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ON THE MYCORHIZAS OF FOREST TREES*

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I. INTRODUCTION

The study of mycorhizas has engaged the attention of numerous investigators since the appearance of Frank's (4) first paper on the subject in 1885. Altogether some sixty or seventy papers have already appeared in print dealing with the subject as a whole, or with certain phases of it. Gallaud (6) has given a very good historical résumé of the work done on mycorhizas up to 1904. It will therefore not be necessary to go into details here. Gallaud recognizes three periods in the study of mycorhizas. The first of these extends from 1840 to 1885. During this time several authors noted the presence of fungi in connection with the roots of various plants, but with very few exceptions they made no special study of them. The second period extends from 1885 to 1894, and its beginning is marked by Frank's work. Frank was really the father of mycorhiza study. He first demonstrated the true morphological nature of ectotrophic mycorhizas and applied the name to them. He also advanced the hypothesis that the fungi in question are symbiotic with the roots of the higher plants. Under the influence of this new idea numerous investigators attempted to verify or refute Frank's work, but with little success. During the third period, from 1894 to 1904, the important papers were mainly of two kinds, systematic and cytological. The systematic papers did a great deal toward extending our knowledge of the distribution of mycorhizas, while, in connection with these, and

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with the cytological papers, which dealt entirely with endotrophic mycorhizas, numerous theories were advanced as to the relation between the host plant and the fungus.

Since the publication of Gallaud's (6) extensive work on endotrophic mycorhizas, several important papers of varying nature have appeared. Bernard (1) in 1909 published his excellent work on the endotrophic mycorhizas of the orchids. This is the most complete and satisfying work yet done on the mycorhizas of any group of plants and is to a large extent verified by the work of Burgeff (2) which appeared a few months later. Other papers will be mentioned farther on.

It is a notable fact that all of the more extensive papers have dealt principally with endotrophic mycorhizas, and that no very intensive work has been done on ectotrophic forms. Mangin (13) in 1910 published a paper dealing with the ectotrophic mycorhizas of forest trees, but it is of an introductory nature, and the figures which accompany it are very diagrammatic.

The work on which the present paper is based was carried on at the University of Michigan during 1911 and 1912. The primary object from the beginning was to work out the seasonal relations of the mycorhizas of our forest trees. During the course of the work, however, a number of other interesting facts were brought to light and they will be presented here. I am indebted especially to Professor F. C. Newcombe, under whose supervision the problem was worked out, for invaluable advice and suggestive criticisms throughout the course of the work. I am also indebted to Professor C. H. Kauffman for his constant interest in the work and for assistance in dealing with the mycological side of the subject.

II. MATERIAL AND METHODS

1. Species of Trees Studied

The species of trees of whose mycorhizas the most extensive study was made are *Carya ovata* (Mill.) K. Koch, *Quercus alba* L., *Tilia americana* L., and *Betula alba* var. *papyrifera* (Marsh.) Spach., which were found to have ectotropic mycorhizas, and *Acer saccharinum* L., and *Acer rubrum* L., which have endotrophic mycorhizas. Some work was also done, for purposes of comparison, on *Larix laricina* (Du Roi.) K. Koch., *Quercus rubra* L., *Quercus velutina* Lam., *Populus grandidentata* Michx., *Fagus grandifolia* Ehrh., *Ostrya virginiana* (Mill.) K. Koch., and *Carpinus caroliniana* Walt., all of which produce ectotrophic mycorhizas, and *Acer saccharum* var. *nigrum* (Michx. f.) Britton, *Juglans nigra* L., *Crataegus* sp., and *Aesculus Hippocastanum* L., which bear endotrophic mycorhizas. No mycorhizas were found on *Cornus florida* L., *Ulmus americana* L., *Sassafras variifolium* (Salisb.) Ktze., and several species of *Salix*. These trees all occur in the woodlots in the vicinity of Ann Arbor, Michigan, or at the Forestry Farm 3 miles west of Ann Arbor.

2. Collection of Material

The collection of material was begun July 1, 1911, and continued till December, 1912. Throughout the warmer parts of the year some collecting was done every week, while during the winter collections were made less often, but specimens were obtained from each species every month, the object being to get series of specimens, extending throughout the whole twelve months, from individual trees of each species. Altogether one hundred and twenty-five collections were made. Great care was taken always to get reliable specimens. As is well known the mycorhizas occur only on the smallest rootlets of the tree and in the more superficial layers of the soil. In a mixed forest, where there are dozens of kinds of roots within a small area, it is oftentimes by no means easy to know to what tree the roots one is working with belong. In the case of a tree with the habits of Acer the task of collecting reliable specimens is not a very difficult one, because large roots of the maple often extend for considerable distances along the surface, or just beneath the surface, of the soil, where they can be easily followed. These always give off numerous short branches which can be easily traced to their ends, where mycorhizas are likely to be found. In the case of such trees as Quercus or Carya, however, it is much more difficult. On these trees the roots are usually interwoven and tangled together, so that it is very difficult to follow one for any great distance. Moreover, the large roots of these trees usually grow out only a few feet from the base of the tree when they turn downward into deeper soil, and it is more distant branches from these whose ends bear mycorhizas, often fifteen or twenty or more feet from the tree. These same trees, however, often give off short roots directly from the base of the trunk, or from the large roots near the trunk, and these very often yield an abundance of mycorhizas. When these can be found they are, of course, easily obtained. When they cannot be found, the only way of obtaining reliable specimens is to go some distance from the tree, dig until mycorhizas are found, and then, after collecting the specimens, carefully trace back the root until it is determined to what tree it belongs.

As equipment for the work of collecting I usually carry a spade, a garden trowel, and a small hunter's axe, besides a knapsack for holding a notebook and bottles for specimens. The specimens, when collected, were either put at once into 1 per cent chrom-acetic acid, or carried to the laboratory in water, and then killed in the acid. During the winter, when the soil was frozen, solid pieces of soil were chopped out with the axe, and those pieces that were thought likely to contain small roots, and, therefore, possibly mycorhizas, were taken into the laboratory and thawed out, when the mycorhizas, if present, could be picked out. Specimens collected in this way were always taken from places where the roots had been identified and their positions marked during autumn. In preparing specimens for study they were embedded in paraffin, sectioned by the microtome, and stained with gentian violet and fuchsin.

3. The Glass Plate Method of Study

Besides the method of study just outlined, the mycorhizas were studied as they grew in their natural habitat by the following method: the humus and leafmold were scraped away from a small area until some mycorhizal roots were uncovered. A round glass plate about eight inches in diameter was then placed over these roots, pressed down firmly, and covered with soil. Care must be taken that no air-spaces are left beneath the glass, and the glass must be well covered; otherwise the roots will wither and die. It is usually necessary to remove the glass when an observation is to be made.

III. OBSERVATIONS AND EXPERIMENTS

A. Ectotrophic Mycorhizas

1. *Descriptive*

Ectotrophic mycrohizas are easily recognized by the characteristic clusters of numerous, short, stubby branches which have been described as coral branching rootlets (fig. 1). When alive, they always have a bright, fresh appearance, even when collected in midwinter. They vary in color from white to bright yellow, brick red, or dark brown. They also vary greatly in the character of the external surface and internal structure. The different forms will therefore be described separately, as follows:

Form 1.—This form is found on *Carya ovata* and is, when fresh, bright yellow in color. A superficial examination with the binocular microscope, or a good pocket lens, reveals a network of mycelium over the surface and numerous short hyphae projecting out from it. A cross section of this mycorhiza (fig. 2), shows the following parts, beginning at the center: (1) The central cylinder; (2) about three circular rows of ordinary cortical cells; (3) a row of radially elongated cells. This row, however, does not extend clear around the section, but, on one side of the section, its cells are cut up into a number of irregularly shaped cells. The cells of this row are entirely separated from each other by fungus tissue which surrounds each cell on all sides. (4) Finally there is a layer of fungous filaments surrounding the section. From the outer part of this layer, which forms a mantle over the entire rootlet, tip and all, there are given off numerous filaments which project out into the surrounding soil. From the inner part of this fungus layer there are given off the filaments which surround the radially elongated cells as described above.

