THE BLACK ROCK FOREST

BULLETIN No. 6

HENRY H. TRYON, *Director*

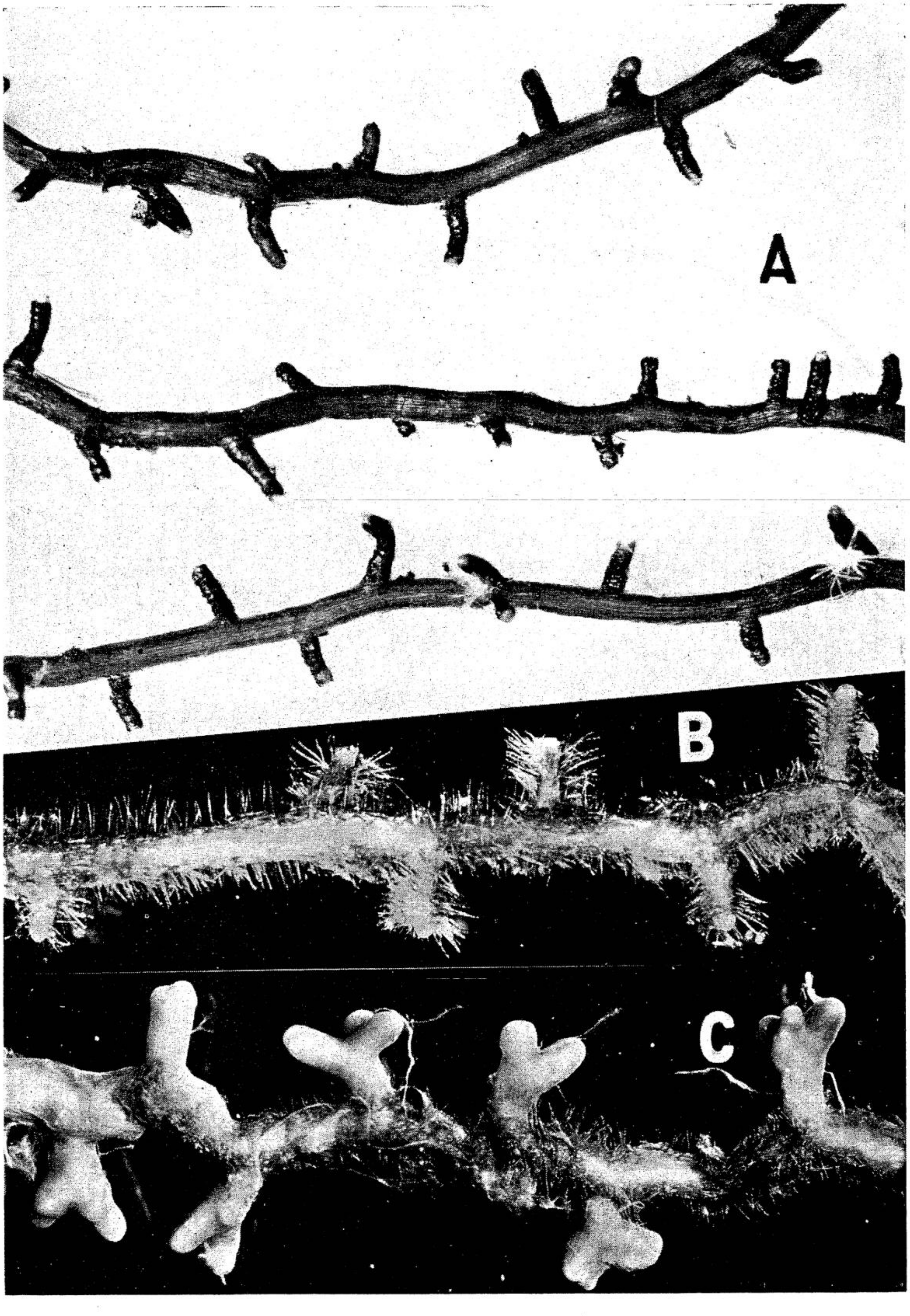
THE PHYSICAL BASIS OF MYCOTROPHY IN PINUS[1]

[1] *Based on a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Division of Biology, Harvard University, and deposited with the Widener Library, Cambridge, Massachusetts, May 1, 1935*

By

A. B. HATCH

CORNWALL-ON-THE-HUDSON, NEW YORK
1937



THE THREE POSSIBLE DESTINIES OF SHORT-ROOTS IN PINE.

A. Non-mycorrhizal in soil where mycorrhiza-formation was inhibited. B. Root-hair covered (in pure culture). C. Mycorrhizal.

From Hatch and Doak (1933).

PRINTED IN THE UNITED STATES OF AMERICA
BY CORNWALL PRESS, INC., CORNWALL, N. Y.

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ACKNOWLEDGMENTS

THE mycorrhizal problem was first suggested to the writer in 1928 by R. D. Forbes, Director of the Allegheny Forest Experiment Station. Under the stimulating guidance of the late Professor J. W. Toumey an introductory survey of the subject was started at Yale University in the same year.

By means of two successive appointments as Fellow of the American-Scandinavian Foundation, the writer was enabled to study at the Royal Academy of Forestry and at Uppsala University, Sweden, under Professor Dr. Elias Melin. Professor Melin gave freely of his intimate knowledge gained during his pioneer studies of the mycorrhizal habit and enabled me to begin with the advantage of his many years of experience. The success of many of the early experiments was due to him.

On returning to America the study was conducted as a joint project of the U. S. Forest Service and the Bureau of Plant Industry at the Allegheny Forest Experiment Station under the sponsorship of Mr. Forbes and Mr. E. N. Munns, then chief of the Division of Silvics, U. S. Forest Service. Coöperation in the pure culture studies was also extended by the Boyce Thompson Institute for Plant Research through the Director, Dr. William Crocker, to whom I am most indebted.

The work was completed at Harvard University under the able and constantly helpful sponsorship of Professor J. H. Faull. Professor Faull provided me with ample facilities and invaluable guidance. I am indebted to him for innumerable kindnesses.

From its inception in Sweden until the completion of the study at Harvard in 1935, active coöperation was extended by Professor P. R. Gast of the Harvard Forest. The plant material resulting from his experiments during 1929-1934 were given to me for the study of the mycorrhizal phases, and he shared with me his interpretation of the experiments we both devised to advance my studies of the effect of nutrition on the development of mycorrhizae. His keen perception and generous help and counsel furthered the work in a multitude of ways.

For the support which made possible the studies at Harvard University I am indebted to the continued assistance of the Arnold Arboretum, the Black Rock Forest, the Harvard Forest, and the Anna C. Ames Memorial Fellowship. The facilities of the Harvard Forest were made available in these coöperative studies through the kindness of the late Professor R. T. Fisher, Director; those of the Arnold Arboretum through the kindness of Professor Oakes Ames, Supervisor. It is also a pleasure to acknowledge the use of the facilities of the Biological Laboratories made available through the Department of Botany, Division of Biology, Harvard University.

Through Professor Fisher arrangements were also made for carrying out a considerable part of the study at the Black Rock Forest where generous support and ample facilities were offered. I worked coöperatively with Mr. H. L. Mitchell, Assistant Director, whose part in the study has been published in Black Rock Forest Bulletins and Papers. I am also grateful to Mr. Mitchell for permitting me to reproduce his figures and refer to his results which are being published simultaneously as Black Rock Forest Paper Vol. I, No. 10.

Aid has also been received from Dr. K. D. Doak, Division of Forest Pathology, Bureau of Plant Industry, Allegheny Forest Experiment Station, with whom joint studies were carried out and to whom I am indebted for the figure in Plate I, B. Dr. F. O. Holmes, Dr. J. Youden, Dr. L. O. Kunkel, and Mr. Reno Marini assisted in the work at the Boyce Thompson Institute for Plant Research. Professor Dr. Torsten Lagerberg, Professor Dr. Heinrich Hesselmann, and Professor Dr. O. Rosenberg, and Froken Anna Pettersson contributed time and (or) facilities for the studies in Sweden. Professor G. Nichols, Professor H. Castle, and Professor Carl Deuber aided in guiding my work at Yale University and Professor Oakes Ames, Professor A. E. Navez, Professor W. H. Weston, Jr., and Professor R. H. Wetmore helped me in numerous ways at Harvard University.

For secretarial aid in preparing the manuscript for publication, I also wish to thank Dean D. S. Jeffers of the School of Forestry, University of Idaho.

I am especially grateful to Constance Talbott Hatch for aid in planning and carrying out many of the experiments and for other able assistance.

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JUNE, 1937.

