiplication usually takes place by the tuber. It produces long rhizomes from its apex or node, upon which stalked off-sets are developed. At the end of autumn the mother body and the pedicel of the off-set undergo degeneration, so that the daughter tubercles are set free. Unless the mother tuber has been infected with the necessary fungus the off-sets decrease in size with each successive generation, until they become so much reduced and deficient in food materials that they are incapable of further multiplication. The fungus necessary for proper development is not a microscopic mould as in the other orchids studied, but *Armillaria mellea*, the well-known "honey fungus." This toadstool is extremely common in our woods where it is a most destructive parasite, "indeed more trees die, in Europe at any rate, from attack by this fungus than through any other parasitic agent†." The fructifications are found generally on or near stumps. If the earth beneath the toadstool be dug up it will be found to contain one or more black strands, resembling bootlaces, which are attached to the base of the stem. These rhizomorphs, as they are called, consist of densely compacted fungus mycelium. Further, the mycelium in the wood of the tree itself is first felted and grows up through the cambium to a considerable height: when the tree is dead and the bark has become loosened the mycelium is transformed into a tangled mass of flattened rhizomorphs. Early mycologists considered that they were here dealing with three different species of fungus—the toadstool (*Agaricus melleus*), the rhizomorph under the bark (*Rhizomorpha subcorticalis*) and the rhizomorph in the ground (*Rhizomorpha subterranea*).

It is with the subterranean rhizomorph that we are here concerned. It forms a cylindrical, smooth, black strand, usually 1 to 1.5 mm. in thickness. Its peripheral portion, the so-called cortex, consists of compact, pseudoparenchymatous, brownish

* S. Kusano, *Gastrodia elata* and its symbiotic association with *Armillaria mellea*. Journ. Coll. Agric. Imp. Univ. Tokyo, iv, pp. 1–66 (1911).

† W. E. Hiley, The fungal diseases of the common larch. Oxford (1919).

mycelium with a comparatively thick wall. The middle layer is composed of a bundle of large thin-walled mycelia with numerous septa. The inner cavity of the strand is traversed by a loose bundle of very fine longitudinal hyphae rich in protoplasmic contents.

When the tuber of *Gastrodia* is attacked by the rhizomorph, infection is effected by a sucker-like branch of the strand which penetrates the cortical cell layers, partly compressing the underlying cells and partly dissolving their walls. This mode of infection is, of course, quite different from the ordinary endophytic mycorrhizal type where infection is effected as a rule by a single hypha (cf. p. 31). It very much resembles the manner in which the parasitic *Cuscuta* attacks its hosts, the rhizomorph creeping over the surface of the tuber and giving off the infection branches at intervals. On entering the tuber the hyphae of the various portions of the strand essentially retain their structure. The infected area of the tuber may be divided into three regions, according to the structure of the cells and the nature of the hyphae contained within them. The external region is composed of two or three layers of cells which contain a densely entangled mass of comparatively thick-walled hyphae; the middle region is similarly composed, except that the hyphae are generally thin-walled and of various breadths and often arranged as a pseudoparenchyma; the innermost region has large cells each containing a few, slender, slightly curved hyphae. The three regions correspond to the zones in the rhizomorph. The hyphae of each region show characteristic alterations. They are permanent in the first region; in the second they undergo self-disorganization; while in the third they are mostly consumed by the cells of the host. The mode of development of the fungus in the middle region simulates the ordinary clumping seen in most orchids, but the course of events is different in that the protoplast is consumed by the hyphae before their collapse takes place. The destruction of the protoplast shows the parasitic properties of the hyphae. The cells of the inner regions are apparently metabolic centres of the orchid where the food materials are elaborated. The nucleus and cytoplasm undergo remarkable alterations, and secondary products appear indicating considerable activities. After the disappearance of the hyphae the nucleus resumes its original form and structure, while the cytoplasm again becomes fibrous and vacuolate. Starch grains disappear from all the mycorrhizal cells, to reappear in the inner region with the cessation of metabolic activity.

The association of tuber and rhizomorph takes place quite occasionally. If a tuber forms mycorrhiza it can give rise to a full grown off-set which remains dormant during the winter and

develops the inflorescence axis in the following year: otherwise no flowers are produced.

So far no results have been published as to the germination of the seeds of *Gastrodia*. One would expect that fungal infection is necessary for seedling development, but whether the fungus is a form like *Rhizoctonia* or whether there is some adaptation by which *Armillaria* becomes operative remains to be seen. In either case the facts will be of the greatest theoretical interest.

The course of events in *Gastrodia* gives some support to the idea that the relation of fungus and orchid is primarily one of parasitism on the part of the former. At times the rhizomorph attacks tubers and destroys them in a manner similar to that in which it treats potato tubers. Usually, however, the fungus is kept well under control and its hyphae prevented from spreading beyond their apportioned region—and even so being absorbed by the orchid cells. It is difficult to see what benefit the fungus can gain under these conditions. The subterranean strands are apparently unable to obtain nutriment from the soil, their function in the usual life of the fungus being that of “runners.” It would seem that *Gastrodia* has turned the attack of these into one of service for transmitting nutriment from the oak stumps to which the fungus is attached, for its own benefit: a colourless saprophyte unable to grow or to flower without the aid of one of the most destructive parasites known!

NUMBER OF SEEDS AND DISTRIBUTION OF FUNGUS.

When one sees the dense masses of seedlings thriving in the culture flasks one contemplates as to the course of events under natural conditions. The enormous numbers of seeds which are usually produced in the capsules of orchids must have struck the most casual observer. “Not that such profusion is anything to boast of; for the production of an almost infinite number of seeds or eggs, is undoubtedly a sign of lowness of organisation. That a plant, not being an annual, should escape extinction, chiefly by the production of a vast number of seeds or seedlings, shows a poverty of contrivance, or a want of some fitting protection against other dangers.” Darwin* estimated that in *Cephalanthera grandiflora* a single capsule contained 6020 seeds and that, therefore, a plant with the usual four capsules would have 24,080 seeds. Similarly *Orchis maculata* had 6200 seeds in a single capsule, and thus a plant having the not unusual number of thirty capsules would produce 186,000 seeds: “As this orchid is perennial, and cannot in most places be increasing, one seed alone of this large number yields a mature plant once

* C. Darwin, Fertilisation of Orchids (1862).

in every few years." In order to retain the number of individuals of a species stationary it is only necessary that one mature plant should be produced during the period of growth of the parent—if more occur the species will tend to oust out all other species. "Linnaeus has calculated that if an annual plant produced only two seeds—and there is no plant so unproductive as this—and their seedlings next year produced two, and so on, then in twenty years there would be a million plants....It would suffice to keep up the full number of a tree, which lived on an average for a thousand years, if a single seed were produced once in a thousand years, supposing that this seed were never destroyed, and could be ensured to germinate in a fitting place*." To give an idea of what the above figures for *Orchis maculata* really mean Darwin worked out the possible rate of increase. "An acre of land would hold 174,240 plants, each having a space of six inches square, and this would be just sufficient for their growth; so that, making the fair allowance of 400 bad seeds in each capsule, an acre would be thickly clothed by the progeny of a single plant. At the same rate of increase, the grandchildren would cover a space slightly exceeding the Isle of Anglesea; and the great grandchildren of a single plant would nearly (in the rate of 47 to 50) clothe with a uniform green carpet the entire surface of the land throughout the globe"—and as *O. maculata* is perennial, the parent plant would still be alive!