Form 2.—A second form, found also on *Carya ovata*, is brown in color when fresh. In microscopic section the same general parts are distinguished as in the form described above. The structural difference is concerned principally with the fungus mantle (fig. 3.) This instead of being made up of easily distinguishable filaments, consists of a pseudoparenchymatous tissue, such as is found in many lichens. The outer surface of this mantle is smooth; that is, there are no hyphae, or very few, projecting out into the soil. From the inner part of the mantle, hyphae extend inward between the cells of the root as in the former case. In the latter case, however, they often extend in farther than the single external layer of cells, and they are all divided up into short cells, so that the pseudoparenchymatous character is observed here as well as in the mantle.

Form 3.—Still another form, also found on *Carya ovata*, is white when fresh. The fungus mantle, in this case, is distinctly filamentous, but the outer surface is smooth as in the last described form (fig. 4). The root istelf and the fungus parts projecting into the root are practically of the same structure as in the first described form.

Form 4.—A fourth form found on *Quercus alba* is white when fresh. The clusters often lie more or less in one plane, as if they had been pressed, and they appear more or less wooly. The filamentous mantle, in this case, is very loosely constructed, and is easily torn and broken up while the microtome sections are being prepared.

Form 5.—A form collected from *Larix laricina* is brown in color and the thick pseudoparenchymatous mantle is smooth on the outside (fig. 6). There are no radially elongated root cells, but the outermost cells of the root have been crowded apart by the fungus until some of them are isolated as islands far out in the fungous mantle. The fungus penetrates nearly to the central cylinder so that nearly all of the cortical cells are entirely separated from each other.

Form 6.—A form found on *Tilia americana* may be called a *heterotrophic* mycorhiza. In external appearance, and in internal appearance, too, for that matter, it looks like an ectotrophic mycorhiza. It has a semi-pseudoparenchymatous fungous mantle which is smooth on the outside. It also has the radially elongated external layer of root cells, characteristic of so many ectotrophic mycorhizas, entirely surrounded and separated from each other by the fungous tissue. The novel thing about this mycorhiza is that, occasionally, the filaments, which extend in between the root cells, enter the next cells beneath, and there appear very much like endotrophic filaments (fig. 7).

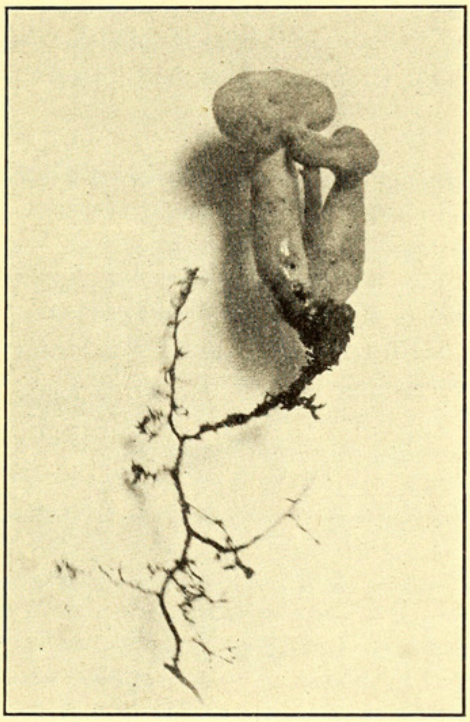
All forms of mycorhizas described above agree in containing no secondary growth. Also no fungous hyphae are ever found in those parts of a root that are not covered by the fungous mantle, so that, if two or more branches of the same root are transformed into mycorhizas, there can be no connection of the fungus of one of these mycorhizas with that of another except through the soil.

2. *Mycorhizal Fungi*

On August 6, 1912, I found the ground around the Tilia, from which I had been collecting regularly, fairly covered with the sporophores of a Russula. On digging up these mushrooms, twenty-two in number, a cluster of fresh young mycorhizas was found immediately below each one, while in three separate cases an actual connection between the mycelium of the mushroom and that of the mycorhizas was easily demonstrated (Text-fig.1). These mushrooms were found only on the area which might be occupied by the roots of the Tilia in question. In searching over the greater part of the woods, only one other specimen was discovered. That one was near another Tilia, and when it was dug up a cluster of mycorhizas was found immediately beneath it.

I have not been able yet to determine the species of this Russula, and it may be an undescribed species. It is a small red mushroom, and it is very acrid. Evidently it belongs to the same group as do

Russula emetica Fr. and *Russula fragilis* (Pers.) Fr., yet it differs from both of these. It is not so fragile as *R. fragilis* and the cuticle does not peel off so easily. The gills are more crowded than in *R. emetica.* Most of all, it differs from these two species in the color of the spores, which in *R. emetica* and *R. fragilis* are white, while in this mycorhiza-forming species they are creamy. A peculiar thing about this Russula



TEXT-FIG. I. Sporophore of Russula attached to mycorhizal root.

is that the sporophores are very apt to be deformed. Whether this is due to the presence of a parasite has not been determined. After this first crop of fruiting bodies had disappeared only four specimens were found during the rest of the season. The same form has been collected several times, however, in another locality, by Professor Kauffman.

On August 12 a dozen sporophores of *Boletus scaber* Fr. var. *fuscus* were growing under the Betula from which I had made regular collections of mycorhizas. When these were dug up, evidence was found in every case, of a connection with the mycorhizas, and in several cases an actual connection was easily seen. Later in the season another crop of these mushrooms was found in the same place, and their connections with mycorhizas were again verified. At the same time, and under the same tree, a considerable number of sporophores of Cortinarius sp. were found, and their connection with mycorhizas was demonstrated.

Finally, on the 16th of September, 1912, a fourth mycorhiza producing fungus was found, this time a puff-ball, *Scleroderma vulgare* Fr. This fungus causes the white mycorhizas which were described above as Form 4, and which occur on *Quercus alba*. A large number of the fruiting bodies were found and the pure white rhizomorphs made it comparatively easy to trace the hyphae from the mushroom to the mycorhiza.

3. *Number of Species of Fungi which may Cause Mycorhizas on the Same Tree*

The mycorhizas of Betula, caused by Cortinarius and Boletus, are easily distinguished from each other, since those caused by Cortinarius are brown, while those caused by Boletus are white. They differ also in the structure of the fungous mantle. In the Boletus-Betula mycorhizas the mantle is very compact and smooth on the outside, as in fig. 4, while in the Cortinarius-Betula mycorhizas the mantle is of a rather loose filamentous structure, and has hyphae projecting out into the soil (fig. 5).

If, now, on other trees, mycorhizas which differ greatly in color and structure are found, it may safely be concluded that they are caused by different species of fungi. Such a case was observed on *Carya ovata*. The first three mycorhizas described in this paper were all found on the same individual Carya, and differed from each other both in color and structure. Moreover, all three forms do not occur on this tree every year. In 1911 all three forms were found, the yellow form being much the most abundant; but in 1912 the yellow form was not present at all, while the white form was very abundant. The brown form was found both years in about the same numbers.