I. INTRODUCTION

Most biologists are accustomed to think of the absorbing roots of trees in terms of those of agricultural plants raised in sand culture. We picture vigorous root tips, protected as they push through the soil by special caps and covered for a short distance back of these by dense outgrowths of root hairs. It is well known that these hairs serve to increase the absorbing surfaces of primary root-ends. They are regarded, almost universally, as the normal and essential organs of absorption in practically all land plants which possess true roots.

Classical though this picture may be, it does not represent, even remotely, the actual conditions in the great majority of trees nor in many other plants. In the forest, roots are invariably invaded by soil fungi, and in some trees these roots are not only modified so fundamentally that they lack root hairs entirely but they also possess no direct contact with the soil from which their nutrients are obtained. Soon after the epidermal cells of these roots are first differentiated they become deeply imbedded in a compact fungal tissue which surrounds them until their death months later (Plates I, A and B; IV, A and B). The comparatively simple processes of absorption by root hairs must here be replaced by a complicated system of nutrient exchange between two widely different members of the plant kingdom. To reach the conduction tissues, soil water and nutrients must be absorbed by fungal hyphae, and then must pass through a parenchyma-like fungal mantle and finally through or around several layers of cortical cells which are separated from one another by a continuous net-work of intercellular mycelium.

Such a compound structure is regarded neither as root

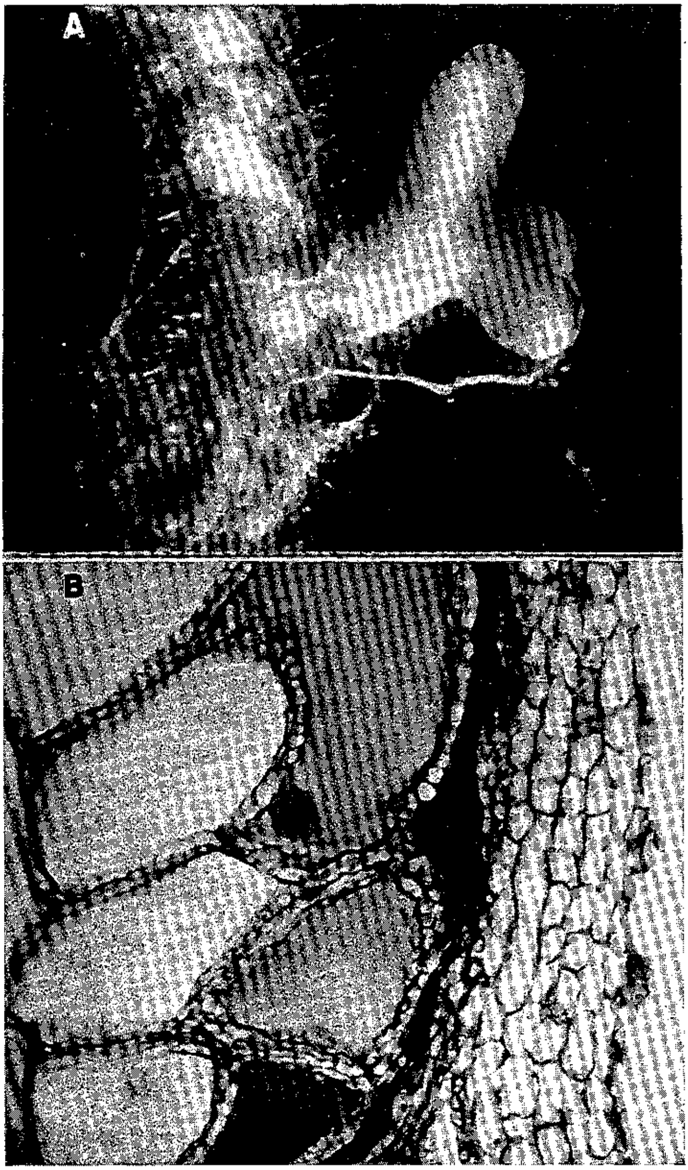

PLATE I. APPEARANCE AND STRUCTURE OF AN ECTOTROPHIC MYCORRHIZA.

A. One short-root of *Pinus Strobus* growing from a root-hair covered long-root showing early stages of dichotomized branching, external view of the fungal mantle, and fungal rhizomorphs extending from the mantle (x 30).

B. Longi-section through a mycorrhiza showing parenchyma-like fungal mantle, dark colored crushed epidermal cells, normal cortical cells of root separated from one another by a fungal mantle one to two hyphae thick. Note the nucleus in one of the cortical cells (x 889).

B Furnished by Dr. K. D. Doak.

nor as fungus (Frank, 1885a), but rather as an association of two different components producing a distinct morphological organ—an organ which is analogous in structure to the thallus of lichens. It is termed a *mycorrhiza,* a compound Greek term meaning *fungus root.* In 1900 Stahl designated the processes of plant nutrition by means of mycorrhizae as *mycotrophy,* or fungus nutrition.

Such dual organs are of two types; namely, (1) *ectotrophic,* those in which the fungal elements are arranged intercellularly and also as a mantle over the root surface and (2) *endotrophic,* those in which the mycelium is characteristically intracellular and appears on the surface of the root as individual hyphae only, at the points where they entered the roots from the soil. Organs of the ectotrophic type are found in all members of the Abietineae, Fagaceae, Betulaceae and Salicaceae, in several individual woody genera, and in a few herbs. They are the type dealt with in this study. In the plant kingdom as a whole, endotrophic mycorrhizae are much more common; they have been found in the majority of the terrestrial plants in which they have been sought (Kelley, 1932; Asai, 1934).

The widespread occurrence of mycorrhizae in trees has recently attracted the attention of foresters, who have questioned their significance and the possibilities of regulating their occurrence. But at present[1] knowledge on the probable significance of the habit in trees is largely theoretical or circumstantial. While many theories have been advanced during the last half century, only two explanations have received any general acceptance; namely, (1) that mycorrhizae represent a pathological condition in which the fungus parasitizes the root more or less at the expense of the tree, and (2) that they facilitate the utilization of organic nitrogen in the humus of forest soils.

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[1] May, 1935.

The pathogenic viewpoint is based, in part, on the erroneous beliefs (Aldrich-Blake, 1930; Hatch and Doak, 1933) that the fungus inhibits normal root elongation and that mycorrhizae are relatively infrequent structures. The conclusion is also inescapable that proof of benefit to trees from association with mycorrhizal fungi has not as yet been provided (May, 1935). In part, the pathogenic viewpoint seems also to be the result of a rigid discipline in thinking about the character of biological interrelationships. Most scientists have rightly developed a conditioned reflex toward anything which even remotely savors of teleology. The conception of antagonistic, reciprocal parasitism, the net result of which may be benefit to both parties, need not come under this category. The highly conservative training of the pathologist in particular often precludes a ready appreciation of how mutual benefit can arise in a basically hostile relationship. I know of no more simple or lucid portrayal of the way in which such a relationship may come into being than that visualized by Wells, Huxley, and Wells (1934, p. 934):

"Fungus and tree-root penetrate where they can and increase their substance blindly at the expense of whatever material they find; selection sees to it that beneficial variations are appropriated as they turn up. The fungus invades, the root resists; there is a balance between the invasion and the defence; there are some advantages on both sides, but damage on both sides also. The advantages reaped by either of the competitor-partners may cancel out its losses, and the net result be nil; or one may achieve a net benefit and to a certain degree exploit the other; or adjustment may be made so that in both the benefit outweighs the damage, and thus a true symbiosis arises."

The second theory, namely, that tree mycorrhizae facilitate the utilization of organic nitrogen from humus soils, has a far more attractive background than the pathogenic view. Nitrates are the common sources of nitrogen in agricultural soils and the absorption of nitrogen in the form of NO_3 may be confirmed by its appearance in quantity in the tissues of plants growing in these soils. In the humus soils of forests, on the other hand, nitrates are rarely found, nor have they been demonstrated in the tissues of trees. These facts, coupled with the known ability of fungi to decompose and utilize the complex organic material of humus, provide a striking circumstantial case in favor of the rôle of these organs as nitrogen gatherers. Furthermore, the struggle in the plant kingdom for nitrogen is severe, and accessory symbiotic organs for acquiring supplementary supplies of this element are frequent in many plant groups (especially legumes).

Leaving the question of the *manner* by which mycorrhizae may benefit trees and turning to that of their gross effect on growth, circumstantial evidence of their usefulness is even more convincing.

