

NUTRITIONAL RELATIONS IN MYCORRHIZAE

(Review of Literature)

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INTRODUCTION

A great deal of information concerning mycorrhizae has accumulated over the last 70 years. Most of the writings deal with the effect of the symbiotic relationship on the component fungus and vascular plant. Also much speculating has been done with regard to the nutritional relationships.

The various all-inclusive theories on the function of mycorrhizae have generally been abandoned for the present, in favor of more basic experimentation on the nutritional aspects of the component parts. As a result of these experiments a great deal of variation in the fungus-plant relationship and their nutritional effect on each other has been discovered. The results suggest that none of the earlier generalized theories can be applied to all mycorrhizae. Experiments have shown that different combinations of plants and fungi affect each other in different ways, depending greatly on the environmental conditions.

The early investigations of mycorrhizae concerned studies in both ectotrophic and endotrophic mycorrhizae. There was much interest in endotrophic mycorrhizae because they are so important in cultivation of orchids. More recent investigations have centered around ectotrophic and ectendotrophic types, probably because of their extensive association with commercial forest trees.

"FUNGUS ROOTS"

Root hairs are generally considered the absorptive organ of land plants. However, Hatch (1937) states the absorption of water and nutrients by practically all land plants is not primarily by root hair contact with soil particles but by fungus hyphae which grow in close association with root tips. This fungus-root association is known as a mycorrhiza.

HISTORICAL

Until approximately 1880 all men who made observations on the association of fungi and roots considered the fungus to be parasitic on the root (Rayner, 1927). Kamienski (1882) however, clearly defined the relation between root fungus and vascular plant as differing from that existing in ordinary cases of parasitism.

Frank (1885) regarded the regular association of root tissues and mycelium as a morphologically distinct organ for which he proposed the name "Pilzwurzel" or Mycorrhiza. He emphasized the close analogy between the thallus of a lichen and mycorrhiza, each with its green and non-green constituent. He concluded a true symbiosis existed between fungi and roots of Cupuliferae. Frank was thus the first man to recognize the association between fungus and vascular plant as a phenomenon of mutualistic benefit or symbiosis. He found this association existing on the roots of many species of trees including the following genera Quercus, Fagus, Salix, Ostrya, Pinus, and Abies.

The now classical papers of Frank published between 1880 and 1895 marked the beginning of major studies on mycorrhizae leading up to the present. His organic nitrogen theory on the function of mycorrhizae, described later in

this paper, provoked much controversy among botanists of that time. As well as doing much of the original work on mycorrhizae himself, Frank influenced others to further investigation, which has contributed greatly to our present knowledge of mycorrhizae.

CHARACTERISTICS OF MYCORRHIZAE

Definition

Boyce (1948 P. 113) defines mycorrhiza as "a compound organ consisting of a plant root and a fungous mycelium, the two elements being constantly arranged in an orderly manner in reference to each other".

Morphology

Frank (1887) described two general types of mycorrhizae which still bear the names he gave them, namely, ectotrophic and endotrophic.

Ectotrophic mycorrhizae consist of a dense fungal mantle surrounding the short roots and completely replacing the root hairs and epidermis. Hyphae of the mantle are continuous with a network of fungus hyphae between the cortical cells of the root. This intercellular network is known as "Hartig's net". The whole results in the short roots becoming thick, blunt and much branched so as to appear coralloid. They are often shiny or somewhat waxy and may be brightly colored.

Endotrophic mycorrhizae have neither a fungus sheath nor "Hartig's net". Hyphae of the fungus, in the form of coils and various shaped swellings, are present in the cortical cells of the vascular plant root. Only a few individual hyphae are seen on the epidermis of the root, and root hairs are present. The entire root appears normal to the unaided eye except the fine branches may be somewhat more shiny than usual. The presence of endotrophic

mycorrhizae can only be determined by microscopic examination of stained root sections.

The more recent literature (Boyce 1948, Schmidt 1947) includes a type of mycorrhizae intermediate between the two described above. This is where characteristics of both types are combined and the term ectendotrophic is applied. Either the same fungus produces both the intercellular and intracellular infections, or two distinct fungi are responsible.

Melin (1917) described mycorrhizae which invade both the cortical and vascular tissues of the root and appeared to retard root development as pseudomycorrhizae. They can usually be distinguished from mycorrhizae because of the complete lack of an orderly arrangement of the invading hyphae.

Occurrence

Hatch (1937 p. 32) states practically all trees possess mycorrhizae. He bases this statement on an extensive review of observations by other workers. Both ectotrophic and endotrophic mycorrhizae exist on forest trees. the former type being most common on the species with roots differentiated into so called "long roots" and "short roots". Boyce (1948) lists Fagaceae, Betulaceae, Salicaceae, and Abietineae as families with the above root differentiation and ectotrophic mycorrhizae. Rayner (1927) states endotrophic mycorrhizae are very abundant in the Orchidaceae, Ericaceae, Ophioglossaceae, Pyrolaceae, Genianaceae, and many genera scattered in various other families. She also reports a fungus-root relationship in Bryophyta, but here the absence of roots precludes use of the term "mycorrhiza". Boyce (1948) states that 75 per cent of all plants have endotrophic mycorrhizae. Endotrophic mycorrhizae are probably often overlooked because they are quite difficult to distinguish from non-mycorrhizal roots.