On *Quercus alba*, again, four forms of mycorhizas were found on the same tree, one of which differs from the other three in color, and all of which differ from each other in structure. Three of these are brown in color and correspond in structure to the first three forms described above. The fourth is the one which was shown to be caused by *Scleroderma vulgare*, and, since the mycelium of this fungus is pure white, it is not possible that it could be the cause of any of the brown forms. There is no doubt, then, that these four Quercus mycorhizas are caused by four different species of fungi.

4. *The Element of Chance in Mycorhiza Formation*

That the transformation of a root into a mycorhiza depends, to a large extent, on chance conditions is shown by the following observations by the glass plate method. Six plates were placed on ectotrophic species of trees, and numbered 1 to 6. Numbers 1 and 2 were on *Tilia americana;* numbers 3 and 4 were on *Betula alba* var. *papyrifera;* and numbers 5 and 6 were on *Carya ovata.* They were all placed during the month of May, 1912, and the roots under each were diagrammed on paper. Plates no. 4 and no. 5 gave no results, because air spaces were left beneath them and the roots dried up. Of the mycorhizas produced under the successful plates the longest was four millimeters and the shortest two millimeters. The lengths of the normal roots produced are given below. There was no indication in any case of the formation of a mycorhizal cluster, all mycorhizas produced being simple and unbranched. The results were as follows:

Plate No. 1, on Tilia.—Nine new rootlets were produced between June 1 and July 15. All of these had practically the same appearance, and there was no reason for supposing that their fates would be different. Four of them were dead before the end of July. The other five remained alive throughout the season. By the first of October two of these had developed normally and had attained lengths of 4.2 and 7.1 cm. respectively, while the other three had developed into typical mycorhizas.

Plate No. 2, on Tilia.—Four new rootlets were produced. Two of them became mycorhizas, and two of them developed as normal roots with lengths of 3.2 cm. and 4.1 cm.

Plate No. 3, on Betula.—Three new rootlets were produced. One of them became a mycorhiza, and two of them developed as normal roots with lengths of 2.5 cm. and 4.9 cm.

Plate No. 6, on Carya.—Three new rootlets were produced and all of them became mycorhizas.

5. *Development of a Mycorhiza*

Apparently the development of a mycorhiza takes place, in the early stages, very rapidly. Probably only a day or two is needed for the formation of a complete mantle and the development of more or less of the "lichen structure," although a considerably longer time is required before the fullest development of the mycorhiza is reached.

For this reason it is very difficult to find specimens of the early stages. The best material for this study was obtained on the seventeenth of August from *Tilia americana.* A section from some of this material is shown in fig. 10. The mantle in this case is still very thin, having the thickness of from two to four hyphae, and it is incomplete, not yet having covered the tip of the root. This shows that infection cannot have taken place at the tip, but must have taken place at some point farther back. Further, there is not yet any "lichen structure" present. In only a few places have the hyphae begun to penetrate between the cells of the root. The formation of the mantle and that of the lichen structure are going on more or less simultaneously although the mantle is in advance. The outer portion of the epidermal wall of the root can still be seen on the outside of the fungous mantle. The fungus has penetrated the epidermal wall at some point back of the root tip, split the wall by dissolving out the middle lamella, just as it does when it penetrates between the cells of the root, and spreads in all directions. Finally a complete mantle will be formed and the outer portion of the epidermal wall will be cast off entirely. Since these mycorhizas are never more than a few millimeters in length, seven or eight at most, and more often only two or three, it is evident that the root does not grow in length after a complete fungous mantle has been formed.

6. *Seasonal Relations of the Mycorhizas*

Beginning my collections about the first of July, I found that throughout the months of July and August good fresh specimens were rather scarce, although the old, black dead ones were very plentiful. Throughout October, November and December good, well-developed specimens were very abundant on all trees studied. This condition persisted during January, February and March, although these were very cold months, and the soil in which the specimens were found was frozen solid. By the middle of April the mycorhizas of Quercus and Carya had begun to die off, and good specimens were becoming rather scarce, although they were still very abundant on Tilia and Betula. By the middle of May those on the two last named species were also getting rather scarce. Throughout June and the fore part of July they were very scarce on all species. In August they began to be more plentiful again, and, by September, they were as abundant as in September of the previous year.

7. *Time of Fruiting*

As far as my observations go, it would seem that the fruiting bodies may be looked for any time after the first of August. In all cases of mycorhiza producing fungi reported in this paper, the fruiting bodies were produced relatively soon after the rootlets were infected.

B. Endotrophic Mycorhizas

1. *Descriptive*

The endotrophic mycorhizas of *Acer saccharinum* and *Acer rubrum* have a very characteristic appearance and are easily distinguished from the normal roots. They are constricted at intervals so that they have a beaded appearance (fig. 12). The beadlets are seldom more than one millimeter in diameter, and usually less than that. A mycorhiza may consist of only one such beadlet or there may be as many as six or more. The cortical cells in these swellings are enlarged much beyond the normal size, so that, in section, the cortical cells in the constriction appear to be greatly compressed (fig. 14). In some cases, only occasional cells contain the fungous filaments, while in other cases nearly every cell of the cortex is infected. I have never seen any of the filaments within the cells of the central cylinder. The filaments are often seen curled about the nucleus (fig. 16), but in other cases they do not appear to bear any relation whatever to the nucleus. They are often seen to pass through the cell wall from one cell to another (figs. 16 and 19), but they never split the cell walls and follow them as do the ectrotophic filaments. Peculiar swellings and various modes of branching are observed (figs. 15 and 18). The vesicles described by several authors are occasionally seen (fig. 17). These are large swellings, usually at the ends of the hyphae, and have thickened walls. The significance of these modifications is not known. Usually when only a few cells of the rootlet are occupied by the endophyte, root hairs are present in variable numbers; but root hairs are entirely lacking in those mycorhizas in which nearly all cells of the cortex are infected.

2. *The Chance of Mycorhiza Formation*

As in the case of ectotrophic mycorhizas, so here the infection of a rootlet depends on chance conditions. The glass plate method of observation did not prove as satisfactory in this case as with ectotrophic mycorhizas, but one of the plates did give some results. Under this plate, which was placed on *Acer saccharinum*, four new rootlets were produced. One of these became infected and developed into a typical mycorhiza consisting of two beadlets, while the other three developed normally and attained lengths of 2.8 cm., 3.1 cm., and 3.4 cm. respectively.

3. *The Infection of Rootlets*

A few sections were obtained in which the fungous hyphae were found within root hairs, or extending from root-hair cells into adjacent cells (fig. 19). This indicates that infection takes place through root hairs just as has been shown by several authors for other endotrophic mycorhizas.

The fungus is found only in the cortex of the beads. I have never seen it in unmodified portions of the root, nor in the constrictions between the beads. Each bead, therefore, must be infected directly from mycelium in the soil. Since the beaded appearance is only found in the case of infected rootlets, and roots which are not infected develop normally, the modified character of the mycorhiza must be due to the stimulus of infection.

4. *Seasonal Relations*

In general the seasonal relations of the endotrophic mycorhizas of Acer are the same as those of the ectotrophic mycorhizas of other forest trees. They are formed during the summer, reach their fullest development in late autumn, and die during the spring. They are, therefore, annual. They do not die off so rapidly in spring, however, as do the ectotrophic forms, and new ones are beginning to be produced long before the old ones are all dead, so that there is never a time when they are as scarce as the ectotrophic forms become.

IV. DISCUSSION

The work presented in this paper may be discussed under five heads: (1) the heterotrophic form of mycorhiza; (2) mycorhizal fungi and their relation to the chance formation of mycorhizas; (3) mycorhiza development; (4) seasonal relations; (5) physiological relations.