About twenty years ago an apparent case of seedling dependence on mycorrhizal fungi was discovered and studied by Melin (1917) in Sweden. Seedlings of pine and spruce, started from wind-distributed seeds in recently drained peat bogs, were found to grow normally only when they became infected with mycorrhizal fungi transmitted by wind or other agencies from the surrounding woodlands. Those seedlings which lacked infection exhibited unmistakable evidences of nitrogen starvation and eventually died.

The second example and later others of similar character concern the initial failures but subsequent recoveries, following artificial soil inoculation, of seedlings in as many as sixteen new forest nurseries and several plantations. Fourteen nursery failures are reported from Western Australia alone where afforestation on a large scale is still in progress. In each nursery seeds of pines germinated normally and produced seedlings which at first were healthy. But except for an occasional group of seedlings the plants soon ceased growth, turned yellow, and thereafter gradually died (Kessell, 1927). Although the trouble was first diagnosed as a nutrient deficiency, it was found that fertilization was of only temporary benefit. An examination of the roots revealed no pathological conditions but it was noticed that, except for the few groups of seedlings which grew healthily, the coralloid roots (mycorrhizae) which nurserymen in other regions had learned to associate with healthy stock were absent. Small quantities of soil from established nurseries in other regions were worked into a few of the seed beds and the pines in these beds recovered and thereafter grew normally. Their recovery was accompanied by the development of mycorrhizae on their root systems. Subsequent inoculations of seed beds in all nurseries saved the entire afforestation project from abandonment.

An identical experience was recently described from Southern Rhodesia where a new nursery in an unforested area was finally saved from abandonment by seed-bed inoculations (Anonymous, 1931). In Java and Sumatra (Roeloffs, 1930) plantations of seedling stock which lacked mycorrhizae have been saved by subsequent interplanting with mycorrhizal seedlings. Still another example is reported from the Philippines where attempts over a period of twenty years to grow *Pinus insularis* from seeds in the lowlands outside of the tree's natural range had invariably failed (Oliveros, 1932). Seedlings transplanted to the lowlands from nurseries located within the highland range of the tree, however, grew more luxuriantly in the new environment than in the old. Not until seed beds were inoculated was it possible to raise the pines from seeds at the low altitudes.

These examples afford striking support for the view that mycorrhizae are beneficial, though it must be admitted that the evidence is circumstantial only. The truth is that there is still lacking incontrovertible proof, such as was adduced long ago for the nodule bacteria of legumes, that mycorrhizal fungi, and they alone, are responsible for the stimulated growth of seedlings in such nurseries. The use of pure cultures of these fungi for inoculating the seed beds can alone supply this essential demonstration.[1] Until such time as this type of information is available all views on the function of mycorrhizae must remain open to question. But in the meantime the indications are plain that a knowledge of mycorrhizae, of the mechanics of nutrient acquisition in trees, and of the rôle of symbiosis in the life of the forest may one day assume a practical importance which was hardly suspected a decade ago.

In addition to uncertainties about the physiological nature of the mycorrhizal relationship is the question of the occurrence of these structures in nature. The impression seems to be quite general among both foresters and botanists that agreement on this point has not been reached. One knows only that some students claim that in forest soils all short-roots are mycorrhizal. Others maintain that, in common with leaf-spot diseases, mycorrhizal infections wax or wane according to seasonal variations which influence the growth and distribution of the fungus. Still others report the finding of healthy trees which wholly lack mycorrhizae. Which of these views is correct? Or if all possibilities actually occur what processes of reasoning have led some students to believe that the relationship is beneficial to the tree and others to conclude that it is detrimental? Quite obviously the whole subject of mycorrhizae is in need of thorough inquiry.

The purposes of this paper are: (1) to assemble a literature which demonstrates that for more than a century investigators of tree roots in natural habitats have found

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[1] Since this was written experiments involving the introduction of pure cultures of mycorrhizal fungi into soils which normally lack ectotrophic mycorrhizal fungi (Hatch, 1936; Young, 1936) have provided proof of the beneficial nature of mycorrhizae in pine. Rayner (1935) has likewise employed pure culture inoculations but in seed beds which already possessed mycorrhizal fungi.—Author, July, 1936.

mycorrhizae to be widespread and habitual, (2) to supply a critical and weighted review of knowledge and theories on the factors controlling the distribution and abundance of ectotrophic mycorrhizae and of their significance in tree growth, (3) to report studies which have led the present writer to discard many current concepts on the mycorrhizal habit (ectotrophic) and to suggest a more adequate explanation of this phenomenon, (4) to indicate some of the problems in need of solution and the more promising methods of attack, and (5) to point out the probable applications of knowledge on mycorrhizae in forestry.

II. REVIEW OF LITERATURE

PREVALENCE OF ECTOTROPHIC MYCORRHIZAE

THE possibility that the mycorrhizal habit may be of significance in plant growth is obviously bound up with its occurrence in nature. If infected roots are scarce they can have little influence on tree growth; conversely, if they are everywhere numerous, it is probable that they exert profound effects either beneficial or injurious depending upon their character. While botanical and forestry literature is replete with evidences of the universality of the habit, this fact is little appreciated by other than specialists. In fact, as Kelley (1932) has pointed out, mycorrhizae are today quite frequently regarded as exceptional if not pathological structures. It is rather surprising then to find that even the original classification of root-systems in many trees into *short-* and *long*-roots (a classification proposed by Th. Hartig in 1852, long before the dual character of short-roots was recognized, and still in use) was based on the differences which today we recognize as those which separate mycorrhizal and non-mycorrhizal roots respectively. Furthermore, there is only one modern student of the habit in trees who does not recognize that these infections are widespread and habitual.

The general lack of appreciation of such facts is in part a result of the tendency to review the theories and controversies of the subject rather than to supply a critical survey of actual observations on the abundance of mycorhizae; in part it is the result of a too literal acceptance of the root-hair picture of absorption as found in many agricultural plants. In this section it will be pointed out that for more than a century the commonest type of root encountered by investigators of trees growing in natural habitats seems to have been mycorrhizal.

The Early Period

In most plant roots the mycorrhizal fungi are endophytic and hence stained sections must be prepared before their presence or absence can be verified. When this was done by early students they usually recognized the presence of the fungus. By far the majority of root studies, however, have not involved the preparation of stained sections and the fact of fungal infection has been completely missed. But fortunately, in those plants which possess ectotrophic mycorrhizae, the presence of the fungus is obvious, to the experienced student, from external appearances. The fungus commonly gives the short-roots a white and more or less glistening appearance and causes them to swell and to branch in various distinctive manner (Plates I, XIII, XIV, XV).

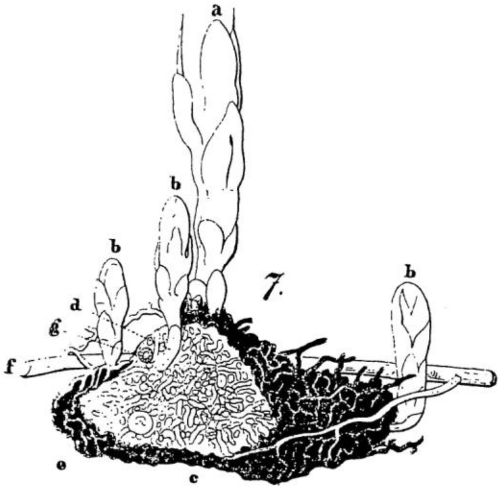
It follows that in trees possessing ectotrophic mycorrhizae, external drawings of the root habits alone will disclose the presence of mycorrhizae. There are many such drawings in the forestry and botanical literature of the nineteenth century. From these drawings and from written descriptions (a) of supposed parasitisms on tree roots (*Monotropa,* truffles, etc.), and (b) of the structure and morphology of tree roots, a wealth of information on the general occurrence of mycorrhizae may be gathered. In many cases this information is much more valuable than that gained from more recent studies because early students were not influenced by preconceived ideas on the character of root systems. Indeed, although they described typical mycorrhizae in every respect, a few investigators did not even recognize that the roots which they regarded as the normal absorbing organs of trees were infected by fungi. To them the fungal mantle was a protective layer of very minute cork cells, and the intercellular-net hyphae of the cortex were curious wall thickenings of unknown function. Quite obviously material of this unbiased character, material assembled from the literature of a period which knew no plant symbioses and which, therefore, was not encumbered by theories of parasitism, of altruism, or of simple physio-chemical relationships, has a value in a review of this kind which is unique.