But even these numbers in our native orchids are much exceeded by those of tropical species. Scott estimated that a capsule of *Acropera* contains 371,250 seeds and, judging from the number of flowers borne by the plant, the total number of seeds for an individual would be 74,000,000: Charlesworth estimated 825,000 seeds for a single capsule of *Cymbidium Traceyanum*: Muller 1,756,440 seeds for a single capsule of *Maxillaria*. It appears to be a general biological rule that where the conditions of successful germination are difficult of attainment a prolific number of seeds (or spores) are produced and, *vice versa*, where the requirements are not of a specialized nature, a smaller number occur.

In the case of orchids it seems not unlikely that the enormous seed production is in some way related to the fungus question. Their small size, their lightness, their net-work integument and the presence in some genera of elaters ensure their effective dissemination. But unless the necessary fungus be to hand no germination occurs—the seed may develop to a certain extent, but it does not produce roots unless the appropriate fungus enters its cells.

So far, however, we know nothing of the distribution of these

* C. Darwin, *Origin of Species* (1859).

fungi in nature except so far as they occur associated with rooted orchid plants. Probably most people are aware that fungi of all kinds are present in the soil, but few realize in what enormous numbers they occur and the manner in which some are restricted to the soil. Hagem* calculated that in a gram of soil from a potato field, 350 spores of *Rhizopus stolonifer* and 250 each of *Mucor sphaerosporus*, *M. nodosus*, *Absidia cylindrospora* and *Zygorhynchus Moelleri* were present; and these numbers are much exceeded by *Penicillium* (90-95 per cent. of spores in uncultivated soil according to Sopp†) and other Hyphomycetes. Traaen‡ calculated that from 10,000 to 120,000 spores of *Geomyces vulgaris* and from 1000 to 20,000 spores of *Humicola fuscoatra* occur in a gram of soil. Much work has been done recently on the biological activities of such fungi, attention being paid chiefly to cellulose destruction and the possibility of nitrogen fixation. It is extremely probable that certain of the forms isolated are capable of acting as mycorrhizal fungi, though none have apparently been recognised as such. Further it is possible to isolate *Rhizoctonia* from the soil in the immediate neighbourhood of orchid plants growing wild (as also from the soil of pots containing cultivated orchids): but notwithstanding the large number of species of soil fungi isolated it does not appear to have been found, or at least recognised, by any investigator. We are thus lacking in data as to the distribution of orchid fungi in the soil. Since, however, Bernard isolated *Rhizoctonia repens* from many European orchids and showed it to be the commonest endophyte amongst cultivated species, it must be of world-wide distribution, since in order to account for the distribution of the orchids it is necessary to assume that this particular fungus must occur practically wherever orchids grow.

ERICACEAE.

A family of plants which is usually linked with orchids as showing the same constancy of fungal infection is the Ericaceae. Frank early realized that the relation between the fungus and flowering plant in these two families is a particularly close one. In certain ericaceous plants he remarked on the absence of root-hairs, the absence of, or reduction in, the amount of cortical tissues, the reduction of the root-cap, and the masses of fungus mycelium in the enlarged cells of the epidermal layer. Ternetz§

* O. Hagem, Untersuchungen über Norwegische Mucorineen II. Skrifter Vidensk.-Selsk. Christiania. I. Math.-Natur. Kl. No. 4 (1910).

† O. J. O. Sopp, Monographie der Pilzgruppe *Penicillium*. Idem, No. 11 (1912).

‡ A. E. Traaen, Untersuchungen über Bodenpilze aus Norwegen. Nyt Mag. Naturvidensk. LII. pp. 19-121 (1914).

§ C. Ternetz, Über die Assimilation des atmosphärischen Stickstoffes durch Pilze. Jahrb. f. wissensch. Bot. XLIV, pp. 353-408 (1907).

was successful in isolating the fungi from certain species and growing them in pure culture, constantly obtaining the same fungus from the same species of flowering plant. All the fungi belonged to the genus *Phoma**—one of the Fungi Imperfecti, but of a totally different group than is *Rhizoctonia*—and were apparently morphologically and physiologically distinct. She showed that infection of *Calluna* took place in the seedling and also found infection in a case of viviparous germination in *Andromeda*.

Rayner† working with *Calluna vulgaris* was able to show that the full development of the seedling was dependent upon the presence of the mycorrhizal fungus—there is here an “obligate symbiosis” of a type very similar to that in orchids. Finding that the sterile seedlings were unable to form a root-system she investigated the matter in the manner made classical by Bernard. The seed coats were found to become infected while the seeds are still in the ovary. Delicate branched hyphae are present in the cells of the ovary wall, in the tissue of the central column and in the funicles of the seeds. Branches of this mycelium grow across from the cells of the ovary wall to those of the seed-coats, extending from one seed to another. The fungus was isolated and grown in pure culture. It proved to be a pycnidial form similar in all respects to the genus *Phoma*. Sterile seeds sown on this develop normally, whereas in its absence the seedlings merely form a few reddish or chlorotic leaves, but no roots. Infection of the seedling root takes place at, or immediately after, it emerges and may begin at the tip by hyphae forcing their way between the cells of the apex, though more usually it occurs simultaneously at several points. The mycelium immediately becomes intercellular and infection spreads rapidly from cell to cell. Some hyphal branches grow out and infect fresh rootlets as they develop; others form a tangled skein of fine hyphae in the superficial cells. One of the most interesting points of the story is, however, that the fungus does not remain confined to the roots but infects the whole of the young seedling. In the subaerial parts the mycelium does not develop so extensively on the surface of the plant, nor do the hyphae become balled up in the superficial cells as in the roots, but are irregularly distributed in the tissues. In the mature plant likewise the fungus is not confined to the roots but is present in the tissues of the stem, leaf, flower and fruit. The hyphae can also be seen ramifying among the hairs or closely applied to the cuticle of

* *Phoma radialis-Oxycocci*, *P. radialis-Andromedae*, *P. radialis-Vaccinii*, *P. radialis-Tetralicis* and *P. radialis-Ericae*.

† M. C. Rayner, Obligate symbiosis in *Calluna vulgaris*. Ann. Bot. xxix, pp. 97-133 (1915).

the epidermal cells: they show no preference for special points of entrance or egress, penetrating with equal ease the cuticularized cells of the epidermis or the base of a hair. The ovary—and later the young fruit—contains mycelium in all parts of the internal tissues. This mycelium infects the seed coats of the developing seeds. The embryo and endosperm of the resting seed are free from infection.