1. *The Heterotrophic Form of Mycorhiza*

A mycorhiza of this sort has, I believe, never been reported before. Stahl (24) reported that the mycorhiza of *Juniperus nana* is sometimes ectotrophic and sometimes endotrophic, and the one on Picea studied by

Möller (14) was usually ectotrophic but occasionally endotrophic; but neither of these authors found both forms in the same rootlet, and they had noevidence that both forms were due to the same fungus. Mangin (13) states that during the ten years he has studied mycorhizas he has never seen an ectotrophic filament within a root cell, but such a condition is found in the heterotrophic form. This mycorhiza may very well be considered an intermediate form between the ectotrophic and endotrophic mycorhizas.

2. *Mycorhizal Fungi and their Relation to the Chance Formation of Mycorhizas*

Very few attempts have been made to identify the fungi which cause ectotrophic mycorhizas, although it would seem an important work. Elaphomyces was reported by Rees (20) as causing ectotrophic mycorhizas on Pinus. Noack (16), who did more than any one else in this field, reported two species of Geaster causing mycorhizas on species of Pinus, two species of Tricholoma on Pinus and Fagus, two species of Lactarius on Fagus and Quercus, and three species of Cortinarius on Pinus, Fagus, and Quercus. Kauffman (9) has shown that *Cortinarius rubipes* Kauff. causes ectotrophic mycorhizas on *Quercus rubra* L., *Acer saccharum* Marsh., and *Celastrus scandens* L. Finally Pennington (18) has reported *Boletus speciosus* Frost and *Tricholoma transmutans* Pk. as mycorhiza formers on Quercus. With one exception, Elaphomyces, these mycorhizal fungi are all Basidiomycetes, and the four that I have added to the known list are also Basidiomycetes. On the other hand, among the identified endotrophic mycorhiza fungi there has been reported only one Basidiomycete, *Armillaria mellea*, reported by Kusano (10) as forming endotrophic mycorhizas with *Gastrodia elata*. It is quite probable, therefore, that many of the common mushrooms are capable of forming ectotrophic mycorhizas with tree roots, but that very few, if any, of them form endotrophic mycorhizas with forest trees. Moreover, all those reported are late summer and autumn mushrooms. No spring mushrooms are known to be mycorhiza formers.

The particular species of fungi and higher plants which may form mycorhizas with each other are not as limited as one might expect. MacDougal (11) states that a higher plant may form mycorhizas with only one or two species of fungi, but his observations were scarcely extensive enough to grant a positive conclusion to that effect. Kauffman (9) has shown that the same fungus may cause mycorhizas on several hosts, and my own work shows that the converse of this is true, at least in the case of ectotrophic species of forest trees.

All mycorhizal fungi, however, are not capable of forming mycorhizas with all species of mycorhizal trees. Individual trees of common mycorhiza-forming species, growing in a soil which is known to be inhabited by mycorhizal fungi, are often found entirely free from mycorhizas. A specific instance of this may be given here. In a woodlot west of Ann Arbor, two trees, a Carya and a Quercus, were found growing a few feet apart, and their roots intermingled. On the Carya, brown colored mycorhizas were found in great numbers, but on the Quercus not a specimen could be found. Apparently the fungus which formed the mycorhizas with Carya was not capable of forming any with Quercus, though this species readily forms mycorhizas. Probably the following year, or at some future time, a fungus capable of forming mycorhizas with Quercus would chance to grow here, and this same tree would then have mycorhizas.

Not only are some trees without mycorhizas likely to be found in such a habitat, but on those trees which have mycorhizas, there are always found some roots which are uninfected, even in the humus layer. This was brought out by my glass plate experiments, and is easily verified by digging up the roots of any mycorhiza forming tree. The reason for this may be that in the case of the uninfected roots, the proper fungus did not happen to be present in the immediate vicinity at the time when the root was susceptible to infection; or there may have been some physiological reason why the roots were not infected. We have no evidence either for or against the latter hypothesis; there is some evidence in favor of the former. In collecting mushrooms it is often noticed that a great abundance of specimens of a certain species may be found in a particular habitat one year, and the next year there may be none at all there. If the mycelium of this mushroom happens to be colored, so that it can be easily found in the soil, it can usually be demonstrated that when the fruiting bodies are abundant the mycelium is also abundant, while if no fruiting bodies are found the mycelium will also be lacking, or will be present only in small amounts. Now, when the mycelium of a fungus, of the proper kind, is in the soil around a tree in great abundance, the chances of the two organisms coming together and forming mycorhizas are very great. On the other hand, if the mycelium is present only in small amounts, fewer mycorhizas will be produced and more roots will develop normally. The same explanation may be applied to the fact that the same tree may have mycorhizas one year and the following year may have none at all. This fact was brought out by Kauffman (9), and has been verified by me in several instances.

3. *Mycorhiza Development*

Frank (4) stated that an ectotrophic mycorhiza is produced by a fungous filament applying itself to the side of a rootlet, and then branching and spreading until it covers the whole rootlet. He evidently believed that the mantle of fungous tissue is put on first, and that the formation of the lichen structure within the rootlet is a secondary process. These statements, however, were probably mere guesses, with no direct evidence to back them. Mangin (13) does not make himself very clear on this question. Judging from his figures, one gets the idea that the fungous mantle is put on over the tip of the rootlet first and progresses from that point until it covers the rootlet. Möller (14), on the other hand, reports, for the mycorhizas of Picea, that infection takes place back of the root tip, and that the lichen structure within the root is formed first, and the mantle put on later. Möller does not give any figures to enforce his statements, and it is of course impossible to judge of the accuracy of his observations. In the course of my work I have never seen any evidence of either of the methods of development indicated by Möller and Mangin, and the method given by me in this paper is certainly correct for the mycorhizas which I have studied.

Möller also reported that the growth of a mycorhiza is resumed in spring after a winter rest, basing his conclusions on the fact that specimens are found in spring with bright, fresh appearing tips. He therefore agreed with Frank that the mycorhiza as a whole grows in length. Such specimens with fresh tips were common on all ectotrophic trees studied by me, in the spring of the year. A number of the mycorhizas, which were already present when my glass plates were first placed, showed this characteristic. These were carefully measured from time to time during a month, at the end of which time they were all dead. In none of them was I able to discover any appreciable increase in length. It appears, then, that the fresh tips are due merely to a freshening up of the mycelium.

The fact that the root is inhibited from further growth by the fungus, offers an explanation of the coral branching clusters of mycorhizas. It is well known that when the growing tip of a shoot is cut off, or otherwise inhibited from further development, the shoot is stimulated to excessive branching. If the growing tip of a root, therefore, is inhibited from further development by a mycorhizal fungus, it is reasonable to expect that branching will result, and, if the branches are in turn infected by the fungus, the ultimate result will be a coral cluster of mycorhizas.

In regard to the development of endotrophic mycorhizas, little can be said. We have shown that infection is necessary to the production of the beads of Acer mycorhizas, and that each bead is produced by separate infection from the mycelium in the soil. Future work must show whether several beads on the same root may be produced simultaneously, or whether they must be produced consecutively. Shibata (22) found well developed nodules on the roots of Podocarpus which were uninfected by the endophyte, and concluded that infection is not necessary to the production of nodules. It has since been shown by Spratt (23), however, that the nodules of Podocarpus are due to a bacterium which inhabits them, and that the endophytic fungus is of secondary importance.