THE LITERATURE OF THE ASSOCIATION OF *Monotropa* SP. AND OF TRUFFLES WITH TREE ROOTS

The curious nature of the absorbing systems of members of the genus *Monotropa,* combined with their lack of chlorophyll, very early led taxonomists to regard these plants as root parasites. That this view is erroneous was first suggested by Graves (Curtis and Hooker, 1826, III), who washed out clumps of *Monotropa hypopitys* from the soil and found that "they were decidedly not attached to the roots of other plants but were only entangled among them." Graves found the roots covered with ". . . a whitish, silky, somewhat fibrous material, connecting them with the decaying leaves and other vegetable substances among which they grow, . . ." Graves also took up large groups of *Monotropa,* transplanted them to other woodland habitats, and found that they continued to grow for six or seven years. The complete independence of *Monotropa* from other cormophytic plants was also demonstrated in 1823 by Hooker (Exotic Flora, 1825, II, p. 85) who raised *M. uniflora* in the Botanical Gardens of Glasgow. The plants grew ". . . in a box of earth which was sent containing other rarities from the neighborhood of Montreal. . . ."

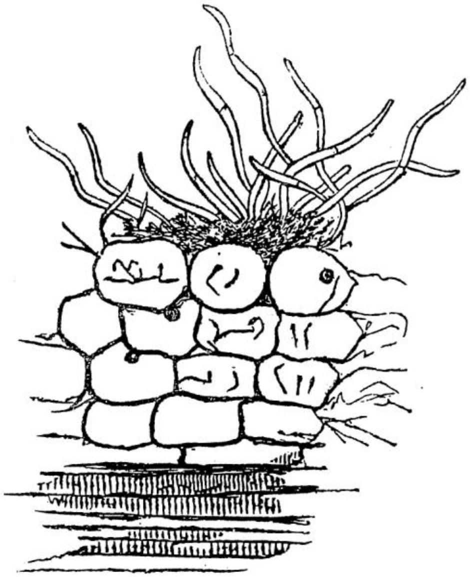
The first to recognize the fungal nature of the outer covering of *Monotropa* roots was the renowned Swedish mycologist, Elias Fries (1832). He designated the fungus *Tuburcinia Monotropa.*

Eight years later in his "Beitrage zur Kenntniss der parasitischen Pflanzen," Unger (1840) reproduced a drawing of this plant (Text Fig. 1) which beautifully illustrates the mass of mycorrhizal roots intermixed with those of *Picea excelsa.* Of this mass Unger wrote: "Der einem Rhizome änliche knollenformige, unregelmässige Körper, woraus die Bluthenschäfte dieser Pflanze entspringen, besteht aus einem Convolute innig verfiltzter Wurzelfaseren, welche zum theil den Parasiten zum theil der Nährpflanze (*Pinus Abies* L.) angehoren. . . ." Neither in his drawing nor in the text does Unger differentiate the roots of the tree from those of the smaller plant and the parasitic contact was believed to be external, in the coralloid mat or "Filz" formed by the two root systems. There can be little doubt that the roots of both plants were typically mycorrhizal.



TEXT FIG. 1. MONOTROPA AND INTERMIXED ROOTS OF TREES.
(After Unger, 1840).

In England during the following years a controversy on the mode of nutrition of *Monotropa* appeared in the "Phytologist," but the work of Graves and Hooker and of Fries was not cited. Of the five participants in the controversy all referred to the nature of the outer covering of the roots and the last investigator, Rylands (1842), established its fungal nature. Luxford (1841), who introduced the subject, referred to the outer covering of the roots as a "bysoid fungus"; Lees (1841) considered it to be in the nature of "suckers"; Wilson (1842) believed it to be "an intrinsic and essential part of *Monotropa*—the part to which I have applied the name root." The matter was apparently settled to the satisfaction of all concerned by Rylands (1842) who, by the use of improved microscopical technique, demonstrated that the covering of these roots was really fungal in nature. Drawings (one of which is reproduced in Text Fig. 2) of a longitudinal section of a root showing this fungal layer were included and the identities of the fungi found in this layer, as determined by M. J. Berkeley, were given.



TEXT FIG. 2. PART OF A LONGISECTION OF A *MONOTROPA* ROOT.
(After Rylands, 1842).

The controversy in England remained unknown on the Continent (indeed, it was not discovered until 1890 by Oliver) and the independent nature of this chlorophyll-less plant was not again clearly established until 1881-1882. In the interim many descriptions and drawings and references to the fungi in the curious roots of these plants continued to appear (Duchartre, 1846; Fries, 1849; Bonorden, 1851; Schacht, 1854; Chatin, 1856-65; Solms-Laubach, 1867-68; Brandt, 1849; and Drude, 1873). In addition to observing the fungal mantle Drude described penetration of the hyphae into the roots and noted that such infections were constant features of these roots. In concluding that the plant was a root parasite Drude described "haustorial-like" branches belonging to *Monotropa* which extended into the roots of beech and spruce, ". . . doch fand ich bald, dass wirklich starkeren Wurzeln der *Monotropa* kurze Adventivwürzelchen aussenden, welche in die dünneren Wurzeln sowohl von Buchen als von Fichten eindringen" (Drude, 1873, p. 44).

In the final papers of this long controversy Kamiensky (1881-1882) satisfactorily demonstrated (for the third time) that *Monotropa* grows quite independently of tree roots. He gave the first full and accurate account of the structure of the roots in which both the mantle and intercellular hyphae were recognized and figured. Roots of trees growing intermixed with those of *Monotropa* were also found to be infected and to exhibit a structure exactly comparable with that in the latter plant; "Les racines de quelques arbres, surtout des hêtres presentent avec racines du *Monotropa* la plus grande resemblence" (Kamiensky, 1882, p. 31). This fact led Kamiensky to attribute Drude's error in concluding that the plant is a parasite to his mistaking infected beech and spruce roots for haustorial branches of the *Monotropa* plant.

The conclusion seems unavoidable that this similarity between tree and *Monotropa* roots, which rendered them identical to the early worker when intermixed, was responsible for the entire controversy. The blending of the two types of roots into one superficially homogeneous mass, so beautifully illustrated and described by Unger (Text Fig. 1), is even more substantial evidence in support of this view than Drude's haustorial branches.

Another relationship (still unsatisfactorily understood) with tree roots, from which a number of references to the mycorrhizae of trees may be obtained, is that between truffles and forest trees. Among the earliest references to this association are those of Tulasne (1841) and of Vittadini (1843). Indications of the abundance of infected roots are not contained in these papers; but in 1862 Tulasne (p. 19-20) proposed that the relationship was a special kind of symbiosis. This conclusion would scarcely have been reached had he not found that the two organisms were rather constantly associated.

In one of the notable papers of this period, ''Du parasitisme probable de quelques especies du genre *Elaphomyces* . . .'', Boudier (1876, p. 115-116) recorded the constant association of tree roots with these hypogeous fungi. He noted that all mycologists who had examined species of *Elaphomyces* with yellow peridia agree in observing that the pockets in which these fungi are found are lined with a reddish network resembling mycelia. But other species form similar linings and these linings were actually found to be infected roots of associated trees, namely, birch, oak, and chestnut, ''. . . d'un reseau rougeatre imitant tres-bien un mycelium, mais forme par les radicelles des arbres et abrisseaux voisins . . .'' (*op. cit.,* p. 115). They were described as turgid, irregular roots which ramify in an abnormal way and become so numerous, crowded, and entangled that they fill all of the spaces between the irregularities on the surface of the *Elaphomyces,* and these modifications of the roots were attributed to the fungus. Boudier noted that the mycelium which covers the surface of the roots does not penetrate beyond the first layer of cortical cells and that the interior remains uninfected. He even observed that the activity of the infected roots was apparently stimulated rather than reduced. Roots of this character were also discovered in places where the fructifications of truffles were absent.

In the same year Condamy (1876) published a series of ten color plates in which the connections between the mycelia of truffles and literally thousands of growing root tips were illustrated. While the details of these roots were not described, association of the mycelia with root tips only adequately indicated that these roots were mycorrhizal.

The frequency with which the mycelia of *Elaphomyces* are found in association with tree roots was again the subject of a paper by Reess in 1880. He described mycorrhizae in some detail and noted penetration of the mycelia within the roots as had Boudier before him.

It was this work by Reess which formed the starting point of the well-known investigations of Frank (1885), which were pursued at the outset with the object of learning whether or not the production of truffles in beech forests could be increased. While Frank's study yielded no information on this question it did lead to publication of the observation that in forests the short-roots of Cupulifers are invariably mycorrhizal. Frank's work is taken up in detail in a subsequent section.

EVIDENCE ON THE PREVALENCE OF MYCORRHIZAE OBTAINED FROM EARLY STUDIES ON THE CHARACTER OF ROOT SYSTEMS

Robert Hartig (1888, p. 122) once remarked, after describing Frank's theory of mutual benefit, ''Diese Ansicht ist offenbar geeignet, alle bisher bestehenden Anschauungen über die Ernährung der Bäume umzudrehen.''