Thus, as in *Neottia*, we are dealing, except in the seed, with a dual organism. The type of association is, however, different from what obtains in the orchids so far studied, where no such distribution has been found—and an analogous constancy apparently only occurs in non-chlorophyllous genera. From the fact that Rayner has recorded the presence of ovarian infection in a number of Ericaceae—Rhododendroideae, Arbutoideae, Vaccinioideae and Ericoideae—it may be that the fungus is similarly distributed throughout the tissues of these plants, and presumably obligate symbiosis is to be inferred.

In no other case has the necessity of the presence of the mycorrhizal fungus for germination been proved. There can be hardly any doubt, however, that such a phenomenon is not restricted to two groups so widely separated as the Orchidaceae and the Ericaceae. What have these families in common? Apart from the similarity in habitat of certain species there seems to be nothing except the smallness of their seeds—and it is naturally to seed characters that one looks in this connection. As we have seen, the seeds of orchids are exceedingly small; reduction in most genera would appear to have reached its limit. In typical Ericaceae the seed is very small, rarely exceeding 2 mm. and often less than half this size. There is a richly developed endosperm in which a straight embryo is embedded one-half to two-thirds the length of the seed, always showing a root, an axis and two cotyledons more or less differentiated. It is also of interest to remark that such genera as *Kalmia* and *Ledum* have a net-work integument to the seed.

PYROLACEAE.

Allied to the Ericaceae is the family Pyrolaceae with the sub-families Pyroloideae and Monotropoideae. In families of flowering plants which show saprophytism and parasitism there usually occur green purely autophytic plants, with typical green leaves and numerous flowers; plants that are purely saprophytic or parasitic, with colourless scales and a reduced number of flowers; and all gradations between. Henderson* instances the

* M. W. Henderson, A comparative study of the structure and saprophytism of the Pyrolaceae and Monotropaceae with reference to their derivation from the Ericaceae. Contrib. Bot. Lab. Univ. Pennsylvania, v, pp. 42-109 (1919).

families Burmanniaceae, Orchidaceae, Gentianaceae and Ericaceae as examples of this. Regarding the Pyrolaceae as a saprophytic sub-family of the Ericaceae we can trace a relation between increasing saprophytism and a more intensive development of mycorrhiza. In the root-tip region we get an ascending series in the amount of fungus present from *Chimaphila umbellata* where the epidermal cells of some roots are without hyphae and other roots with hyphae, but not in every cell, to *C. maculata* with a greater number of the epidermal cells filled with hyphae; in *Pyrola rotundifolia* and *P. elliptica* all the cells are infected, and there is the beginning of intertwined hyphae round the root tip; then in *Monotropa Hypopitys* an increase in the width and extent of the sheaths and a division into two zones—an outer loosely woven mass of hyphae and an inner more compact one—and finally in *M. uniflora* a still greater width of the fungal sheath. In the least saprophytic species the epidermis soon dies off, carrying with it the fungal hyphae as in *Chimaphila* and *Pyrola*, whereas in *Monotropa*, especially *M. uniflora*, the epidermis is still living and filled with hyphae when the root is quite old.

Corresponding with this increase in saprophytism there is an increase in the number of seeds produced and a reduction in their size and structure. "The endosperm in the Pyrolaceae consists of relatively few large cells—the embryo of about twenty-five to thirty cells with no trace of cotyledons. In the Monotropaceae the number of endosperm cells is still less and the cells are larger, the embryo also is very small, composed of only nine or five cells." As these seeds also have their integument in the form of a net-work there is an exceedingly close superficial resemblance to those of orchids.

Comparing the members of the Pyrolaceae as a whole with the Ericaceae it would seem exceedingly probable that their seeds are even more dependent upon infection by the mycorrhizal fungus than are those of their chlorophyllous relatives. It will be interesting to learn at what stage infection takes place and whether or not a close approximation to the more advanced orchid type obtains. It is probable that the fungus will be found to be generally distributed in these plants as in *Calluna*.

BURMANNIACEAE AND GENTIANACEAE.

The other two families in which mycorrhizas are typically developed are the Burmanniaceae and the Gentianaceae*—in

* "Most of the Orchideae are humus-plants, and it is noteworthy that dicotylous saprophytes, such as the Pyrolaceae, the gentianaceous *Voyria*, and others, show a reduction of the embryo like that of the Orchideae. In *Monotropa* the embryo has but nine cells. The germination of the seeds of these dicotylous saprophytes is unknown. It takes place only in the presence

fact Stahl considered that from this point of view the latter family are as important as the Orchidaceae. Moreover, in these families the seeds are small and numerous, with little reserve food material and no chlorophyll. Further there are the typical gradations from green plants to colourless saprophytes and correlated with this is an increase in number and decrease in size of the seeds, with a change in the embryo until we end in the most reduced examples with little differentiated or formless masses, and an increasing amount of fungus in the roots. The seeds of the saprophytic genera have a net-work integument and in appearance bear a very close resemblance to those of orchids. The Burmanniaceae are closely related to the Orchidaceae, and we should expect that showing so many characters in common there would also be a resemblance in the important one of obligate fungal infection for germination. In the Gentianaceae there are many isolated records of difficulties in obtaining seed germination in some of the genera, and it is common knowledge that many Gentians are difficult to raise from seed. It would seem extremely probable that in this family also the mycorrhizal fungus is necessary for seedling development.

Ceillier* has worked out in detail the relation between the presence of mycorrhiza and small seeds. In certain cases as in Juncaceae the seeds are small and little differentiated, but as they possess chlorophyll they are able to begin photosynthesis immediately on sowing. Small seeds with much reduced embryos also occur in parasitic forms such as *Cuscuta*, *Orobanche*, etc. No fungus is present in these genera, but apparently germination is not successful unless contact is made with the organs of the requisite host. It may be that the stimulus necessary in these cases is analogous to that requisite to bring about root formation in plants with obligate mycorrhizas.

ORIGIN OF SAPROPHYTISM.

What is the trend of evolution in plants of which the roots are normally infected with endophytic fungi? A general survey of families in which endotrophic mycorrhizas are typically developed shows that it is the rule for these families to have small seeds ill-adapted for successful germination. It has also been proved for orchids and for *Calluna* that the seeds need to be infected by the mycorrhizal fungus before the seedling can

of very special surroundings. Probably the fungi which are found in the roots in symbiosis are essential. The smallness of the seeds allows of a large number being formed, and thus the probability that one of the seeds at least will reach favourable conditions for germination is increased," Goebel, *Organography of Plants*, Part II, pp. 254 [1898] 1905.