Most mycorrhizal fungi are members of the Hymenomycetes, the family Boletaceae in particular having many mycorrhiza-formers. At least one species in the Gasteromycetes is known to be a mycorrhiza-former (Rayner, 1945). No experimental evidence has proved Ascomycetes present in mycorrhizae (Melin, 1953).

Some fungi such as many species of Boletus are specific as to the tree with which they will form mycorrhizae (Wilde, 1946). Others such as species of Amanita may be found associated with a wide variety of tree species.

Mycorrhizae are much more abundant in humus layers of forest soils than in any other soil (Frank 1885, Hartig 1888, Stahl 1900, and Peklo 1908). However, mycorrhizae will develop abundantly on tree roots in open field soils (Hatch 1937). This indicates the forest humus layer is not essential for formation of mycorrhizae.

FUNCTION OF MYCORRHIZAE

Probably the first recorded theory on the function of mycorrhizae was the one put forth by Kamienski in a paper published in 1886. Speaking of Monotropa hypopithys L., he said that owing to the presence of a thick sheath of mycelium the absorbing surface of the roots has no direct contact with the soil, and all soluble food materials entering the plant from that source must pass through the mycelial envelope. He held that the hyphae grew upon the surface of roots merely as on a convenient base offering a larger surface than the surrounding soil. Physiologically the mycelial mantle was assumed to function as the epidermis of the root, and the hyphae extending outwards from it as root hairs. He did not prove experimentally that Monotropa required the mycelium on its roots for satisfactory growth.

Nitrogen Theory

Frank's theory on the physiological relationship between the fungus and the vascular plant is stated in his second publication in 1885 (1885b). He says mycorrhizae are formed only on soils containing humus, and are probably on roots of all trees under certain conditions. The root fungi carry to the trees not only the necessary water and salts but also soluble organic matter derived from the humus. The fungus mantle, described as the absorbing system of the tree, was also believed responsible for formation and intake of suitable compounds of carbon and nitrogen. His experiments, using Fagus seedlings germinated in sand and water cultures, showed survival and growth were much better in the presence of both humus and mycorrhiza-forming fungi. From this he concluded the fungus component transformed carbon and nitrogen components of humus into forms utilizable by the tree. Nitrogen was not necessarily nitrated and was obtained by the plant in organic form. His proposal was strengthened by the fact that nitrate nitrogen was found neither in forest humus soils nor in trees with mycorrhizae, yet it was present in trees without mycorrhizae. He was probably justified in concluding from these experiments that the proper nutrition of the seedlings depended on the presence of their appropriate root fungus. However, his interpretation of the fungus symbiont as forming carbon and nitrogen compounds utilizable by the tree was not proved.

Frank extended the above theory to endotrophic fungi. Here, however, owing to the lack of the fungus mantle it was impossible to relate the root fungi directly to absorption as in ectotrophic mycorrhizae. He noted the extensive digestion of intracellular fungal hyphae by the vascular symbiont and saw an analogy between the role of the fungus here and that of the insect in insectivorous plants. However, he conducted no experiments with endo-

trophic mycorrhizae.

Frank's theory, known as the "Nitrogen Theory", in essence was that mycorrhizae are more efficient organs for absorbing nitrogen from humus than are non-mycorrhizal roots because their fungal components are capable of utilizing the complex organic sources of nitrogen contained in these plant remains. Many workers have tried to prove this (Melin 1927, McArdle 1932, Rayner 1934, and others) but all failed to find the relationship between nitrogen availability and the abundance of mycorrhizae.

The Mineral Salt Theory of Stahl

Stahl (1900) noticed that abundance of ectotrophic mycorrhizae decreased with increase in soil fertility. In his experiments he found that plants with extensive root systems and other features tending to increase absorption, as measured by the transpiration current, had few mycorrhizae. He had previously noted that fungi, as evidenced by the high nitrogen and mineral salt content of their fructifications, are extremely efficient in extracting nutrients from organic materials. Higher plants, lacking the ability to utilize humus, are of necessity forced to compete with fungi for the mineral salts which the latter liberate from the humus. Therefore, if the plants are equipped with mycorrhizae, they would be favored in this competition. This severe competition between vascular plants and the more efficient soil fungi accounted, in Stahl's opinion, for the increased abundance of mycorrhizae in humus of forest soils which were comparatively poor in nutrient salts. It was supposed that those plants with very extensive root systems could obtain sufficient nutrients without the aid of mycorrhizae. This accounted for those plants which grow well without mycorrhizae.