Undoubtedly all previous ideas on the absorption process in trees *had* been modeled after those for agricultural plants. Yet in 1888 there was already in existence an extensive literature which pointed to the distinctiveness of the process in tree roots,—a literature to which Theodor Hartig, Robert's father, had been an outstanding contributor. But Robert Hartig did not appreciate the significance of these contributions. The task of interpreting this early literature was first accomplished by George Sarauw in 1893a in his monograph, ''Rodsymbiose og Mycorrhizer.'' Much of the valuable material for establishing the general character of the mycorrhizal habit which Sarauw assembled in this scholarly review has long been overlooked or forgotten. In view of the importance of this material in any survey which endeavors to clear up uncertainties about the mycorrhizal habit, its recapitulation here needs no apology.

In modern texts of silvics, plant physiology, and anatomy, drawings are provided of the root tips of corn or of other agricultural plants as they appear when grown in moist air or in sand culture. Forestry texts of the nineteenth century were equally constant in displaying the roots of pine or of fir gathered from trees grown in forest or nursery environments.

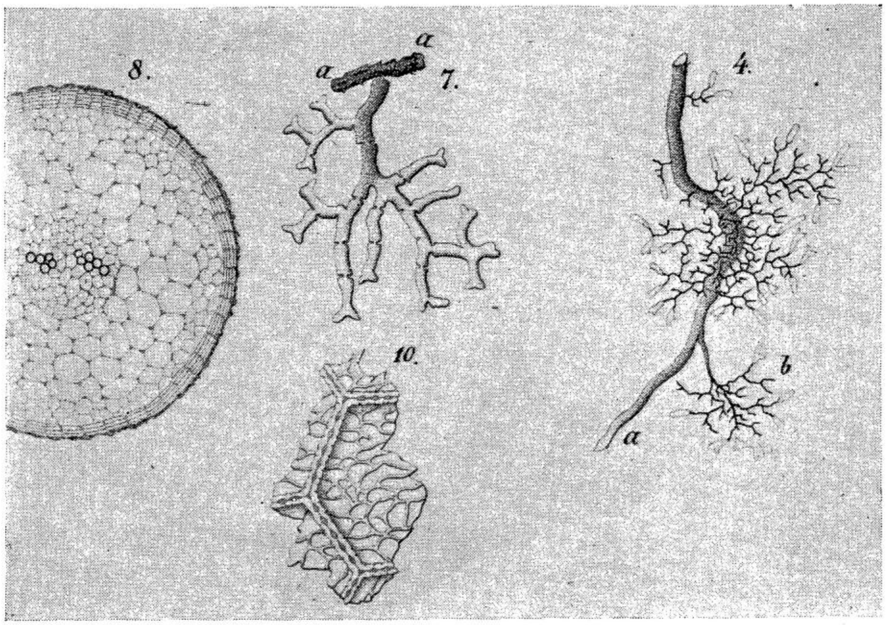
One of the most widely used forestry texts in Germany during this period was the ''Lehrbuch für Förster,'' a text which was carried through eleven editions (1807-1879) by three successive generations of the Hartig family. Starting in 1840 and appearing in all subsequent editions, a drawing of the absorbing roots or ''Saugwurzeln'' of fir was included which undoubtedly shows mycorrhizae. The same drawing was also included in the separate editions of Th. Hartig's ''Luft-, Boden- und Pflanzenkunde in ihrer Anwendung auf Forstwirthschaft.''

Another German text of the period, ''Der Baum'' (Schacht, 1853; Plate 1, Fig. 18; Plate 2, Fig. 4), includes drawings of whole seedlings of *Abies pectinata* and of *Pinus sylvestris*. Modern studies have shown that in the absence of infection by mycorrhizal fungi (that is, in sand culture) the short-roots both of fir and of pine remain simple and unbranched. When infected by mycorrhizal fungi, they become swollen and branched, racemosely in fir, but dichotomously in pine. Schacht's drawings show the short-root tips of both to be white and swollen. Those of *Abies pectinata* are branched racemosely, those of *Pinus sylvestris* in some cases are branched dichotomously. There can be little doubt that both seedlings were mycorrhizal.

TEXT FIG. 3. ROOTS OF *ABIES PECTINATA*, SHOWING CORALLOID MYCORRHIZAL SHORT-ROOTS.
(From Schacht, 1860).

In subsequent editions of Schacht's text (both the German and French) these figures were replaced by a more extensive drawing of the roots of *Abies pectinata* (Schacht 1860-1865; Plate 1, Fig. 20). Again the white swollen tips and racemose branching of the short-roots beautifully typify the mycorrhizae of this fir (Text Fig. 3).

The most frequently cited illustration of mycorrhizal roots in this period are those of Th. Hartig (Text Fig. 4) which appeared in his "Vollständige Naturgeschichte der förstlicher Culturpflanzen Deutschlands'' (1840-1852, Plate 18, Figs. 4-10). In these both the fungal mantle and the intercellular mycelial net are well illustrated by sectional drawings. But contrary to current opinion (Rayner, 1927, p. 6) Th. Hartig did not regard the fungal elements in these roots as parasites. He did not even suspect that the structures which he described were anything other than normal inherited anatomical parts of the short-roots. Although he once more referred to the intercellular net in 1863 and reproduced the drawings (Text Fig. 4) again in his ''Anatomie und Physiologie der Holzpflanzen'' in 1878, the character of the fungal net which now bears his name was never understood by Th. Hartig.



TEXT FIG. 4. LONG-ROOTS OR "TRIEBWURZEL" (4, a). SHORT-ROOTS OR "SAUGWURZELN" (4. b and 7). CROSS SECTION OF A SHORT-ROOTS (8). INTERCELLULAR MYCELIUM OR "ANASTOMOSIRENDES GEFLECHT" (10).
(From Th. Hartig, 1852).

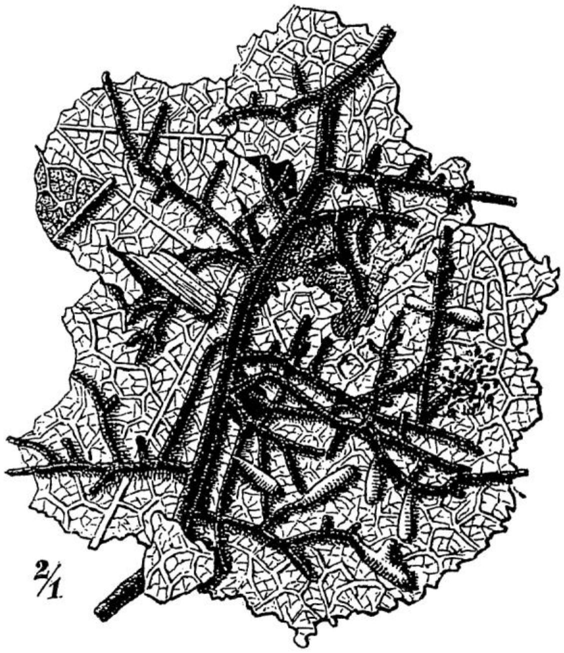
As Sarauw (1893a, p. 51) pointed out, the very fact that these figures were included in the forestry publications of the period is excellent evidence that mycorrhizae (although not recognized as dual structures) were regarded as the normal and typical absorbing organs (Saugwurzeln) of trees. Sarauw also noted that in the entire silvicultural literature of the period not a single illustration of the roots of older trees is found that does not show evidences of the presence of the fungal mantle. This fact was regarded by him as of profound significance in establishing the generality of the habit. ''Ikke uvigtigt for Betydningen af Spørgsmaalet om Mykorrhizernes Optraeden forekommer det mig derfor at vaera, at der i den hele tidligere til Skovbruket egentlig knyttede Literatur ikke findes nogen Afbildning af Sugerødder hos aeldre Skovtraeer uden Svampeskeden.''

Before the true nature of Hartig's ''Anastomosirenden Geflicht'' (intercellular network of mycelium) was finally established, three other students of root anatomy had observed and recorded these curious wall structures in absorbing roots (Nicolai, 1865, p. 62; Van Tieghem, 1871, p. 187; and Resa, 1877, p. 26). It is of interest that Resa, who regarded them as a series of intercellular spaces surrounding the cortical cells, reported that they were not confined to the short-roots but occurred also in some long-roots.