* R. Ceillier, *Recherches sur les facteurs de la répartition et sur le rôle des mycorrhizes*. Thèse, Paris (1912).

produce roots. Further it is in these families that typical saprophytic species occur (if we concede that the Pyrolaceae are saprophytic Ericaceae): in fact the presence of fungi in the roots of saprophytes is so common (the apparent exception being *Wulfschlaegelia*), that MacDougal regards these seed-plants as being "saprophytic symbionts*." Without the necessary data it is doubly unsafe to theorise, but it suggests itself that in families adapted to a mycorrhizal habit there is a tendency for the seed to become dependent upon the fungus for successful germination, and there is a correspondingly greater production of seed. It has been customary to associate increasing saprophytism with the greater development of mycorrhizal fungus. May it not be rather that saprophytism has arisen by the mycorrhizal fungus taking over some of the functions necessary in germination and relieving the flowering plant of the need of excessive food production for the developing seed and thus of the necessity for carbon assimilation? (The great amount of fungus in the roots of saprophytes militates against the idea that the root may be simply a lodging-place for the fungus to be at hand for germination and of no use in nutrition.) We see in *Calluna* an almost perfect device for the infection of the seed, and the fungus is generally distributed. The most general infection so far found in orchids is in *Neottia*, which, as has been pointed out above, is most comparable with *Calluna*. But *Neottia* is saprophytic. In chlorophyllous orchids it almost looks as if when the necessary stimulus is given for seed germination precautions are taken to prevent general infection, the primary root even being free. In orchids digestion of the endophyte may also be a means of preventing general infection (though in *Neottia* this property can be easily recognised). Does such a general infection as we get in *Calluna* ultimately lead to saprophytism of the type seen in the Pyrolaceae? Are the events described above in the germination of certain orchids an effort to prevent general invasion and the "perfect symbiosis" of *Neottia*?

LOLIUM.

A case which recalls to mind that of mycorrhiza—especially having regard to recent discoveries—is that of the grass *Lolium*. The fact that the grains of *Lolium temulentum* contain a layer of fungal hyphae situated between the aleurone layer and the fruit and seed coat was first demonstrated by Vogl in 1898, and since then has been many times investigated in different species

* Johow (1889) places all the known saprophytic flowering plants in the six families Orchidaceae, Burmanniaceae, Triuridaceae, Piroleae, Monotropaeae and Gentianaceae. (The Triuridaceae are a small family of tropical saprophytes with the two genera *Sciaphila* and *Triuris* and about forty species.)

of the genus. The latest worker is McLennan* who used *Lolium perenne* for her researches. The fungus is far more common in the genus than has hitherto been thought, and it is remarkably constant. Every seed examined (169 of *L. temulentum* and 115 of *L. perenne*) showed infection. The fungus is endophytic, occurring within the cells. It is present in the embryo sac at, or immediately after, fertilization: thus there is a material difference from what happens in orchids and *Calluna*. The fungus increases in quantity at the expense of the nucellus and the cells of the carpel wall. As the endosperm is formed the fungus is absorbed as a source of food supply for the developing embryo. The ovum is infected before any divisions have taken place in it.

The hyphae already in the very young embryo follow the development of the stem-apex and remain localised in their growth until germination takes place. The growth of the fungus keeps pace with that of the plant: the hyphae, however, are mainly restricted to the growing apex, but can be seen extending for a short distance down the stem. Even at this stage the intracellular nature of the fungus can be demonstrated. Some of the parenchymatous cells of the grass are invaded and used as a food supply by the hyphae. When the inflorescence is formed the fungus is especially abundant at the base of the carpels. The cells so affected do not increase in size, and are only to be distinguished from normal unaffected cells by their different staining properties. It is not till the ovule is well advanced that any great increase in the fungal partner takes place. The fungus has not yet been isolated†. It has been suggested that it is probably a degenerate member of the Ustilagineae (Smuts) or of the ergot type. The former would seem the more likely. Smuts attack grasses very generally and often it is the flower that is infected and later the seed, and thus the whole plant. On general grounds it would appear that the line of development to the stage found would be the gradual subjection of a parasitic fungus such as *Ustilago* rather than the further development of a typical mycorrhiza. An examination of *Lolium* roots shows that no typical endophytic fungus is present—in fact these are peculiarly absent in the Gramineae, though recorded by Schlicht for *Holcus lanatus* and *Festuca ovina* and by Tubeuf for certain moorland grasses—and the area of infection seems limited to the region of the stem apex. Thus, though it would appear at first sight that the progress of evolution had

* E. McLennan, The endophytic fungus of *Lolium*. Part I. Proc. Roy. Soc. Victoria, xxxii (N.S.), pp. 252–301 (1920).

† Fuchs (Hedwigia, LI, pp. 221–239 (1911)) claims to have proved that the fungus is a species of *Fusarium*.

been along a line similar to the *Calluna* type leading to infection of the embryo as apart from the seed-coat, and consequent continuous infection, it is more likely that in the typically non-mycorrhizal grasses such a union has been brought about by a subjection of a seed parasite.

RELATION BETWEEN FUNGUS AND FLOWERING PLANT.

Throughout the preceding pages incidental remarks have been made regarding the relation between the two constituents of the mycorrhizal association. The subject is one of extraordinary interest and of extreme difficulty. It does not seem possible to regard all such associations as being of the same nature or as having arisen in the same way.

As we have seen, Rylands was the first to record fungi in association with roots, though his account is not very clear: his idea that the fungus performs no essential function in the economy of *Monotropa* is one that has had few supporters.

Reissek, who in many ways seemed before his time in his attitude towards the subject, regarded the regularity and permanence of the presence of fungi in orchid roots as of great importance. He apparently considered that they were not absolutely necessary for the life of the plant and suggested that the orchid could generate without the root fungus in the same way that the greater number of flowering plants are able to propagate without flowers.

The gradual realization of the dual nature of lichens brought in its train the conception of symbiosis, but the increasing knowledge as to the nature of fungus-roots played a not inconsiderable part in the growth of the idea.

From the year 1862 Tulasne began to consider the relation between the False Truffle (*Elaphomyces*) and the roots of trees as one not of simple parasitism as he had previously (1841) thought, but one by which both organisms benefited in some way. Pfeffer in 1877 took up this idea of mutual benefit and made it more precise. Other workers—Treub, Goebel, Kamienski—also regarded the relation between fungus and root as of this description. It is to the work of Frank, beginning in 1885, that we owe a proper conception of the widespread phenomenon and a clearly outlined theory of symbiosis between fungus and root. Naturally as more facts both of observation and experiment were obtained Frank's original theory was somewhat modified—originally it was that plants with ectotrophic mycorrhiza did not themselves draw nutriment from the soil, but that the mycelial filaments which completely envelop the absorbent roots procure for it all its nutriment. Such roots always lack absorbent root hairs. The absence of these organs of absorption corre-

sponding to the presence of mycelial filaments suggests that the latter take up the functions of the former. Later, the view taken was that the fungus does not necessarily nourish the roots, but draws its nutriment from the humus of the soil and passes on a portion of this to the roots. In other words the presence of the fungus allows the root to make use of certain substances of the humus that it would be incapable of utilizing in its absence. Another hypothesis which figures largely in the literature of the subject is that of Stahl*. This author endeavours to show that the rôle of the fungus consists in furnishing the plant with mineral nutriment. Comparing plants with and without mycorrhizas he points out certain differences which always appear to indicate a much greater circulation of water in the latter. Thus their roots are strongly developed, they possess numerous root hairs, their leaves transpire energetically and are often provided with water stomata. Further, their tissues are ordinarily rich in starchy matters and poor in sugar, i.e. in a condition favourable for transpiration. The fact that mycotrophic plants transpire less† and are in consequence less well fed in nutrient soils leads to the idea that the service which the fungus renders to the host consists in remedying the insufficiency of transpiration. Stahl imagines that the fungus hands over the products of assimilation of the salts rather than the salts themselves. There exists between phanerogams and fungi growing in the humus of forests, heaths, moors, etc., a competition for the salts which the vegetable débris already contains in a concentrated form. The advantage in this struggle would apparently be on the side of the fungi owing to their mode of life. Plants with very active transpiration are alone capable of struggling with success against fungi in soils rich in humus: plants with feeble transpiration are only able to subsist in these conditions by the help which their symbiotic fungus brings.