A serious objection to the Stahlian theory has been the non-acceptance by physiologists of the assumption that absorption of water (measured by the

transpiration current) and of mineral salts dissolved in it are related processes. However, there is uncertainty about this relationship as it occurs in plants.

Hatch - Stahlian Theory

Hatch (1937) conducted extensive pure-culture experiments based on the mineral-salt theory of Stahl. By withholding mineral salts in various combinations he was able to show that "poverty" in mineral salts was the chief factor responsible for variations in the production of ectotrophic mycorrhizae on pine seedlings (p. 82). This strengthened the theory of Stahl. Also, the fact that Hatch obtained reduced development of mycorrhizae by means of the addition of mineral salt solutions lacking nitrogen to his deficient soils, tends to discount the "Nitrogen Theory".

Hatch demonstrated that ectotrophic mycorrhizal roots have a very much larger absorbing surface area than non-mycorrhizal roots. The absorbing surface area is increased because mycorrhizal short roots are larger than non-mycorrhizal short roots and because the surface area of the hyphae composing the fungus mantle is acquired for absorption. He found the mycorrhizal relationship in pine to be a symbiotic mechanism on which the trees were dependent for their existence in all but the most fertile agricultural soils.

Endotrophic Mycorrhizae

Bernard was one of the early pioneers in the study of endotrophic mycorrhizae, the fungal component of which is intracellular in the root. He demonstrated (1904) that certain orchid seeds could be germinated and the seedlings grown only in the presence of their mycorrhiza-forming fungi. He observed that the intracellular hyphae, which are usually dense and variously branched, sooner or later became reduced to an amorphous mass within each cell.

He interpreted these as degenerating cells, whereas Magnus (1900), considered them as signs of increased activity of the root cell. Magnus stated there are two types of infected cells - those which digest hyphae and those in which the hyphae may persist and remain ready for future infection of other cells. Bernard (1909) regarded the cells with persistent hyphae as antibodies which acted to reduce new infection from outside.

Knudson (1922) confirmed Bernard's observations on the necessity of a fungus component for growth of members of the Orchidaceae. However, he claimed the fungus component was necessary only because it produced sugar from the organic medium for the plant; also it acidified the medium, which created the correct pH for orchids. He based this on his ability to germinate and grow orchids satisfactorily without mycorrhizae by using sugar and nutrient inorganic salts in the growing medium. However, this still does not explain the invariable presence of the endophytes in orchid roots or the specificity of the fungus forms associated with particular orchid species.

Rayner (1920, 1922, and 1925) has shown species of the Ericaceae also to be dependent on mycorrhizae for successful growth. She has shown in Galuna sp. that mycorrhiza-formation may be inhibited by certain conditions of the rooting medium.

Ternetz (1907) proved that some root fungi of ericaceous mycorrhizae can assimilate atmospheric nitrogen in culture. This fact has since been carefully checked and verified by Duggar and Davis (1916). This ability of nitrogen-fixation by fungi, strangely enough, takes place in soils that are usually too acid for nitrogen-fixing bacteria.

RECENT NUTRITION INVESTIGATIONS

Melin and his students, working with the mycorrhiza-rich trees of Sweden, have conducted extensive research on the nutrition of mycorrhizal fungi in pure culture. Their investigations have also extended to the nutritional aspect of the mycorrhizal relationship, and this work is presently being continued.

MYCORRHIZAL FUNGI IN PURE CULTURE

For a fungus to be authoritatively accepted as a mycorrhiza-forming species it must be proved capable of forming mycorrhizae with a higher plant in pure culture (Modess, 1941). This, together with observation of the occurrence of the fungus in nature as a mycorrhizal component with the same higher plant, provides conclusive evidence that it forms mycorrhizae. The mycorrhizal fungi referred to in the following pages have satisfied the above criteria, and are thus known to be mycorrhiza-formers.

Enzyme Variation

There is considerable variation in the nutrient requirements and enzyme systems of mycorrhizal fungi. Norkrans (1950), has found of five species of Tricholoma, all of which are mycorrhiza-formers, that only three species could utilize fructose effectively as a carbon source. Only one of the five species readily decomposed cellulose, but two of the remaining four decomposed cellulose in the presence of glucose. These last two apparently form the enzyme cellulase but not when grown on cellulose alone. None of the species tested formed cellulase when grown in media without cellulose, which indicates the cellulolytic enzymes were adaptive. According to her findings, the enzyme systems of mycorrhizal fungi, even within the same genus, vary considerably.