4. *The Seasonal Relations of Mycorhizas*

Möller (14) is the only previous worker who has made any serious attempt to study the seasonal variations of mycorhizas. He worked with one and two year old seedlings of Picea. His results agree with mine so far as the time of mycorhiza formation and development is concerned. He seems, however, to have missed the fact that the *mycorhizas are annual.* To sum up the results of my observations, it may be said that new mycorhizas are formed throughout the summer months. They reach their fullest development in late autumn, and persist in this condition throughout the winter. In late spring they die. They are, therefore, annual. No doubt the character of the season may hasten or retard the death of old mycorhizas and the formation of new ones, so that these processes may take place earlier some years than others. Since the formation of new mycorhizas usually begins before all of the old ones are dead, there is no time of year when no specimens at all can be found. The best time to collect for demonstration purposes, or for class use, is in late autumn just before the soil freezes up, since they can be found most easily and abundantly at this time.

Cannon (3) found that certain desert perennials annually produce a crop of fine roots, which he calls deciduous roots, at the beginning of the rainy season. During the dry season these roots all die. The annual mycorhizas really represent a second kind of deciduous roots. It must be pointed out, however, that, in the case of the mycorhizas, it is the infection by the fungus which determines whether a particular root shall become deciduous or shall develop into a perennial root, while the roots described by Cannon are all deciduous.

5. *Physiological Relations*

The question as to whether mycorhizas represent symbiotic associations or parasitic associations has been much debated, and more or less evidence, applying to particular cases, has been presented on both sides. It is worth while in the first place to consider just what the difference is between symbiosis and parasitism. By parasitism is usually understood a condition in which one organism obtains nourishment at the expense of another living organism. Symbiosis has been defined as a condition in which two organisms live in intimate relationship with each other in such a way that both are mutually benefited by the association. In the case of plants it is usually taken for granted that this benefit has to do with obtaining food. In a case of perfect symbiosis, therefore, the two symbionts must get equivalent amounts of food from each other, and the interchange must be brought about in such a way that neither plant is harmed, by a loss of a particular kind of food, more than the other. It is probable that such an ideal condition never exists in nature. We cannot, of course, suspect any symbiont of altruistic motives in supplying another plant with nourishment. Plants do not give food to each other: they take food from each other. In other words, each symbiont is a parasite on the other, and the difference between parasitism and symbiosis is only relative. Symbiosis is a double parasitism; a constant struggle for supremacy between two organisms. It is obvious, then, that a sort of association may exist which in some cases would rightly be called parasitism, and in other cases would be called symbiosis, depending on the relative potency of the individuals concerned.

There are several hypotheses which might apply to the mycorhizas: the higher plant may be parasitic on the fungus; the fungus may be parasitic on the higher plant; the association may represent a true symbiosis; or the individual cases may vary from symbiosis to parasitism.

Frank (4) first advanced the hypothesis that the ectotrophic mycorhizas represent symbiotic associations in which the fungus serves as a conveyor of food from the humus to the root. He really had no direct evidence of this, but conceived the idea principally because of the apparent similarity to the lichens. This comparison of the tissues of the mycorhizas to those of a lichen is very apt, provided that we recognize the vital differences between the two organisms. If the individual cells of the root were all separate plants, the similarity of the mycorhiza to a lichen would be very striking, but those cells are merely minute parts of a complex organism. As soon as a single cell is separated from a root, it becomes a helpless, functionless body, and soon dies. Yet that is exactly what the mycorhiza forming fungus does to the cortical cells of the root. We have shown that in all well developed ectotrophic mycorhizas the external cells of the cortex are entirely surrounded and separated from each other by the fungus hyphae, while, in some cases, nearly all of the cortical cells are similarly involved. These cells, which are thus separated from each other and from the root, can certainly never function again as root cells. The root, therefore, cannot get anything from the fungus through these cells. The only way it could get anything from the fungus, in such cases, would be by the direct contact of the fungus with the central cylinder.

Stahl (24) accepted Frank's hypothesis and attempted to prove it. After a large number of observations on plants of very diverse nature, he stated that a well-developed root system, and active transpiration, accompanied by the excretion of much water, and the presence in the leaves of an abundance of starch, calcium oxalate, and nitrates, characterize mycorhiza free plants. Plants with mycorhizas, on the other hand, he found to have a less active transpiration current, and the carbohydrates in the leaves are in soluble form. Stahl imagined a great struggle to be going on among plants growing in a humus soil to obtain the requisite amount of nutrient salts. Plants with a strong transpiration current can get along very well by themselves, but those with a weak transpiration current can get the necessary amount of food only by making use of mycorhizal fungi. On a similar basis he explains the usual absence of mycorhizas in habitats which are lacking in humus but rich in nutrient salts. The struggle there is not so great and the plants do not need mycorhizas. Also, according to this hypothesis, in a habitat which is abundantly supplied with water, the transpiration current is usually rapid enough so that plenty of mineral food can be obtained without the aid of mycorhizas. On the other hand, obligate mycorhiza forming plants are to be found, says Stahl, in habitats which are rich in humus or poor in mineral salts, since, with either of these conditions present, it is difficult for the plants to get sufficient mineral food.

Schatz (21) carried out a number of cultural experiments in an attempt to prove Stahl's hypothesis with respect to the absorption of nutrient salts, but his first cultures gave negative results, and his later cultures, though giving positive results, are mostly too few in number to be convincing, and, judging from the photographs, were not very decisive.

Yet, granting that such competition among plants exists, it is not clear that it has anything to do with the formation of mycorhizas. The natural habitat of most mycorhizal fungi is in humus soil. Very few grow in the habitats that are characterized by mycorhiza free plants, and it is obvious that, if the fungi do not occur there, mycorhizas could not be formed. Moreover, the fact that healthy trees of mycorhiza forming species are sometimes found entirely free from mycorhizas, even when growing in humus soil, shows that the trees can get along very well without mycorhizas. Again, the time of year at which the mycorhizas are developed is against the hypothesis that they aid the higher plant in the absorption of mineral salts from the soil. The mycorhizas are developed and the fungi concerned are most active during late summer and autumn. At this time of the year the trees are relatively inactive and do not need so much food as earlier in the season, when the mycorhizas are absent. On the other hand, it has been shown by Preston and Phillips (19) that there is more food stored in roots, and so available for the fungus, at this time of year than in spring and early summer; and this is just the time when the fungus needs an abundance of food, for it is preparing to produce its fruiting bodies.

There seems to be no good evidence, then, that the root gets any food through, or from, the fungus, the evidence indicating, rather, that it does not. On the other hand, there is no question but that the fungus gets some food from the root. Just how it does this is not known, since no haustoria penetrating the cells have ever been found, but it undoubtedly gets something of value from the middle lamellae of the cell walls, which it dissolves, and, since the nutrient solutions of the cell sap pass from cell to cell through the walls, the fungus has an opportunity to obtain food from that source.

We are forced to the conclusion, therefore, that the tree is not benefited by association with the fungus, and that the ectotrophic mycorhizas are not symbiotic associations, but are instances of the parasitism of fungi on the roots of trees. Usually this parasitism is comparatively harmless, although the fungus kills all rootlets which it attacks. On the average tree only a small percentage of the roots are transformed into mycorhizas, since the mycorhizas are present only in the superficial layers of the soil and all the deeper roots are allowed to function normally. For this reason the tree can endure the mycorhizas without any serious inconvenience, just as it can endure a considerable number of insect galls on its leaves. In the case of seedlings, however, the mycorhizas may become quite serious. The roots of a seedling are all in the superficial layers of the soil and, therefore, subject to the attacks of fungi. If all the rootlets are transformed into mycorhizas, the supply of mineral salts will be cut off from the seedling and it will be killed. Such a case has actually been reported by Nadson (15). The seedlings of Quercus studied by him were dying off, and he was unable to find any possible reason for their death except the ectotrophic mycorhizas which were present in unusual abundance.