Magnus (1900) from his anatomical investigations regarded the digesting cells as serving for absorbing the nutriment of the fungus: the lodging cells, on the other hand, are set apart for the nourishment of the fungus on the cell contents and for its hibernation. This idea would give the classical balance of symbiosis—each component benefiting to an approximately equal degree.

Gallaud regards the communication of the endophyte with the exterior in endophytic mycorrhizas as insufficient to assure to the plant the absorption of nutritive substances. From a

* E. Stahl, *Der Sinn der Mycorrhizenbildung*. *Jahr. f. wissensch. Bot.* xxxiv, pp. 539–668 (1900).

† The difficulty in drying orchid plants for herbarium purposes is a result of this.

study of numerous types of infection he holds that the fungus when in the root leads a life independent of the exterior and that it must therefore obtain all its nutriment from the plant. Comparing its mode of life with that of fungal parasites such as *Peronosporaceae* he decides against its parasitic nature and regards it as a special form of saprophyte—an internal saprophyte.

Ternetz working with the fungi from *Ericaceae* records as a result of careful experiments that they are able to fix free nitrogen. From a theoretical point of view this is of extreme interest, fitting in well with what is known concerning the bacteria in the root nodules of the *Leguminosae*, but so many discordant results have been recorded in such studies that it would be well not to accept these without confirmation. Incidentally it may be again remarked that Burgeff was unable to show any such fixation in orchids.

Owing to the totally different complexion that Bernard's work put upon the mycorrhiza question, his views are of particular interest. He regards the fungus in orchids as a parasite: an orchid suffers from a benign cryptogamic malady. Symbiosis for him represents the immunity realized by phagocytosis.

Burgeff on theoretical grounds considers that both orchid and fungus must benefit by increased power of reproduction. He is in general agreement with Stahl as to the nature of the benefit the flowering plant receives. The union arose originally from the ability of the fungus to take up carbon compounds from the soil. The function of the fungus in germination is to introduce a solution of carbohydrates into the seed by means of its enzymes.

Most recent workers on ectotrophic mycorrhizas regard the fungus as parasitic. Fuchs* attempted to inoculate the roots of *Abietineae* by adding fungus spores to the soil. He did not succeed in his experiments, but regarded the vehemence with which the young plants cut off the infected cells as an effort to prevent the attacks of a parasite.

Weyland† introduced the microchemical method of studying the question and it is probable that from such studies a clearer idea of what is really taking place will be obtainable, by the determination of the localization of nutriment. He considers that the fungus in an ectotrophic mycorrhiza is really a parasite and has nothing to do with symbiosis.

* J. Fuchs, Ueber die Beziehungen von Agaricineen und anderen humusbewohnenden Pilzen zur Mycorrhizenbildung der Waldbäume. *Bibliotheca Botanica*, LXXVI (1911).

† H. Weyland, Zur Ernährungsphysiologie mykotroper Pflanzen. *Jahr. f. wissensch. Bot.* LI, pp. 1-80 (1912).

Weevers* working from a chemical point of view on the presence of ammonia and ammonium salts in plants established the fact that although ammonium salts were found in abundance in the tubercles of the Leguminosae they were in small quantity or absent in mycorrhizal plants. He holds therefore that if fungus-roots really assimilate nitrogen it must be brought about in a manner different from that in the Leguminosae. Weevers is rather of the opinion that mycotrophic plants are, with the help of their fungus partner, able to utilize fully the organic compounds of the soil.

McDougall†, working with ectotrophic mycorrhizas of forest trees formed by the association of toadstools with the roots, considers that they are not in any sense symbiotic associations but must be considered as instances of parasitism by the fungi.

Rexhausen‡ studied ectotrophic mycorrhizas by the micro-chemical method. He considers that the fungus and the root together form an osmotic unit for the absorption of nutrient salts. These are probably made soluble for the root by the fungus. This gathering up of nutrient salts is first used by the fungus for its own benefit. The mycorrhiza is not a fixed symbiotic condition, but is dependent upon the biological condition of the soil. Where the conditions are not suitable for the growth of the fungus it acts as a parasite on the root and may damage it severely, as it cannot be kept in check. Where the fungus is well nourished it can be easily withstood by the root. Thus in good soils the mycorrhiza gradually disappears or, at all events, the fungus part becomes less.

It will be apparent from the above that many somewhat diverse theories have been put forward to account for the fungus-root association and many modifications have been proposed. No purpose would be served here by entering on a detailed criticism: the only general one we would suggest is that no benefit can result from pushing the old idea of mutual and equal advantages of the two components to its extreme. Referring only to orchids it seems most reasonable to regard the condition as having arisen from parasitic attacks by the fungus. This seems beyond doubt in the exceptional case of *Armillaria* and *Gastrodia*. The ability of the fungus to transport nutrient solutions has been made use of by the flowering plant. As in the case of Leguminosae and their nodules the tables have been

* T. Weevers, Das Vorkommen des Ammoniaks und der Ammonsalze in den Pflanzen. Recueil des Travaux botaniques Néerlandais, XIII, pp. 63-104 (1916).

† W. B. McDougall, On the mycorrhizas of forest trees. American Journ. Bot. 1, pp. 51-74 (1914).

‡ L. Rexhausen, Über die Bedeutung der ektotrophen Mykorrhiza für die höheren Pflanzen. Beit. z. Biol. der Pflanzen, XIV, pp. 19-58 (1920).

turned and the "host" has become the aggressor, even attracting the fungus to the embryo. We are short of definite facts—there is a conflicting mass of detail on such an important point as the relation between the endophyte and the soil—and until these are obtained one theory seems as good as another.

It would be indeed strange if the difference between ectotrophic and endotrophic mycorrhiza should resolve itself into a case of the fungus being parasitic on the flowering plant in the former, while in the latter the flowering plant is parasitic on the fungus.

I am indebted to Mr E. H. Ellis for the photomicrographs, with the exception of Figs. 1 and 4, for which I must thank Mr R. J. Tabor.