Lindeberg (1948) has found most tree mycorrhizal fungi which he studied produced no, or very little, polyphenol oxidase which is a litter-decomposing enzyme. Litter-decomposing Hymenomycetes that are not mycorrhiza-formers do produce the above enzyme. However, he found two mycorrhiza-formers, Boletus subtomentosus (Schaeff.) Fr. and Lactarius deliciosus (Linn.) Fr., which decomposed litter, and thus apparently had characteristics of both groups. Although most ectotrophic mycorrhizal fungi do not contain cellulose-decomposing enzymes, some do, and these are considered by Melin (1953) as facultative mycorrhizal fungi. That is, these fungi may exist either in a mycorrhizal association or as saprophytes on forest litter. Of the soil fungi considered, therefore, there are some which may both form mycorrhizae and decompose litter; these are known as facultative mycorrhizal fungi.

When facultative mycorrhizal fungi are living in the mycorrhizal association they do not produce cellulase as long as soluble carbohydrates are available from the roots. However, the cellulose of the cell wall against which the mycorrhizal fungus grows, may induce formation of cellulase when the root no longer supplies an excess of sugar (Norkrans, 1950). This may in part account for the occurrence of ectendotrophic mycorrhizae and for mycorrhizal fungi becoming parasitic under certain conditions as Gosselin (1944) claims occurs with Polyporus circinatus Fr.

Growth Substance Deficiencies

Melin and Norkrans (1942), and Melin (1954) have found that all species of ectotrophic mycorrhizal fungi studied, with one exception, are deficient in thiamine when grown in pure culture in synthetic nutrient solutions. The exception is Mycelium radialis atrovirens Melin which does not form typical mycorrhizae, but frequently grows over typical mycorrhizae and penetrates intracellularly in long and short roots (Hatch, 1934). Melin (1946) states

that this fungus is autotrophic, or self sufficient, both to thiamine and biotin.

Different isolates of the same species of thiamine-deficient fungi may have different demands for thiamine, and there are great variations between species (Melin, 1954). Variation also exists in demand for the two components of thiamine. The deficiency for thiamine may be influenced by environmental conditions such as oxygen and composition of the medium (Mikola, 1948).

Melin (1946) found that the growth of mycorrhizal fungi was greatly increased by adding small amounts of forest tree litter-water-extracts to synthetic nutrient media containing sugar, salts, and thiamine. The organic matter of the litter contained sugars which alone increased the growth rate. To eliminate these sugars as a factor so that he could find the effect of the growth substances present, he introduced the extract to the culture solutions in suboptimal amounts. He then found the ash constituents alone increased the growth rate of two mycorrhizal species. Lindeberg (1944) has evidence that calcium and manganese are the ash constituents mainly responsible for the growth promoting effect in these two species. Apparently one or several growth-substances are present in the extract which Melin suggests are vitamin-like substances. His experiments with vitamins show the increase was not due to inositol, thiamine, biotin, riboflavin, pantothenic acid, nicotinic acid, adermin, p-aminobenzoic acid, ascorbic acid, or indolyl acetic acid. Norkrans (1950) using different mycorrhizal fungi than Melin, found pantothenic acid increased the growth of Tricholoma imbricatum Fr. in pure culture and nicotinic acid increased the growth of Tricholoma fumosum. Addition of amino acids to growing media containing ammonium nitrogen have been found to greatly increase the growth rate of most ectotrophic mycorrhizal fungi (Melin, 1953). Most species studied have been strongly favored by

addition of glutamic acid. Apparently some amino acids can be utilized in greater quantities than the fungus can synthesize them from ammonium nitrogen. Possibly the required amino acids are made available only when the mycorrhizal association exists. There is much variation in the demands of mycorrhizal fungi for amino acids.

There is a remarkable similarity in the growth stimulus given mycorrhizal-forming fungi by the addition of amino acids and the addition of litter extract to synthetic media. It appears that one or several of the amino acids used may be present in the litter extract; however, this is apparently difficult to determine.

Since litter-decomposing fungi, like the mycorrhizal fungi, are thiamine deficient (Melin, 1946), it seems unlikely that the acquisition of thiamine is the factor leading to the association of mycorrhizal fungi with roots. There is the possibility that the litter-decomposing fungi are able to obtain a substitute for thiamine from litter, which allows them to grow on it. The mycorrhizal fungi, however, may be unable to utilize this substitute for thiamine and are forced to associate with tree roots to obtain it.

Melin (1954) has shown that tree mycorrhizal fungi are not only deficient in thiamine, amino acids, and B-vitamins but also in some unknown substance which is present in the root. He grew mycorrhizal fungi in synthetic media with thiamine and with amino acids and B-vitamins. To each of these two amended media he added aseptic excised pine roots before the fungi were added to the flasks. The results of his growth experiment are reproduced in Table 1.

The growth of all mycorrhizal fungi was greatest in the flasks containing excised roots. In the flasks without roots, the mycelium of some of the fungi was barely visible. It appears, therefore, that some root constituent other

TABLE 1. GROWTH PROMOTING EFFECT OF EXCISED PINE ROOTS ON VARIOUS MYCORRHIZAL BASIDIOMYCETES.
RELATIVE VALUES. FROM MELIN, 1954.