In the case of endotrophic mycorhizas the situation is more complex, because the mycorhizas of different species of plants differ so greatly. No doubt an important reason why so many hypotheses have been offered to explain the physiological relations of endotrophic mycorhizas is that the different authors worked with very different sorts of plants, and the same hypothesis could not apply to all. Bernard (1) and Burgeff (2) have shown conclusively that the seeds of certain orchids will not germinate except in the presence of their mycorhizal fungi. This fact, of course, proves the symbiotic nature of the orchid mycorhizas studied by these men, but it would be unsafe to draw any conclusions from this with respect to other families of plants, or even to all orchids. Magnus (12) demonstrated just as conclusively that in the endotrophic mycorhizas of Neottia certain cells of the root digest the fungus hyphae, and so derive benefit from the association. Shibata (22) later found the same thing to be true of Podocarpus mycorhizas. Groom (7), who did his work on Thismia a few years earlier, failed to find that the hyphae were digested by the host cells, but he did find evidence that the root obtained food in some way from the fungus, and it is probable that a repetition of the work would show that digestion does take place. No cytological work has been done on the Acer mycorhiza, but it must be said that the endophyte in these mycorhizas is very similar to that described by Shibata, and acts much the same, so that it is quite possible that the same physiological processes occur.

Janse (8) advanced the hypothesis that the endophytes of endotrophic mycorhizas have the ability to fix free nitrogen, just as do the bacteria in leguminous tubercles. He had no direct evidence of this, and the few cultural experiments which he performed gave negative results, but his statements induced other workers to take up the problem from that point of view. Nobbe and Hiltner (17) performed very extensive cultural experiments in which they grew plants of Podocarpus, both with and without mycorhizal nodules, in nitrogen-free sand, and obtained results which were very favorable to Janse's hypothesis. These results, however, can no longer hold, since Spratt (23) has shown that nitrogen fixing bacteria inhabit the same nodules. Later Ternetz (25) found nitrogen fixation in cultures of fungi which she obtained from endotrophic mycorhizas, but since her method of isolating the fungi, by allowing them to grow out of the roots in hanging drop cultures, is open to criticism, the work should be subjected to verification before final acceptance. It can at least be said there is no evidence that would warrant us in suspecting the Acer endophytes of having nitrogen fixing ability.

Stahl's hypothesis of the absorption of salts from the soil certainly could not apply to the Acer mycorhizas, since there are very few communications of the endophyte with the soil. Also, these mycorhizas are not always accompanied by a low water supply, since I have collected well developed, living mycorhizas from the mud at the bottom of a kettle-hole which is full of water during all wet seasons. Not only is it impossible that the endophyte is of importance in the absorption of salts, but the rootlet itself is inhibited from absorbing them to any great extent, since it is deprived of root hairs. The endophyte, of course, gets all, or a large percentage of its food from the root. It must, therefore, be considered as an internal parasite. It is possible, however, that, in those cases, in which the endophyte occupies only a small percentage of the cortical cells, the root may receive sufficient benefit from the digestion of fungous hyphae to justify applying the term symbiosis to the association.

SUMMARY.

1. Six forms of ectotrophic mycorhizas, including a heterotrophic form, are described.

2. Four species are added to the known list of ectotrophic mycorhiza forming fungi: *Russula* sp. on *Tilia americana*, *Boletus scaber* var. *fuscus* on *Betula alba* var. *papyrifera*, *Cortinarius* sp. on *Betula alba* var. *papyrifera*, and *Scleroderma vulgare* on *Quercus alba*.

3. At least four, and probably more, different species of mushrooms may form mycorhizas on the same tree.

4. The infection of a young root and its transformation into a mycorhiza depend on the chance presence of a fungus which is capable of forming mycorhizas with that particular species of root, or on some chance condition of the root or the fungus.

5. Infection for the formation of ectotrophic mycorhizas takes place by a fungous filament penetrating the outer portion of the epidermal wall of the root and then branching and spreading in all directions, by dissolving the middle lamellae, until a complete mantle, covering the rootlet, is formed. While this mantle is being formed, other branches of the mycelium are penetrating between the epidermal cells of the root by dissolving the middle lamellae and splitting the cells apart.

6. As soon as a complete mantle of mycelium is formed over the root any further growth of the root in length is inhibited. Because of this fact the root is stimulated to produce branches. These branches are in turn infected, and the result is a coral like cluster of mycorhizas.

7. The fruiting bodies of an ectotrophic fungus are usually produced soon after the mycorhiza is formed.

8. Both ectotrophic and endotrophic mycorhizas are normally annual. They are formed during summer, reach their fullest development in late autumn, persist unchanged throughout the winter, and die in spring.

9. The roots of Acer are infected through root hairs in the production of endotrophic mycorhizas. The modified bead-like character of the mycorhizas is due to the stimulus of infection.

10. The matter presented in this paper indicates that the endotrophic mycorhizas of the maples are sometimes symbiotic associations, and sometimes associations in which the fungus can only be considered as an internal parasite of the roots. The ectotrophic mycorhizas of

forest trees, on the other hand, are not in any sense symbiotic associations, but must be considered as instances of the parasitism of fungi on the roots of trees.

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EXPLANATION OF FIGURES IN THE PLATES IV–VII

All figures, except 1, 11, and 12, were drawn with camera lucida.

FIG. 1. A root with ectotrophic mycorhizas.

FIGS. 2–6. Cross sections of ectotrophic mycorhizas. Fig. 2, form 1, from Carya ovata. Fig. 3, form 2, from Carya ovata. Fig. 4, form 3, from Quercus alba. Fig. 5, form 4, from Quercus alba. Fig. 6, form 5, from Larix laricina.

FIG. 7. Portion of cross section of heterotrophic mycorhiza from Tilia showing hyphae (*a*) which have entered the cortical cells of the root.

FIG. 8. Longitudinal section of ectotrophic mycorhiza of Quercus showing root tip covered by fungous mantle.

FIG. 9. Enlarged section of ectotrophic mycorhiza of Quercus alba showing hyphae (*a*) penetrating between the cortical cells of the root.

FIG. 10. Section of immature ectotrophic mycorhiza of Tilia americana showing root tip still uncovered and hyphae (*a*) just beginning to penetrate between the root cells. *b* = outer portion of epidermal wall.

FIG. 11. Diagram showing cortical root cells (*a*) surrounded by fungous tissue (*b*).

FIG. 12. Root with endotrophic mycorhizas.

FIG. 13. Tangential section of endotrophic mycorhiza of Acer rubrum.

FIG. 14. Longitudinal section of endotrophic mycorhiza showing the constriction between two beadlets.

FIG. 15. Enlarged cells containing endophyte.

FIG. 16. Cells with endophyte curled around nucleus.

FIG. 17. Cell containing a vesicle.

FIG. 18. Cell with endophyte showing swellings of hyphae.

FIG. 19. Hyphae within root-hairs.