Another series of papers, concerned chiefly with the occurrence, character, and functions of root hairs provides additional indications of the frequency with which mycorrhizae were encountered during this period. The first to recognize that fungi are present in tree roots was the Italian investigator Gasparrini (1856) who discovered them while studying the nature and function of root hairs. He regarded the organism in the short-roots of *Pinus Halepensis* as an alga; but in *Corylus avellana* and *Castanaea vesca* it was regarded as fungoid. In all cases, however, Gasparrini was apparently confused as to the nature of the fungal mantle, which he regarded as a part of the root.

The infrequency of root hairs in many forest trees attracted the interest of Hartig (1840), Gasparrini (1856), Schacht (1859-1860), and Schwartz (1883). While Schacht found them occasionally on all trees except *Picea excelsa,* Schwartz reported them to be uniformily absent. In first-year seedlings, on the other hand, according to Hartig, Gasparrini, and Schacht, root hairs were abundant, and this fact was subsequently substantiated by Klebs (1885, p. 543), Reess and Fisch (1887, p. 22), Frank (1888, p. 255), V. Tieghem (1888, p. 343), etc., and by modern investigators. It is also known through modern studies that root hairs are usually produced on long-roots. With mycorrhizal short-roots, however, the nature of the fungal covering obviously excludes the possibility of root hairs appearing. Furthermore, it is now known that seedlings in their first year (Frank, 1885, p. 132; Möller, 1903, p. 325; Melin, 1917, p. 367, and 1927) frequently lack or form but few mycorrhizae. These facts strongly suggest that the early reports of the infrequency of root hairs on forest trees, except in one-year seedlings, may have had their origin in the unrecognized fact that fungal mantles replace root hairs on short roots in plants over one year in age. It is interesting to note in this connection that Schwartz (1883, p. 168) found abundant root hairs on *Taxus baccata,* a plant possessing endotrophic mycorrhizae, in which the formation of hairs is not suppressed by fungal infection.

Sarauw (1893a, p. 172-173) believed that references to root hairs with more than one cell also shed some light on the frequency with which early workers encountered fungus-infected roots. As early as 1794 Franz von Paula Schrank (p. 52) reported all root hairs of trees examined by him to be several-celled. Link (1837, I, p. 359, 369, 381; II, p. 33-35) separated roots according to those with one- and those with two-celled hairs, and both Treveranus (1835, p. 376-377) and Gasparrini (1856, p. 42) believed root hairs, in some cases, were more than one-celled. Th. Hartig (1840-1878, Plate 1, Fig. 14) also illustrated several-celled root hairs. But root hairs in most Phanerogams are simple elongations of cortical or epidermal cells and never possess septa (Schwartz, 1883, p. 176). Sarauw (p. 172), accordingly, was certain that in all cases where authors had referred to root hairs with more than one cell, they had confused septate fungal hyphae with root hairs. This recalls (Sarauw, 1893a, p. 173), a remarkable admonition by C. A. Agardh (1829-30, p. 120) "... that the fine hairs which appear on certain parts of the roots where the soil is not tightly packed are fungus-like formations and should not be confused with plant organs"[1] (my translation).



TEXT FIG. 5. MYCORRHIZAL ROOTS OF BEECH GROWING BETWEEN LEAVES.
(From Müller, 1878, 1886).

Still without recognizing their true nature, a number of excellent drawings and descriptions of mycorrhizal roots of beech were published by Müller in 1878 (p. 31-43, 63, 141-143, Figs. 4-7). The roots were branched racemosely

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[1] The ease, for example, with which fungal hyphae can be confused with root hairs, even when the association of fungus with roots is known, is well illustrated in a drawing of a beech root by P. E. Müller (1878). Müller recognized his error after the appearance of Frank's paper of 1885 and reproduced his earlier figures again (Müller, 1886), but with a correct legend.

between decaying leaves, to which they were tightly bound by fungal hyphae (Text Fig. 5). In Müller's opinion the fungus was useful in that it brought about more intimate contacts between absorbing roots and the leaves from which nutrients were drawn (*ibid.,* p. 42-43). Although Müller did not recognize the real character of the association, his descriptions and drawings (Text Fig. 5) adequately establish the fact that the associations were habitual.

Another factor in directing attention to fungus-infected roots in this period was the current interest in the phylogenetic significance of the dichotomous habit in roots. Janczewsky (1874) believed dichotomy, in all Phanerogams, to be the result of parasitic invasions by microorganisms. Thus in *Pinus Strobus* L. he accounted for the constant forking of short-roots by an equally constant infection by a "meistentheils epiphytischen Pilz" (Janczewski, 1874, p. 116). Although it has been established by pure culture experiments (Melin, 1925; Hatch and Doak, 1933; and Plate III, G, of this paper) that dichotomy in pine can also be produced in the absence of the fungus by certain conditions of the nutrient environment, the fact remains that in nature this type of short-root branching is almost invariably the result of fungus infection.

The question of the cause of dichotomy having been introduced by Janczewski, it was taken up by Bruchman (1874) who recorded his observations on a number of conifers in his paper "Ueber Anlage und Wachsthum der Wurzeln von Lycopodium und Isoetes." Fungus mycelia were found in the periblem of all dichotomized roots of *Pinus sylvestris* L. and also in a "Krust" of dead cortical cells which covered the entire surface of these roots (that is, the fungus mantle). Dichotomy was found to be absent, however, in roots of *Picea excelsa, Juniperus communis* and the dicotyledonous trees examined, although they also exhibited the same type of fungus infection (In these, fungus infection is now known to stimulate racemose branching).

In this entire early literature there is no reference which so convincingly establishes the common occurrence of the mycorrhizal habit in trees as the one by Th. Hartig in his "Vollstandige Naturgeschichte der förstlichen Culturpflanzen Deutschlands" (1851). In describing the figures in Plate 18 of that volume, Hartig gave his first clear classification of pine root systems (see also "Lehrbuch für Förster," 1840, 1851). According to their characters the tertiary roots of pine were separated into two groups, "Triebwurzeln" and "Saugwurzeln," which are the equivalent today of long-roots and short-roots respectively. The two types, reproduced here in Text Fig. 4 (4, a and b), were described by Hartig as follows:

"*a.* Triebwurzel, d. h. derjenige Theil der Faserwurzeln, welcher die Verlängerung und Ausbreitung der Wurzeln vermittelt."

"*b.* Saugwurzeln, d. h. die den Triebwurzeln entsprossenden vielfach verästelten feinsten Wurzelästchen. An der Spitze einer jeden, den Winter über braun gefärbten Faser erzeugt sich im Frühjahr ein verdickter, Spargel ähnlicher Trieb, das eigentliche Organ des Organ des unterirdischen Pflanzenlebens. Diese Spargelspitzen erhalten sich bis zur Mitte des Sommers und scheinen dann zu verschwinden, indem die äussere, sehr dicke Zellenmasse abstirbt, eintrocknet und braun wird, worauf der jüngste Saugwurzeltrieb von den vorhergehenden nicht mehr zu unterscheiden ist. Daher schreibt sich die, selbst von Botanikern vielfach aufgestellte Behauptung: dass die Wurzeln ihre jüngsten Triebe jährlich abwürfen und reproduzirten, wie die Drone Blätter erzeugt und abwirft."

"Fig. 8. Querschnitt eines Saugwurzelastes im Frühjahr. Die ausserste Umhüllung bildet eine aus radialen Zellen bestehende Korkschicht, von welcher ein durch eigenthümlichen Bau der Wände ausgezeichnetes grosszelliges Parenchym eingeschlossen wird. Es zeigen hier nämlich die Zellenhäute ein dem Blattadernetze ähnliches anastomosirendes Geflecht. Fig. 10, welches ich für das Bild verästelter, den ganzen Umfang der Zelle umgebender Intercellulargänge halte, deren Zweck eine erhöhte Zellenthätigkeit sein mag. Diese meines Wissens bisher nicht weiter beobachtete Organisation erlangt gerade an diesem Orte eine besondere physiologische Wichtigkeit.''

While these descriptions contain several inaccuracies, the evidence is conclusive that Hartig's Saugwurzeln were mycorrhizal short-roots and that the distinctive characters which separated these from his Triebwurzeln were merely those which separate mycorrhizal and non-mycorrhizal roots.

In passing to the next period, a period in which controversy on the character of the mycorrhizal habit was often heated, we can anticipate that the contention of several workers that mycorrhizae were infrequent structures did not long survive the test of field examinations.