Fungus	Incubation Time, Days	Thiamine		Amino Acids & B-Vitamins	
		Without Root	With Root	Without Root	With Root
<i>Boletus bovinus</i> (L.) Fr.	13	--	--	42	100
<i>Boletus edulis</i> Bull. ex Fr.	14	--	--	26	100
<i>Boletus elegans</i> Fr.	14	--	--	34	100
<i>Boletus subtomentosus</i> L. ex Fr.	11	41	60	66	100
<i>Boletus variegatus</i> (Sw.) Fr.	10	14	64	20	100
<i>Amanita muscaria</i> (L.) Fr.	22	31	56	44	100
<i>Amanita pantherina</i> (Dc. ex Fr.)	21	--	--	27	100
<i>Cortinarius glaucopus</i> (Schaeff. ex Fr.) Fr.	21	--	--	7	100
<i>Pholiota caperata</i> Pers. ex Fr.	22	--	--	6	100
<i>Rhizopogon roseolus</i> (Corda) Th. Fr.	10	8	55	16	100
<i>Russula xerampelina</i> Schaeff. ex Fr.	38	0.2	100	1	100

than thiamine, amino acids, and B-vitamins, is necessary for maximum growth of these fungi. It is also seen from the table that the roots cannot supply amino acids or B-vitamins in sufficient quantity for maximum growth of the fungi.

Similar experiments with excised tomato roots yielded similar results indicating the metabolite concerned is not specific for pine roots, but is produced also in roots of other plants.

A possible weakness in this experiment is that fungi in liquid culture seem to have a tendency to grow faster if there is any object to which the mycelium may become attached. However, the same results were obtained when Melin (1954) used the method of Beyerinck which employs solid instead of liquid media. Experiments using excised roots do not prove conclusively that the same results would be obtained in living trees.

Macdougall and Dufrenoy (1944), have shown that auxin occurs abundantly in the fungal hyphae of pine mycorrhizae. They assumed that the coralloid branching of the short roots was due to the action of the auxin translocated from the hyphae. Slankis (1951), using pure cultures found experimentally that certain auxins did control the length and thickness of the short roots of pine as well as the degree of their coralloid branching. In view of these findings it seems that there may be an auxin deficiency in pine roots and that hyphae of a mycorrhizal fungus may satisfy this deficiency.

Considering the nutrition of endotrophic mycorrhizal fungi, Hamada (1940) found most of them able to utilize cellulose. Very little investigation has been done on the growth requirements of this type of mycorrhizal fungi.

NUTRITIONAL RELATIONSHIPS BETWEEN MYCORRHIZAL FUNGI AND ROOTS

Ectotrophic Mycorrhizae

Bjorkman (1942) has found that pine roots with an excess of soluble carbohydrates have more mycorrhizae than those with a deficiency of soluble carbohydrates. He assumes that the amount of sugar in the roots of trees is the only important factor governing presence or absence of mycorrhizae. According to him mycorrhizal fungi obtain their assimilated sugars directly from roots, and these sugars are the most suitable source of energy and carbon for the fungus associates. If there is no excess of soluble carbohydrates in the roots of the vascular plant, few if any mycorrhizae are formed. Evidence for his hypothesis is as follows: Three-year-old pines which had been strangulated for one summer had very few new mycorrhizae, whereas the non-strangulated control plants had very well developed mycorrhizae. Chemical analyses showed the strangulated plants had much less reducing substance than the controls.

MacDougal and Dufrenoy (1945) disagree with Bjorkman because they found excised mycorrhizal roots were able to live for many seasons. They maintain that the carbohydrates found in the roots were obtained from the soil by means of the fungus.

Ectotrophic and ectendotrophic mycorrhizal components gain entrance to vascular plant roots by means of pectolytic enzymes which dissolve the middle lamella of cortical cells (Melin, 1953). Both mycorrhiza-forming and litter-decomposing fungi contain these enzymes and the reason the litter-decomposing fungi do not enter roots is not clear. Melin suggests that the resistance of trees to many fungi may be due to excreted substances from the roots which are inhibitory only to certain species.

In agreement with the Hatch-Stahliian theory, McComb and Griffith (1946) found that mycorrhizal seedlings absorbed more inorganic elements than non-mycorrhizal ones. They believe, however, that the greater capacity for absorption is due to the higher metabolic activity of mycorrhizal roots. To determine whether metabolic activity is greater in mycorrhizal than non-mycorrhizal roots Kramer and Wilbur (1949) used radioactive phosphorus in solutions containing excised roots with and without mycorrhizae. Their later measurements of radioactivity in the excised roots showed the mycorrhizal roots had accumulated much more phosphorus than the non-mycorrhizal roots. This seems to strengthen the Hatch-Stahliian theory especially since they found the main accumulation of P^{32} in the fungal sheath and not in the host tissue.