FIG. I

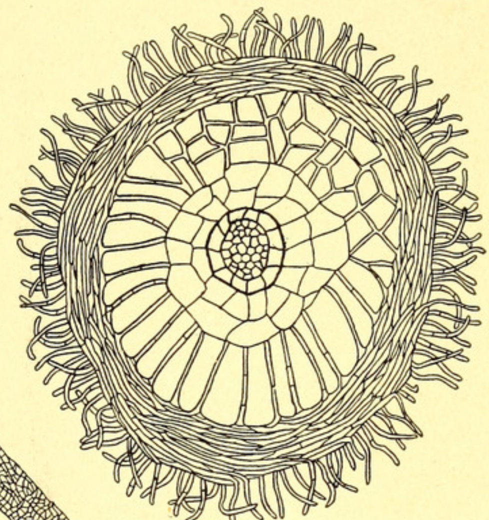
FIG. 2

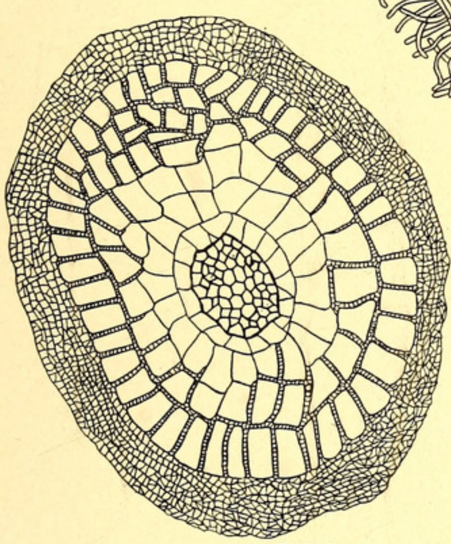
FIG. 3

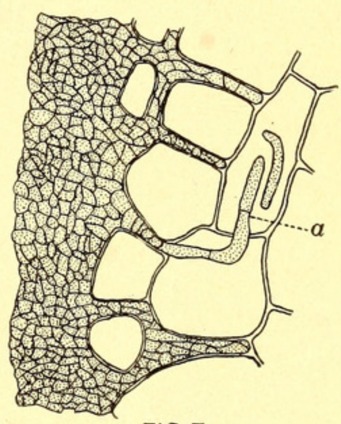

FIG. 7

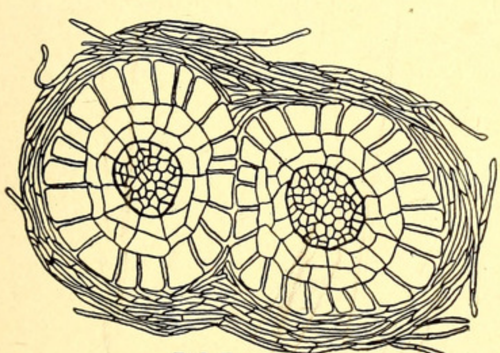
FIG. 5

McDOUGALL: MYCORHIZAS OF FOREST TREES.

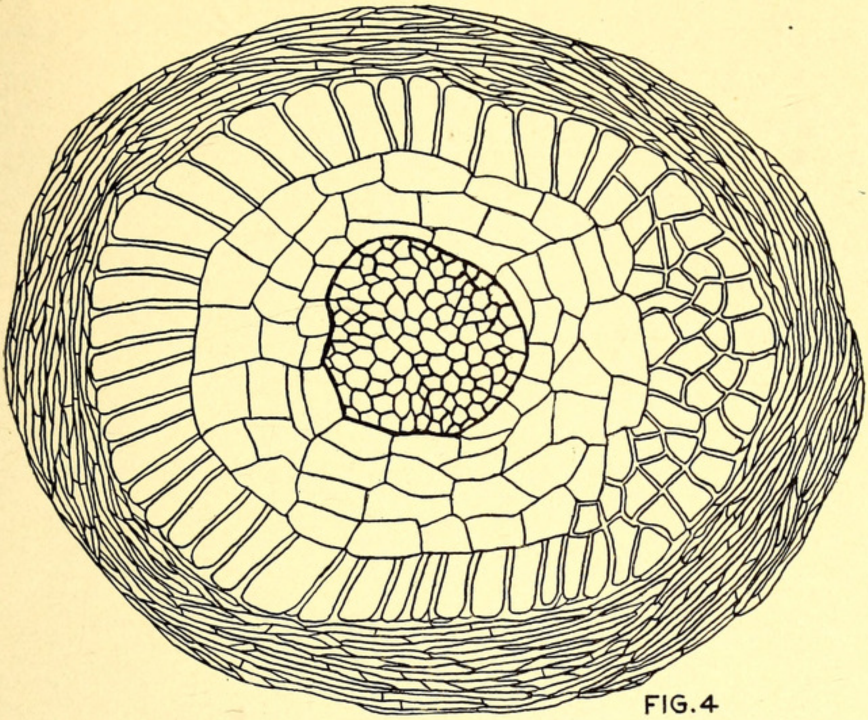

FIG. 4

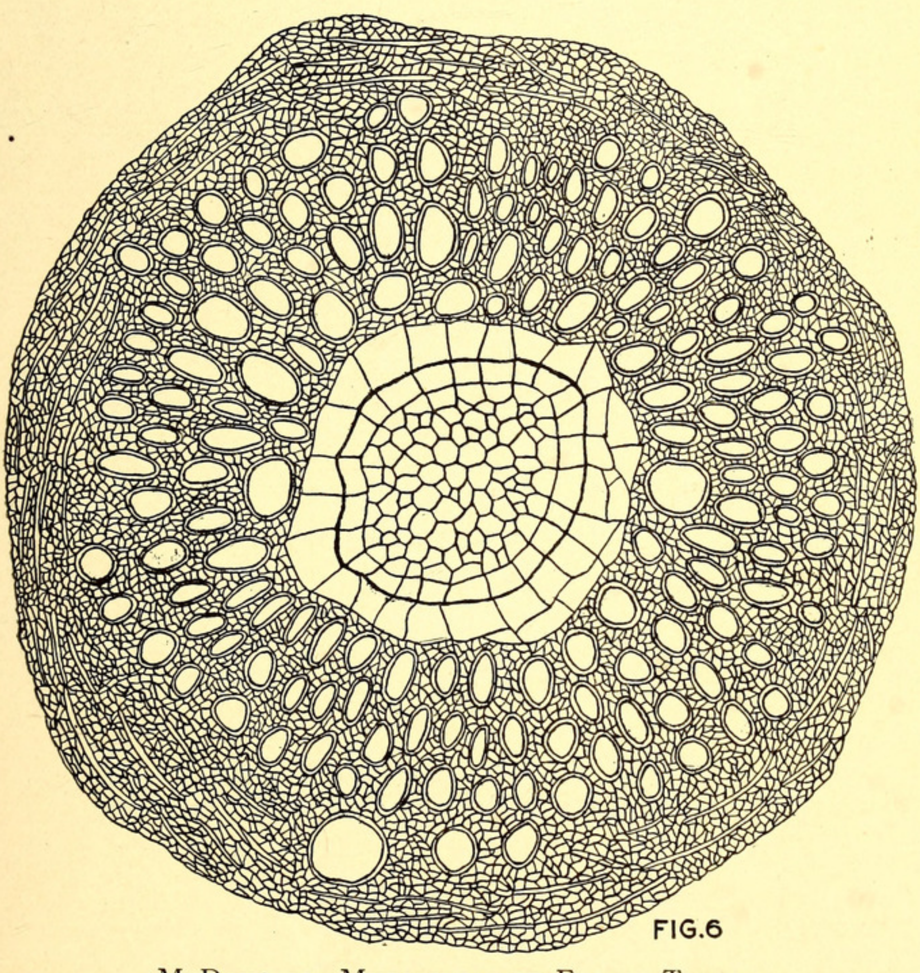

FIG. 6

McDOUGALL: MYCORHIZAS OF FOREST TREES.