EVIDENCE ON THE GENERAL OCCURRENCE OF THE MYCORRHIZAL HABIT, 1882-1935

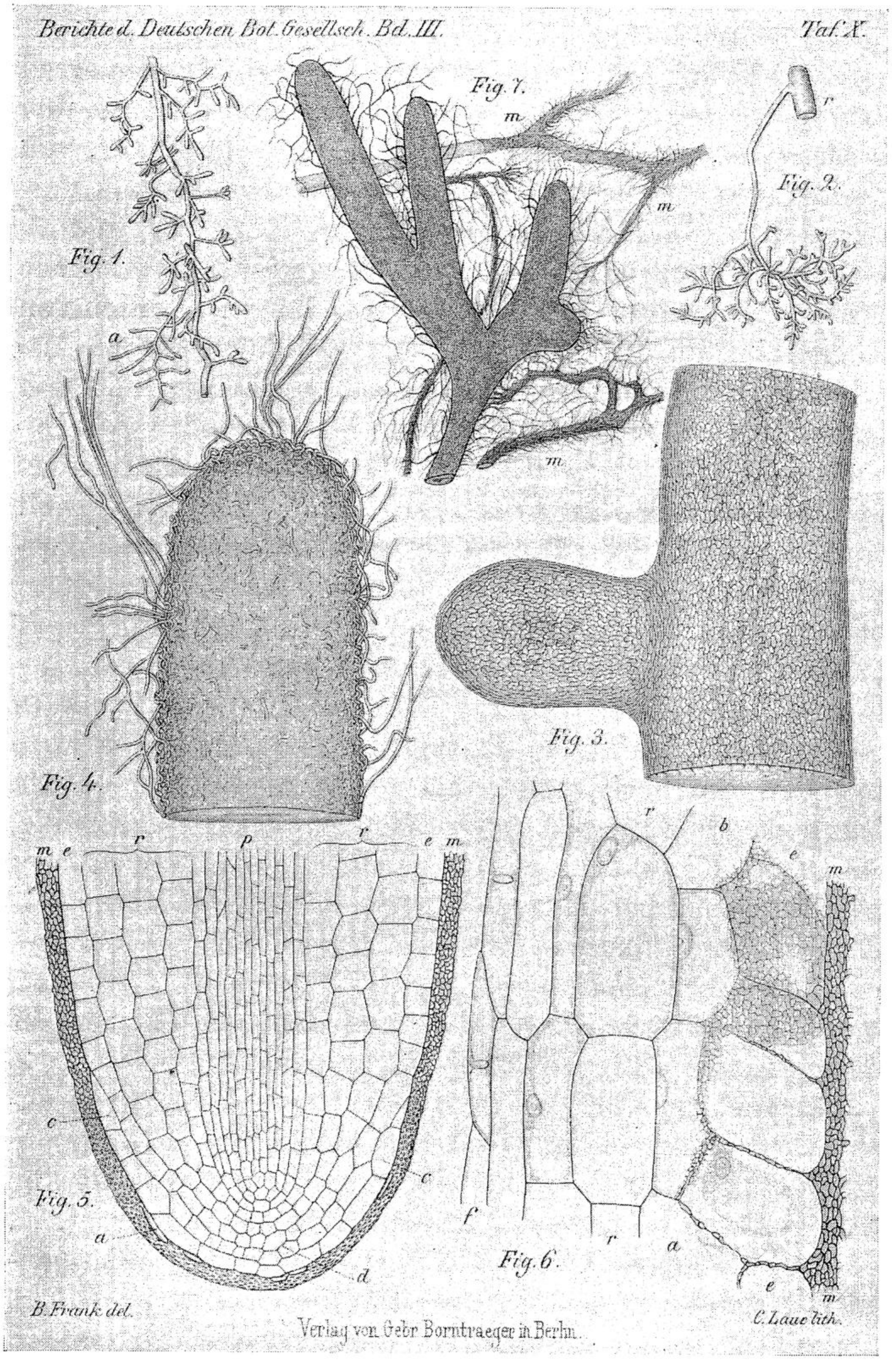
A significant factor in creating and perpetuating uncertainty about the commonness of the mycorrhizal habit has been the custom of reviewing theories rather than the observations on which these theories were based. All too frequently the views of authors opposed to the beneficial theory of mycorrhizae possessed backgrounds of hasty, local, or inaccurate field studies or else they were theoretical only. In any survey of the literature these publications are given undue weight unless observational data rather than theoretical conclusions are made the basis of comparison. In the present section an attempt is made to confine the review to direct field evidence only. When possible, obvious fallacies in the mode of collecting these field data are pointed out.

The Discoveries of Gibelli and Frank (1883-1885)

The first publication which yielded extensive comparative information on the occurrence and distribution of mycorrhizae was Gibelli's (1883) report on the "ink disease" of chestnut in Italy. Mycorrhizae were encountered while Gibelli was examining diseased roots of *Castanea* for the causal organism of this disease. Short-roots possessing the typical mycorrhizal structure were described and illustrated with precision. Both the mantle and the intercellular net were recognized. To determine whether the fungi in these roots produced the disease, Gibelli examined the roots of healthy chestnut trees and found fungus infection equally abundant. Next his examinations were extended to trees other than chestnut, and in species of *Quercus, Corylus, Ostrya,* and *Carpinus,* mycorrhizae were reported to be normally present. Finally, Gibelli examined the roots of many species of woody plants from various parts of Italy and found constant infection in all Cupulifers. In a large number of other woody plants, however, infection was not discovered (i.e., in species of *Fraxinus, Acer, Ulmus, Pinus, Juniperus, Cornus, Ligustrum, Viburnum, Pyrus,* etc.). The fact that Gibelli found some plants (Cupulifers) constantly infected and others (*Fraxinus, Acer,* etc.) completely fungus-free was largely responsible for his belief that the infections were without effect on tree growth; that the fungus was tolerated only because it caused no injury.

The similarities between Gibelli's observations and the earliest ones of Frank (1885a, 1885b) are very striking despite the fact that the two investigators reached opposite conclusions. Thus in Cupulifers (species of *Carpinus, Corylus, Fagus, Quercus,* and *Castanea* were examined) Frank found all short-roots invariably converted to mycorrhizae: "Um die Wurzeln der Cupuliferen in den vorscheidenen Lebensaltern zu untersuchen habe ich von Eichen, Rothbuchen, Hainbuchen, und Haseln aus verschiedenen Gegenden jedesmal zowohl 1-, 2-, und 3-järhrige Pflanzen, als auch mit Saugwurzeln versehene Wurzelstücke älterer Bäume kommen lassen, nämlich von Eichen bis zu 120 jährigen, von Rothbuch bis zu 120 jährigen, von Hainbuchen bis zu 100 jährigen und von Haseln bis zu 40 jährigen Alter. Es ergab sich das Resultat, dass bei diesen Bäumen die Saugwurzeln in allen Lebensaltern in der Form der Mycorrhiza entwickelt sind, dass der Pilz während des ganzen Lebens die Wurzel begleitet" (1885a, p. 134). A considerable number of other plants were also examined and in some (species of *Salix, Populus, Pinus* and *Tsuga*) mycorrhizae were determined to be less frequent than in Cupulifers, while in others they were wholly absent. In the latter group were included the herbaceous and woody vegetation of the beech woods (species of *Oxalis, Mercurialis, Anemone, Asperula, Viola, Convallaria, Hedera,* and *Acer*) as well as a large number of trees (species of *Betula, Alnus, Ulmus, Morus, Platanus, Juglans, Pyrus, Sorbus, Crataegus, Prunus, Rhamnus, Cornus, Franxinus, Syringa, Sambucus, Taxus, Juniporus,* and *Larix*). Several of these duplicate the genera listed by Gibelli.

The fact that all short-roots in Cupuliferae were ensheathed by mycelium (Text Fig. 6) led Frank to the obvious conclusion that water and nutrients entering the tree by these roots were obtained from the fungus, ". . . gewisse Baumarten, vor allen die Cupuliferen, ganz regelmässig sich im Boden nicht selbständig ernähren, sondern überall in ihrem gesammten Wurzelsystem mit einem Pilzmycelium in Symbiose stehen, welches ihnen Ammendienste leistet und die ganze Ernährung des Baumes aus den Boden übernimmt" (*op. cit.*, p. 128-129). The constancy of this association, as Frank found it, is further



TEXT FIG. 6. MYCORRHIZAE OF *FAGUS* (4, 7) AND *CARPINUS* (1, 2, 3, 5, 6).
(From Frank, 1885a).

shown by his statement on the taxonomic value of these roots, "Ja diese Symbiose ist dieser Pflanzenfamilie so treu, dass man fast versucht sein könnte sie als systematisches Kriterium gelten zu lassen. . . ." (*op. cit.,* p. 136.)