That mycorrhizal roots of pine absorb more nutrients from a substrate than non-mycorrhizal roots was demonstrated by Hatch (1937). The means of molecular or ionic translocation of nutrient elements through mycorrhizal roots, however, has not been satisfactorily explained. Melin and Nilsson (1950) exposed only the fungal associate to a phosphate solution containing radio-active phosphorus and demonstrated that pine seedlings in pure culture took up phosphorus through the mycorrhizal component. Further, the phosphorus had been translocated from the roots to the stems and needles. Isotopic nitrogen in the ammonium group was also found to be transferred from a solution to the roots, from where it was translocated to the stems and needles (Melin and Nilsson, 1952). These experiments furnish the first critical evidence that nutrients pass through fungal hyphae into the root of the vascular plant. These workers have also demonstrated (1953) using the isotope technique mentioned above, that nitrogen from N^{15} - labelled glutamic acid was transferred to pine roots by a mycorrhizal fungus in pure culture. Their

evidence indicates the glutamic acid was not deaminated by the fungus and may have been translocated to the root cells from the mycelium in organic form. MacDougal and Dufrenoy (1946) obtained evidence indicating ectendotrophic pine mycorrhizae act as absorbing organs which take up all nutritive elements from the soil. They conclude that these elements are metabolized in the hyphae and then translocated to the cortex of the higher partner.

These experiments support the early views of Frank, but conclusive evidence that organic nitrogen is transported into the roots via the hyphae is still lacking.

Endotrophic Mycorrhizae

MacDougal and Dufrenoy (1944) and Downie (1941) have shown that nutrients are conveyed by endotrophic mycorrhizae to the roots of vascular plants. MacDougal and Dufrenoy conclude that auxin is produced in the fungal associate and translocated from there to the roots. These workers together with Gaumann, Braun, and Bazzigher (1950) have confirmed the early finding of Bernard that the tissues of orchid tubers contain an unidentified substance which prevents parasitism of the fungus.

SOME CONDITIONS AFFECTING THE FORMATION OF MYCORRHIZAE

Bjorkman (1942) found that the amount of light influences the formation of ectotrophic and ectendotrophic mycorrhizae. He states that no mycorrhizae develop in the dark or in less than ten per cent of full daylight. With an increase in light, mycorrhizal production increased until the maximum was reached at approximately 25 per cent of full daylight. He found addition of glucose compensated to a certain extent for the low light intensity.

Bjorkman agreed with Hatch (1937) that deficiencies of phosphorus and

inorganic nitrogen in the soil increase the formation of tree mycorrhizae. He found, however, that amounts of potassium and calcium did not affect mycorrhiza-frequency. This disagrees with the findings of Hatch.

As stated previously, Bjorkman concluded that infection by mycorrhizal fungi may be conditioned by the excess of soluble carbohydrates present in the roots of trees. If this is true, factors affecting carbon assimilation of the tree such as light, nitrogen, and phosphorus indirectly influence mycorrhiza-frequency. According to Bjorkman this theory explains the variation in mycorrhiza-frequency in different forest types.

Many of the amino acids and vitamins previously mentioned as being required by certain mycorrhizal fungi are known to exist in forest soils (Melin, 1953). The distribution and concentrations of these would undoubtedly influence the presence and amount of mycorrhizae.

Most tree mycorrhizae have been found to be acidophilic (Melin, 1953), but different species do require different hydrogen-ion concentrations. The soil reaction therefore, probably influences the occurrence of mycorrhizae.

Rayner (1934) and Melin (1946) describe certain forest soil and litter substances toxic to mycorrhizal fungi. The occurrence of these substances and the effect they have on various mycorrhizal fungi must influence the abundance of some mycorrhizal fungi.

PROBLEMS IN MYCORRHIZAE

It is evident that investigation of mycorrhizae and of their component parts in pure culture brings to light many aspects of physiology in mycorrhizae. More studies of this nature should be undertaken. If an understanding of the symbiotic relationship under controlled conditions can be obtained, then an

approach can be made on the much more complex problem of this relationship in nature.

More information should be obtained on the interactions of mycorrhizal fungi and non-mycorrhizal or litter-decomposing fungi. Studies on the physiology of litter-decomposing fungi may reveal differences between them and mycorrhizal fungi which account for the necessity of a symbiotic relationship for the latter.

A sound understanding of the nutrient exchange and relative aggressiveness of the component parts of mycorrhizae would contribute greatly to solving the problem of entrance of root rots in trees. In this connection studies on the causes and nature of disturbances of mycorrhizal equilibria in nature are also required.

SUMMARY

1. There are two main types of mycorrhizae - ectotrophic and endotrophic. These differ in their method of association with root cells.
2. Ectotrophic mycorrhizae are known to occur in most species of forest trees.
3. Endotrophic mycorrhizae are especially abundant in the families Orchidaceae and Ericaceae.
4. It is generally agreed that mycorrhizae increase the nutrient absorbing capacity of plants.
5. The fungus and higher plant components of mycorrhizae are normally in a state of equilibrium.
6. Most ectotrophic mycorrhizal fungi lack the ability to utilize cellulose.
7. Most endotrophic mycorrhizal fungi can utilize cellulose.