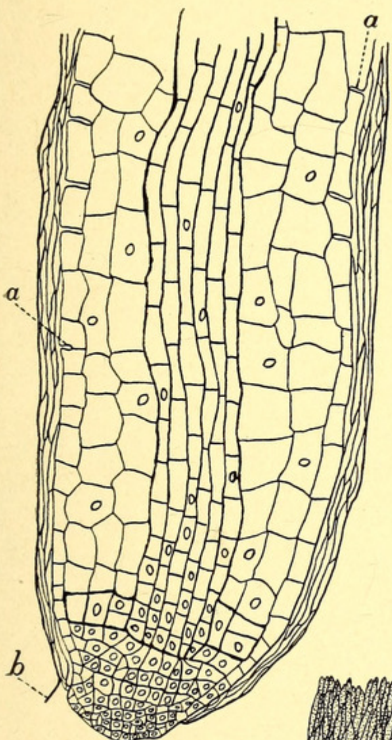

FIG. 10

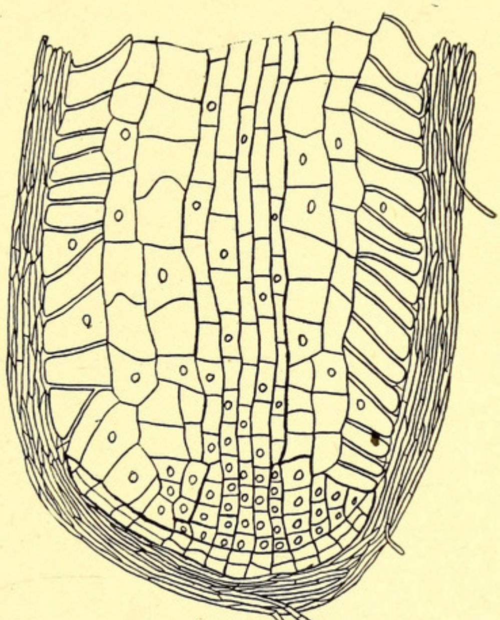

FIG. 8

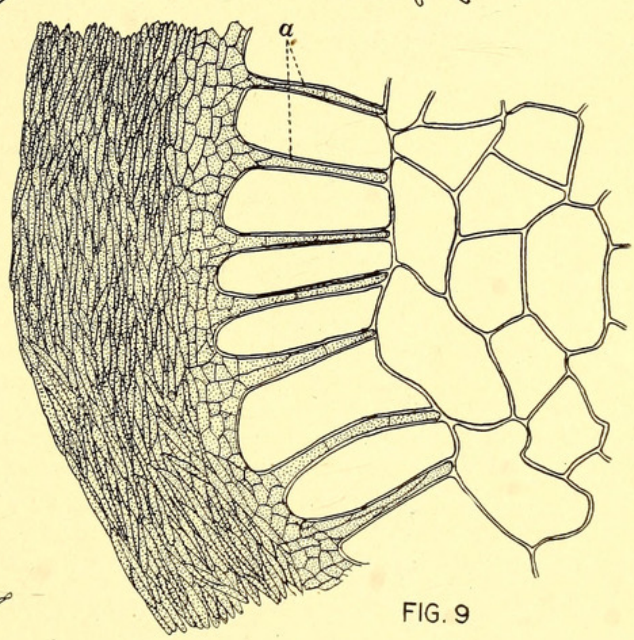

FIG. 9

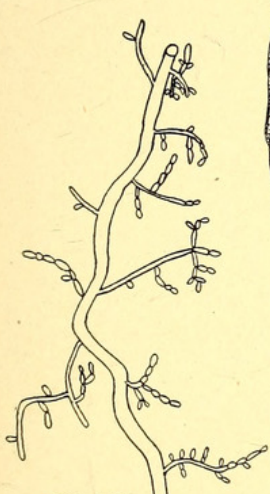

FIG. 13

McDOUGALL: MYCORHIZAS OF FOREST TREES.

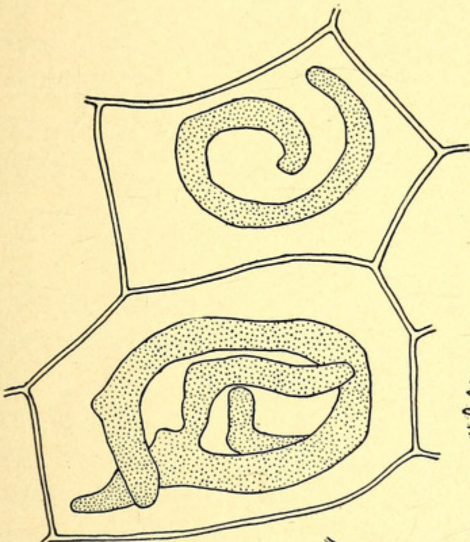

FIG. 15

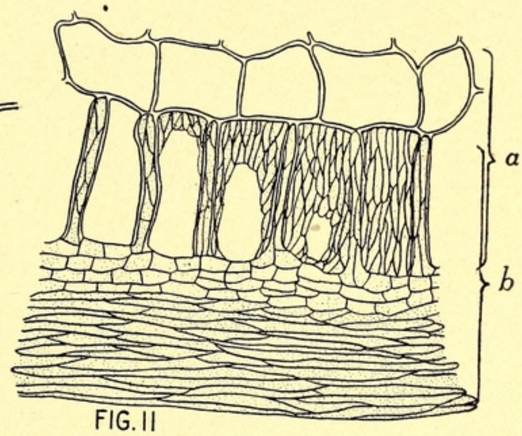

FIG. 11

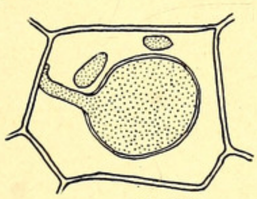

FIG 17

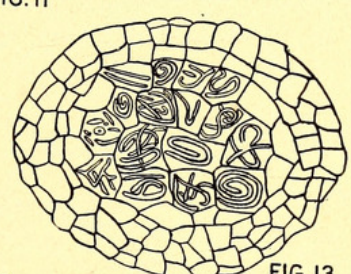

FIG. 13

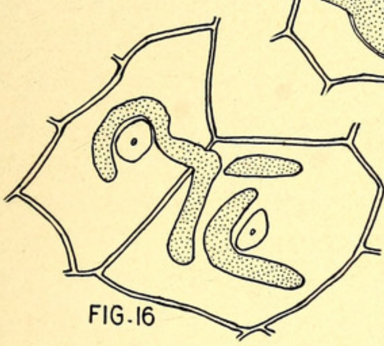

FIG. 16

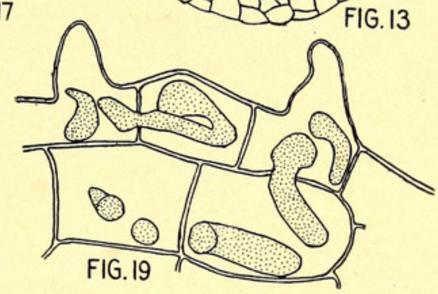

FIG. 19

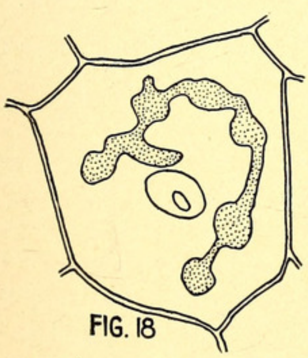

FIG. 18

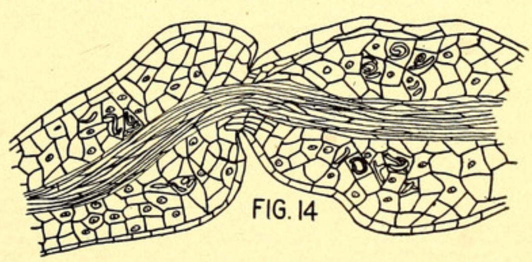

FIG. 14

McDOUGALL: MYCORHIZAS OF FOREST TREES.