While the above was in the press an important paper by H. Christoph entitled "Untersuchungen über die mykotrophen Verhältnisse der 'Ericales' und die Keimung von Pirolaceen" appeared in Beih. Bot. Centralbl. xxxviii, pp. 115–157 (1921). In it the author controverts the results obtained by Rayner concerning the necessity of the root-fungus for seed-germination (cf. p. 48). It should be noted, however, that he has not seen the full description of Dr Rayner's researches, but apparently only an abstract of her preliminary account. Christoph concerned himself with the manner in which the fungus reaches the roots of the Ericaceae, whether from the soil or from the seed coat. His first series of experiments were performed with cuttings. He took both large and small green side shoots from plants of *Calluna vulgaris* both wild and cultivated. These were planted in shallow pots in humus heath soil—the soil in the one pot being sterilized and that in the other not. In both experiments a number of cuttings struck and succeeded in establishing themselves. The roots of the cuttings in unsterilized soil became slightly infected, but no fungus could be found in those growing in sterilized soil. On replanting and transferring the latter cuttings to sandy soil they still remained free from fungal infection and continued in that condition for two and a half years.

Similar experiments with cuttings of *Erica carnea* gave analogous results. Both series succeeded and those planted in sterile black heath soil, and after one and a half years transferred, remained free from fungus infection for two and a half years.

The plants without fungi in their roots were in just as good a condition as those which became infected and Christoph is of the opinion that the fungus is of no assistance to the plants and must be regarded as a harmless parasite.

A second part of the paper deals with germination experi-

ments with these two species. The results of thirteen experiments are summarized, though the complete account is not published.

Different soils were tried, both sterilized and unsterilized. Seeds of *Calluna* and *Erica* were sown in these, some having their coats sterilized, some being used just as they were taken from the capsules. The results were similar in both series of experiments, except that *Erica carnea* germinated only in the absence of light. Germination occurred in all experiments, e.g. sterilized seeds germinated in sterilized soil. Only those seedlings growing in unsterilized soil become infected with fungus whether the seeds are sterilized previously or not: in certain cases seeds which were taken from capsules in which a fungus was very obvious did not give rise to infected seedlings when sown in sterilized soil. The author concludes that infection of the root always comes from the soil and never from the seed coat.

Regarding infection in the capsule, Christoph states that so long as the carpels are still green and the seed white a fungal infection of the tissue can never be observed.

The author succeeded in extracting the fungus from the roots of the plants, but was unable to obtain spores in pure culture and was therefore unable to identify it. That it was probably the appropriate fungus was shown by infecting seedlings of both *Calluna* and *Erica*.

The Ericales are considered to be facultative mycotrophic plants, since specimens growing in normal conditions always have fungus in their roots. In very dry places, however, plants of *Calluna vulgaris* and of *Erica carnea* are often without fungi; and in pot cultures allowed to become dry the fungus soon disappears.

The third portion of the paper deals with the Pyrolaceae. Working with *Pyrola uniflora*, *P. secunda*, *P. minor* and *P. rotundifolia* it was found that the hyphae of the infecting fungi had clamp-connections (and were therefore probably Basidiomycetes). The conclusion reached is that here also no true "symbiosis" exists—infection depends upon many external factors, of which temperature, soil, moisture and aeration are the chief. Coralloid roots are not brought about by infection: there is a special development of the large epidermal cells and these, owing to their function of absorption, are specially suited for fungal development.

In *Monotropa* the fungus possesses no clamp connections.

The author was successful in germinating seeds of *Pyrola rotundifolia* which he chose, as they were the largest of the four species. The best results were obtained from: 1. Strong concentrated soil-extract. 2. Addition of peptone solution. 3. Sowing

on humus from habitat of plant—on sterilized soil there was no germination. 4. Keeping cultures in the dark. 5. Moderate moisture.

It is suggested that the carbon compounds of the highly concentrated soil extract, acting in combination with the peptone, brought about germination by chemical action.

Parallel experiments with peptone solution alone, soil extract alone, and with a mixture of both gave a slight germination in peptone solution, a stronger one in soil extract, but much the best is a mixture of the two.

With regard to the question of infection of the seedling root from the capsule it is obvious that there is great discrepancy between the accounts of Rayner and Christoph, and until the results of one or other worker be confirmed it is not possible to draw from them theoretical conclusions. That cuttings of *Calluna* can strike and come to maturity in sterilized soil without root infection is somewhat unexpected on account of Rayner's clear description of the distribution of the fungus in the plant; in cultivated orchids it is quite likely that after the seedling stage fungal infection is not necessary.

Concerning the germination of *Pyrola rotundifolia* seeds the account is not full enough to draw from it any theoretical conclusions. The fact that the seeds can be brought to germinate by chemical means is not surprising: it is analogous to what has been found by Bernard in *Cattleya*. There was apparently no attempt made to try the effect of the root-fungus on germination.

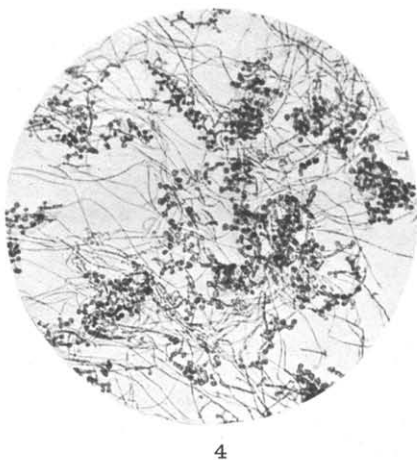
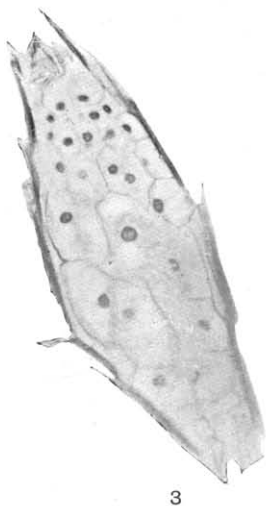
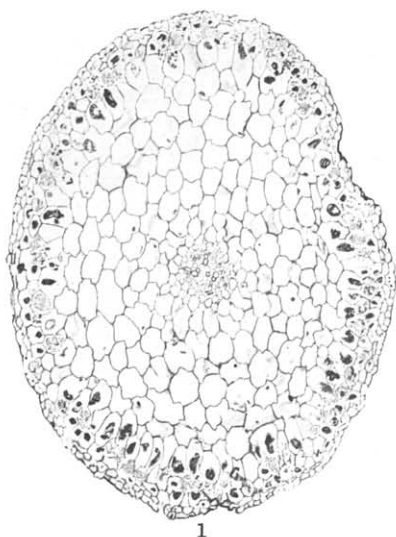
EXPLANATION OF PLATES.

PLATE II.