8. Water extracts of forest tree litter increase the growth rate of ectotrophic mycorrhizal fungi in pure culture.
9. Most ectotrophic mycorrhizal fungi are deficient in thiamin, amino acids, and other B-vitamins in pure culture.
10. There is evidence that some mineral nutrients and organic compounds pass into tree roots directly through the hyphae of mycorrhizal fungi.
11. Some environmental conditions affecting the formation of mycorrhizae are light intensity, soil nutrient deficiencies, and toxic substances in the soil.
12. The growth rate of ectotrophic mycorrhizal fungi even in the presence of vitamins, and amino acids, in pure culture is increased by the presence of pine and tomato roots.

REFERENCES

- Bernard, Noel. Recherches experimentales sur les Orchidees. Rev. gen. de Bot. 16, p. 405. 1904a. (Not seen).
- Bernard, Noel. Remarques sur l'immunité chez les plantes. Bull. de l'Institut. Pasteur, 7, p. 369. 1909. (Not seen).
- Bjorkman, E. On the condition for the formation of mycorrhiza in pine and spruce. Symb. Bot. Upsal. 6 (2): 1-190. 1942.
- Boyce, J. S. Forest Pathology. 550 pp. McGraw Hill Book Co., New York. 1948.
- Downie, D. G. Notes on the germination of some British orchids. Trans. Proc. Botan. Soc., Edinburgh 33: 94-103. 1941.
- Duggar, B. M. and A. R. Davis. Studies in the physiology of the fungi. I. Nitrogen fixation. Ann. of the Missouri Bot. Garden, 3, p. 413. 1916.
- Frank, A. B. Ueber die Ernährung gewisser Baum durch Pilze. Ber. d. d. bot. Gesell. 3, p. 128. 1885a. (Not seen).
- Frank, A. B. Neue Mittheilungen ueber die Mykorrhiza der Baume u. der Monotropa Hypopitys. Ber. d. d. bot. Gesell. 3, p. 27. 1885b. (Not seen).
- Frank, A. B. Ueber neue Mykorrhiza-formen. Ber. d. d. bot. Gesell. 5, p. 395. 1887b. (Not seen).
- Gosselin, R. Studies on Polystictus circinatus and its relation to butt rot of spruce. Farlowia 1 (4): 525-568. 1944.
- " Gaumann, E., R. Braun, and G. Bazzigher. Uber induzierte Abwehrreaktionen bei Orchideen. Phytopathol. Zeitschrift, 17: 36-62. 1950.
- Hamada, M. Studien ueber die Mykorrhiza von Galeala septentrionalis Reichb f. - Ein neuer Fall der Mykorrhiza - Bildung durch intra-radical Rhizomorpha. Jap. Jour. Bot. 10: 151-211. 1939.
- Hartig, R. Die pflanzlichen Wurzelparasiten. Allgem. forst. in Jagd-Zig, p. 118. 1888b. (Not seen).
- Hatch, A. B. A jet black mycelium forming ectotrophic mycorrhizae. Svensk, Bot. Tidskr. 28: 369-383. 1934c.
- Hatch, A. B. The physical basis of mycotrophy in Pinus, Bull. Black Rock Forest 6: 1-168. 1937.
- Kamienski, F. Les organes vegetatifs du Monotropa Hypopitys L. Extrait des Mem. de la Soc. Nat. d. Sc. nat. et math. de Cherbourg, 24, p. 5. 1882. (Not seen).