- Fig. 1. Transverse section of root of *Habenaria* just above the root tip. The dark masses show where digestion of the fungus is taking place. $\times 36$.
 Fig. 2. Seed of *Cymbidium*, stained and mounted whole. The embryo is seen as an oval black patch within the network integument. $\times 56$.
 Fig. 3. Longitudinal section of a seed of *Odontoglossum*. The anterior end shows smaller cells, the posterior end larger cells. (The integument has been ruptured in making the preparation.) $\times 215$.
 Fig. 4. Fungus from *Odontoglossum* (*Rhizoctonia lanuginosa* Bern.) at the beginning of sclerotium formation. $\times 36$.
 Fig. 5. The same more highly magnified showing chains of "spores." $\times 215$.

PLATE III.

- Fig. 6. Seed of *Odontoglossum* sown seven days on a culture of the fungus: stained and mounted whole. $\times 56$.
 Fig. 7. The same more highly magnified. $\times 215$.
 Fig. 8. Longitudinal section of a seed of *Odontoglossum* nine days after sowing. The fungus has entered the larger cells at the suspensor end of the seed and formed balls of hyphae. (The integument has been broken in cutting the section, cf. Fig. 6.) $\times 215$.
 Fig. 9. Section of protocorm of *Odontoglossum*. The growing point of the stem can be seen at the upper end and the first and second leaves (Section

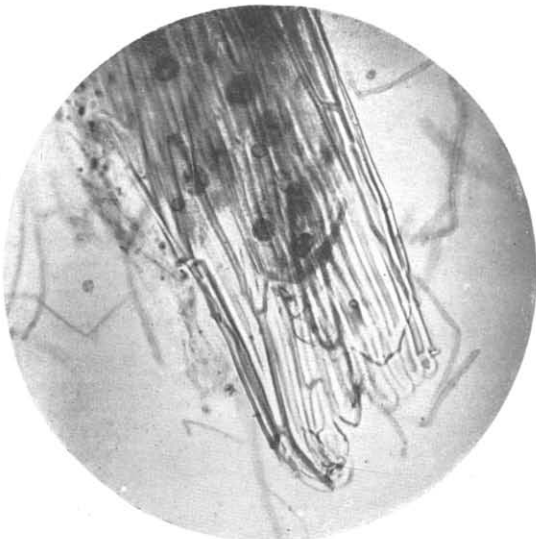




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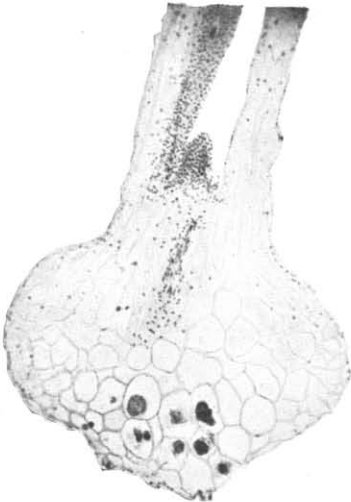
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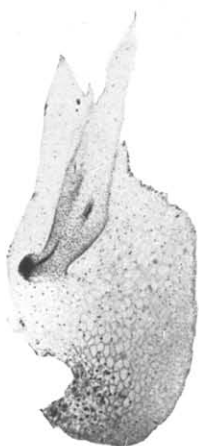
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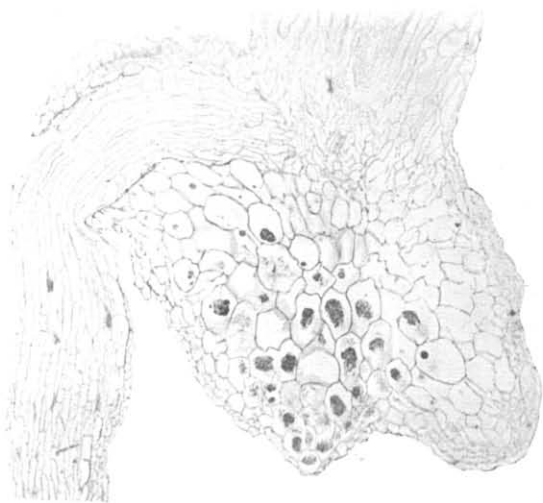
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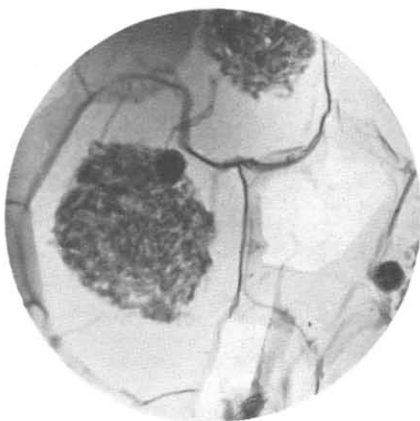
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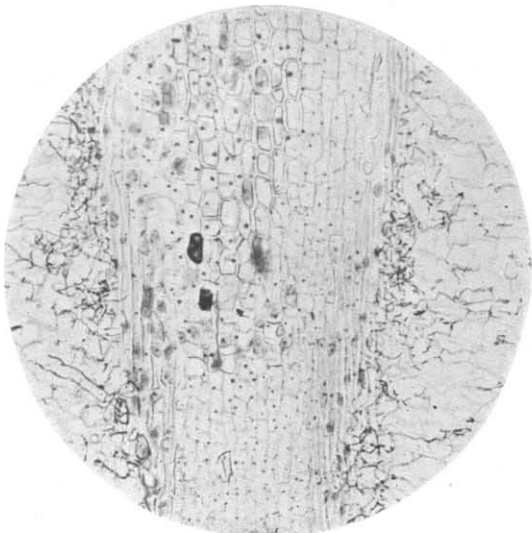
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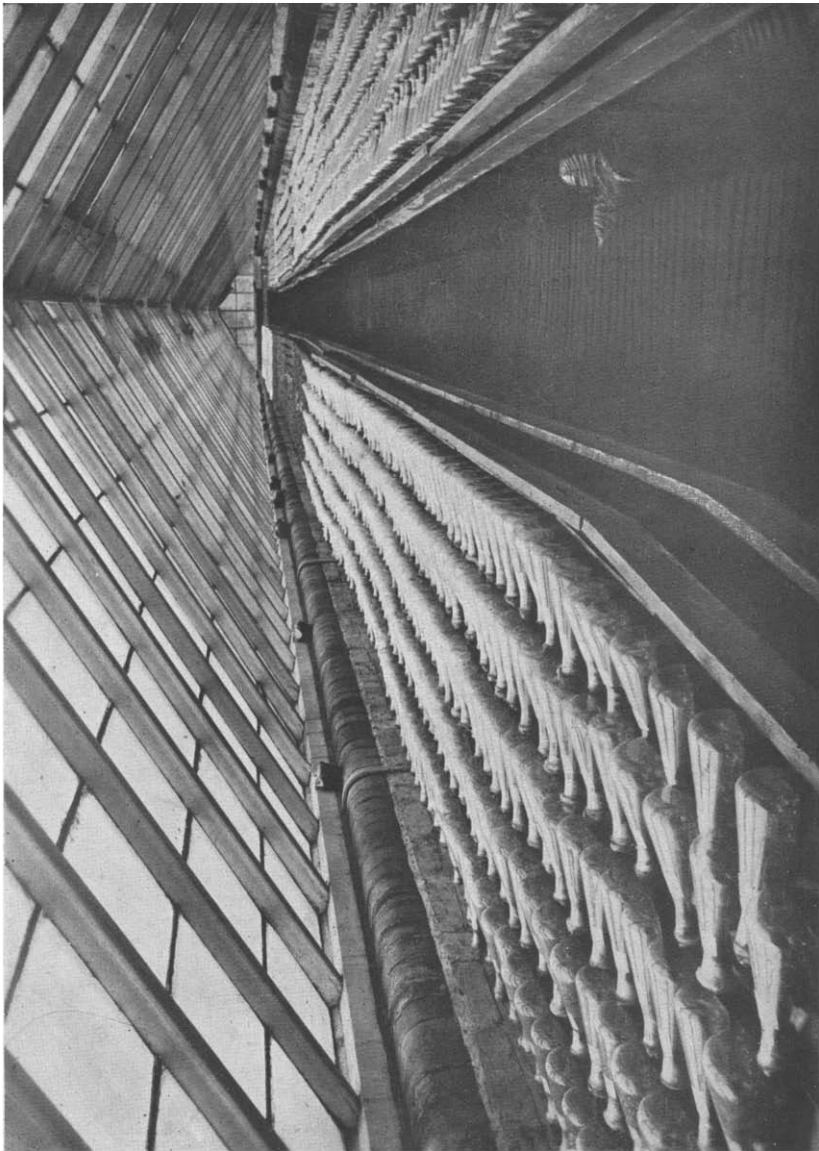
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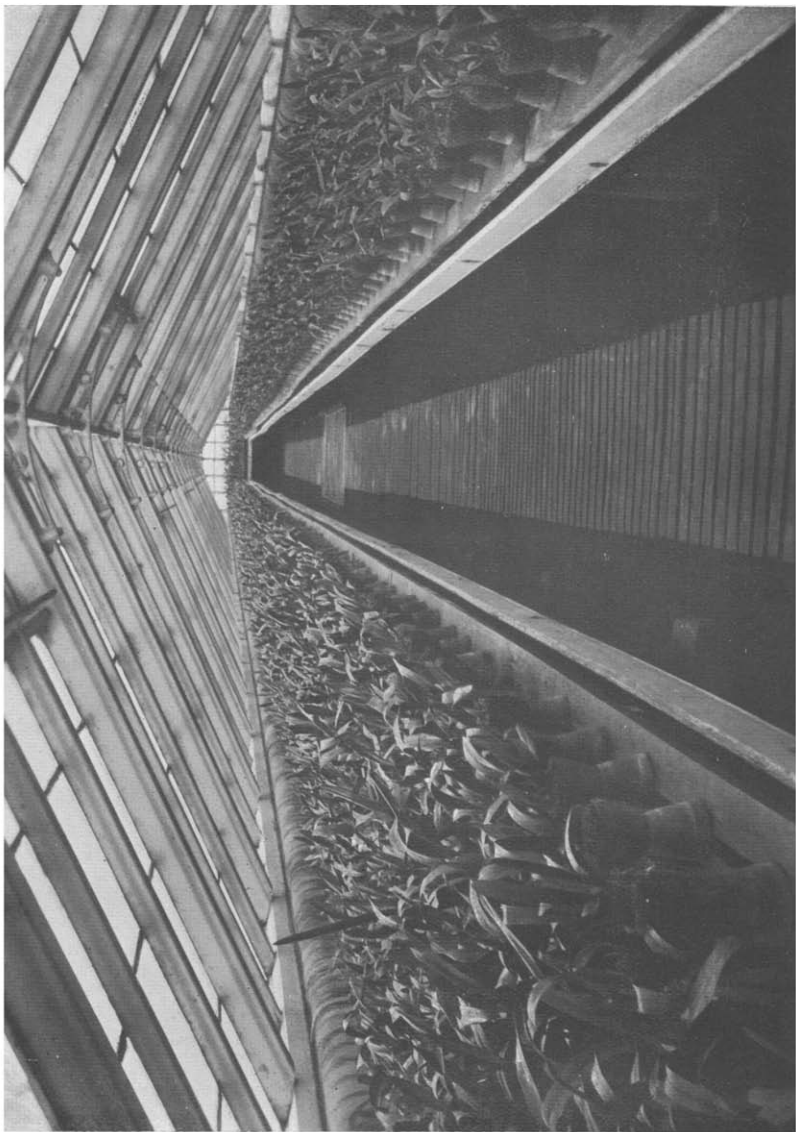
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not quite median). The fungus in many of the cells is already digested. $\times 56$.

- Fig. 10. Later stage of *Odontoglossum* showing the beginning of the formation of the central stele and root. The stem growing-point is well developed. The fungus in the cells is mostly digested. $\times 36$.

PLATE IV.

- Fig. 11. Protocorm of *Vanda* showing the young root absorbing its way out of the side of the protocorm away from the fungal zone. $\times 18$.
 Fig. 12. *Odontoglossum* seedling after the formation of the first root. The root is not infected from the protocorm. (The dark patches in the root are raphides.) $\times 36$.
 Fig. 13. Cells from fungal zone of Fig. 12 more highly magnified. The fungus is "clumped" (cf. Fig. 8). A fungal hypha is seen passing through the cell-wall. $\times 215$.
 Fig. 14. Longitudinal section of the root of *Habenaria* near the tip. Digestion is more prominent in the older (upper) portion of the root. $\times 18$.
 Fig. 15. Longitudinal section of an aerial root of *Epidendrum* showing infected cells in the centre and mycelium in the velamen. $\times 18$.

PLATE V.

Odontoglossum house; first stages of seedling raising by the "pure culture" method.

PLATE VI.

Odontoglossum house; seedlings one year old grown by the "pure culture" method.

PLATE VII.

Odontoglossum house; seedlings three and four years old.

MYCORRHIZA IN THE ERICACEAE.

By M. Cheveley Rayner.

There has recently been published a paper by Christoph⁽¹⁾ containing an abridged account of researches carried out by him on the relations between plant and mycorrhizal fungus in the Ericaceae. The species studied were *Calluna vulgaris* and *Erica carnea* and that part of the investigation with which the present critical review deals is concerned with (a) the behaviour of "cuttings" of the shoot, and (b) seed cultures of the former plant.

With the researches on "cuttings" I do not propose to deal at present as it is hoped that a paper may be ready for publication in the near future detailing the results of my own experiments in the same direction. With regard to the work on seed cultures of *Calluna*, the experimental results recorded by Christoph are so remarkably at variance with those published in 1915⁽²⁾ and since that time repeatedly confirmed by me, that I welcome the present opportunity of discussing them in some detail.

As a starting-point of my own investigations may be taken the simple observation that seed of *Calluna* germinated on moist filter paper in a germinator yields seedlings which root freely in the seed dishes, the roots showing regular fungal in-