- Kamienski, F. Ueber symbiotische Vereinigung v. Pilzmycelien mit den Wurzeln hoherer Pflanzen. Arb. d. St. Petersburg, Naturf. Gesell., 17, p. 34. 1886. (Not seen).
- Knudson, L. Non-symbiotic germination of Orchid seeds. Bot. Gazette, 73, pp. 1-25. 1922.
- Kramer, P. J. and K. M. Wilbur. Absorption of radioactive phosphorus by mycorrhizal roots of pine. Science, 110: 8-9. 1949.
- Lindeberg, G. Uber die physiologie Lignin abbavender Boden hymenomyceten Symbol. Botan. Upsal. 8 (2): 1-183. 1944.
- Lindeberg, G. On the occurrence of polyphenol oxidases in soil-inhabiting basidiomycetes. Physiol. Plant. 1: 196-205. 1948.
- MacDougal, D. T. and J. Dufrenoy. Criteria of nutritive relations of fungi and seed plants in mycorrhizae. Plant Physiol. 21 (1): 1-10. 1946.
- MacDougal, D. T. and J. Dufrenoy. Symbiosis on Aplectrum, Corallorhiza, and Pinus. Plant Physiol. 19: 440-465. 1944.
- Magnus, W. Studien an der endertrophen Mykorrhiza von Neottia Nidusavis Jahr. f. wiss. Bot. 35: 205. 1900. (Not seen).
- McArdle, R. E. The relation of mycorrhizae to conifer seedlings. Jour. Agri. Res. 44: 287-316. 1932.
- McComb, A. L. and J. E. Griffith. Growth stimulation and phosphorus absorption of mycorrhizal and non-mycorrhizal northern white pine and Douglas fir seedlings in relation to fertilizer treatment. Plant Physiol. 21 (1): 11-17. 1946.
- Melin, E. Studier over de norrlandska myrmarkernas vegetation med sarkild hausyn till deras skogs-vegetation efter torrlagning. Akad. Avhandl. Uppsala, 1917.
- Melin, E. Studier over Barrtradsplantans utveckling i rahumus. Meddel. f. statesn Skogsforsoksanst 23: 433-494. 1927.
- Melin, E. Investigations of the significance of tree mycorrhizae. Gustav Fischer Jena, Germany. 152 pp. 1925.
- Melin, E. The effect of forest litter extracts on the growth of soil fungi with special reference to the mycorrhizal fungi of trees. Eng. Summ. Symbol. Botan. Upsal. 8 (3): 116-123. 1946.
- Melin, E. Physiology of mycorrhizal relations in plants. Ann. Rev. Plant Phys. 4: 325-346. 1953.
- Melin, E. Growth factor requirements of mycorrhizal fungi of forest trees. Svensk Botan. Tidskr. Bd. 48, H I, 86-94. 1954.

- Melin, E. and H. Nilsson. Transfer of radioactive phosphorus to pine seedlings by means of mycorrhizal hyphae. *Physiol. Plant.* 3: 88-92. 1950.
- Melin, E. and H. Nilsson. *Svensk. Botan. Tidskr.* 46: 281-285. 1952. (Not seen).
- Melin, E. and H. Nilsson. Transfer of labelled nitrogen from glutamic acid to pine seedlings through the mycelium of *Boletus variegatus* (Sw.) Fr. *Nature* 171: 134. 1953.
- Melin, E. and B. Norkrans. Über den einfluss der Pyrimidin und der Thiazol-komponente des Aneurins auf das Wachstum von Wurzelpilzen. *Svensk Botan. Tidsk.* 36: 271-286. 1942.
- Mikola, P. *Commun. Inst. Forest Fenniae* 36: 1-104. 1948. (Not seen).
- Modess, O. Zur Kenntnis der Mykorrhiza-bildner von Kiefer und Fichte. *Symb. Botan. Upsal.* 5: 1-146. 1941.
- Norkrans, Birgitta. Studies in growth and cellulolytic enzymes of *Tricholoma* with special reference to mycorrhiza formation. *Symb. Botan. Upsal.* 11: 1-126. 1950.
- Peklo, J. Die epiphytischen Mykorrhizen nach neueren Untersuchungen. I. *Monotropa Hypopitys* L. *Bull. intern. Acad. d. Sc. de Boheme*, 1908. (Not seen).
- Rayner, M. C. The ecology of *Calluna vulgaris*. II. The calcifuge habit. *Jour. of Ecol.* 9: 60-74. 1921.
- Rayner, M. C. Nitrogen fixation in the Ericaceae. *Bot. Gazette*, 73: 226-235. 1922.
- Rayner, M. C. The nutrition of mycorrhiza plants: *Calluna vulgaris*. *The Brit. Jour. of Exp. Biol.* 2: 265. 1925.
- Rayner, M. C. Mycorrhiza an account of non-pathogenic infection by fungi in vascular plants and bryophytes. *New Phytologist* 15: 239 pp. 1927.
- Rayner, M. C. Mycorrhiza in relation to forestry. *Jour. Soc. For. Great Britain*, 8: 96-125. 1935.
- Rayner, M. C. *Trees and Toadstools*. Faber & Faber, Ltd., London. 1945.
- Schmidt, Edwin L. Mycorrhizae and their relation to forest soils. *Soil Sci.* 64 (6): 459-468. December, 1947.
- Slankis, V. Über den Einfluss von-B-Indolylessigsäure und anderen Wuchsstoffen auf das Wachstum von Kiefernwurzeln. *Symb. Botan. Upsal.* 11: 1-63. 1951.
- Stahl, E. Der Sinn der Mykorrhizenbildung. *Jahr. f. wiss. Bot.* 34: 539. 1900. (Not seen).

- Ternetz, C. Über die Assimilation des atmosphärischen Stickstoffes durch Pilz.
Jahrb. f. wiss. Bot. 44: 353. 1907. (Not seen).
- Wilde, S. A. Forest Soils and Forest Growth. 241 pp. Chronica Botanica Co.
1944.