The Controversy of 1885-1887

There is probably no incident in the history of mycorrhizal investigations which has been more often related than the storm of criticism which immediately followed the publication of Frank's first paper. Within two years of its appearance opinions for and against the theory were expressed by twelve other authors. Of these Grosglick (1885), Penzig (1885), and Kummer (1885) confined their arguments to criticisms of Frank's theory, to his quotations, and to his claims of priority. Of the remaining nine, three only, Kamiensky (1886), Henschel (1887), and Robert Hartig (1886) opposed Frank's claims, at the same time citing original observations of their own on the relative abundance of mycorrhizae in nature.

Kamiensky examined tree roots in the vicinity of St. Petersburg and maintained that fungus roots were by no means as numerous as Frank claimed. Henschel (p. 117) correlated seedling health (in *Picea excelsa*) with the abundance of mycorrhizae and observed that, "Der Grad der Schwächung nimmt in den Verhältnisse zu, als die Pilzwurzelbildung überhandnimmt." It was Robert Hartig, however, who raised by far the most weighty protest. He reported that in his recent studies of the *Rosellinia* disease of oaks, several twelve-year-old trees had been excavated from the gardens of the forest experiment stations at Munich and on these, ". . . weder an Eiche, noch Roth- und Hainbuch, Haseln, p.s.w. . . . eine Spur der Mycorrhiza zu beobachten war . . ." (*op. cit.,* p. 351). Another point raised by Hartig was the time of infection of the roots: ". . . seien gerade zu der Zeit, in welcher die Bäume am meisten Wasser und Nährstoffe aufnehmen, nämlich im Sommer zahlreiche, neugebildete Wurzelspitzen völlig pilzfrei, die dann erst in Herbst und Winter befallen und zum grössten Theil getödtet werden.'' (It should be stated that Hartig misjudged the real situation in this statement. New mycorrhizae are produced during periods of active root growth and these periods fluctuate according to weather conditions. Except in moist summers root growth is less active in summer than in spring and autumn.) Hartig remained skeptical of Frank's claims that all short-roots are normally mycorrhizal, and being the leading forest pathologist in Germany at that time his opinion carried weight.

The remaining six of the authors who took part in this first controversy, namely, Woronin (1885), P. E. Müller (1886, 1887, 1902, 1907), Lecomte (1887), Paul Sorauer (1886), Mattirolo (1886, 1887) and Reess (1885, 1887) wholly or partially supported Frank's statements of the generality of the habit with first-hand observations in the field. Thus, Woronin who disagreed with Frank's beneficial theory did not question his statements on the frequency of mycorrhizae, and noted that Frank's ''Pilzewurzel—est mir schon seit 2 Jahren gut bekannt. Den betreffenden Wurzelpilze fand ich an Coniferen, an Salicineen (verschiedens Salix-arten) und sogar an einigen Gräsern'' (Woronin, 1885, p. 205). These observations were made in Finland in connection with Woronin's studies of edible species of *Boletus,* a genus which is now known to be one of the most common producers of mycorrhizae. Mattirolo (1886, 1887, 1888, three publications containing the same material) had similarly observed mycorrhizae in Italy over a period of years in connection with his studies on truffles. Lecomte (1887) confirmed Frank's opinion for parts of France: ''J'ai trouvé souvent le *mycorhiza* sur les racines de divers arbres et en particulier sur celles du Hêtre, du Chataugniers, du Chêne et du Noisetier, dans la region Montagneuse du departement des Vosges'' (p. 38). ''De jeunes *Corylus Avellana* que j'ai eu l'occasion d'observer, avaient toutes leurs radicelles recouvertes par le Mycorhiza. . . .'' P. E. Müller (1886, 1887) re-examined the material he had published in 1878 (see p. 25) and again reproduced the figures of that work, which beautifully illustrate mycorhizae. He not only confirmed the abundance of mycorrhizae for Denmark but strengthened Frank's views with observations on the extensiveness in the soil of the mycelia producing the mycorrhizae. In Germany, Paul Sorauer (1886) appears to have confirmed Frank's claims of the generality of the habit, since he illustrated mycorrhizae of *Pinus Strobus* L. and wrote as follows of the habit in general: ''. . . bei den Cupuliferen sich fast ausnahmslose Regel erweist (nur Keimpflanzen und Wasserkulturen lassen pilzfreie Wurzeln erkennen), findet es sich minder allgemein bei den Coniferen . . . und noch weniger oft bei den Salicineen.'' Finally Reess (1886, 1887), whose earlier contribution to this subject in connection with his studies of *Elaphomyces* (1880) has been noted in the previous section, continued his examinations and found mycorrhizae in many species of trees, especially pines and other conifers, in Cupulifers and other broad-leaved trees, and in *Monotropa.* On their abundance, however, Reess did not fully agree with Frank, ''Von der Kiefer kann ich Schätzungs sagen, dass ich im Elaphomycesreichsten Bezirk um Erlagen nicht weniger pilzefreie jungen Wurzeln gefunden habe, als Pilz befallene.'' (1886, p. 295.) Reess also noted the complete absence of mycorrhizae in a whole series of other trees.

Toward the end of 1887 Frank contributed another classical paper, ''Ueber neue Mycorhizerformen.'' He described roots with exclusively intracellular infection, and designated them *endotrophic* mycorrhizae. It is this type of mycorrhizae which is found in *Fraxinus, Acer, Ulmus, Cornus, Pyrus, Viburnum, Platanus, Juglans, Taxus,* etc. and most of the other woody plants, except members of the Cupuliferae, Betulaceae, Abietineae, and Salicaceae, which Frank, Gibelli, Reess and other workers first listed as lacking mycorrhizae. Accordingly, the first and indeed many subsequent reports that such plants lack mycorrhizae resulted from inability to recognize infections by external appearances. Today there is not a single woody plant (excepting only parasites) which is definitely known to lack mycorrhizae in all environments.

Thus of the fourteen biologists who expressed opinions on ectotrophic mycorrhizae between 1883 and 1887, eleven reported field observation; eight of these recorded mycorrhizae in great abundance in certain groups of trees, and only three maintained that they were scarce. In contrast, if this analysis were on the basis of theoretical interpretations it would be found that only four of the fourteen authors supported the beneficial viewpoint (Frank, Müller, Lecomte, and Paul Soraur).

In 1888 v. Tubeuf, a student of Robert Hartig, entered the lists in support of the "injurious" views of his teacher. His observations were made on seedlings sent in to Hartig's laboratory and they apparently favored neither viewpoint.

It is highly illuminating now to follow this controversy to its conclusion. Of the group supporting the view that the habit is normal and widespread, Frank (1888-1894) and Müller (1889-1913) published additional observations. Two investigators who first supporting the opposite view also published in subsequent years, namely, Robert Hartig (1888-1891) and v. Tubeuf (1889-1903).

In 1892 in his "Lehrbuch der Botanik," Frank summarized his observations as follows (p. 259): "Ich habe nachgewiesen, dass die Mykorhizen eine beständige Erscheinung an allen Bäumen unserer Wälder sind, soweit sie zu den genannten Familien gehören,"—(Cupulifers, Betulaceae and conifers)—, "und dass auch in allen Ländern und Erdtheilen, wo nach dieser Symbiose gesucht worden ist, sie sich regelmässig gefunden hat, sowie dass auf jedem Naturboden schon in den ersten Lebensjahren des Baumes die betreffenden Pilzmycelien auf die Wurzeln desselben gelangen, und dass der Baum auch während seines ganzen Lebens, soweit auch sein Wurzelsystem sich vergrössern mag, mit allen seinen Saugwurzeln in dieser Symbiose sich befindet.''

In the numerous references to mycorrhizae which Müller included among his papers on forest soils in Denmark over a period of three decades, the generality of the habit was never questioned. As a typical example of his observations, a statement on the mycorrhizal conditions of the seedlings in his experiments with Weiss (reported in 1907) may be quoted: ''. . . so habe ich kräftig entwickelte korallenförmige Mykorrhizen niemals an einer kleinen und schwachen Pflanze gefunden sowie ich . . . abgesehen von den Gefässversuchen . . . auch keine grossen und kräftigen Pflanzen ohne Mykorrhizen beobachtet habe'' (p. 196). Both authors (Frank and Müller) continued to observe mycorrhizae in great numbers and to regard the habit as a normal one. Turning now to the advocates of the opposite viewpoint we find that gradually they too came to recognize that the habit was more general than they had originally claimed.

In 1888 Hartig wrote as follows: ''Diese Wurzelpilze kommen in dem humosen Boden der Waldbestände sehr allgemein vor, und gebt kaum eine Eiche, Buche, Hainbuche oder einen Nadelholzbaum an denen man nicht zahlreiche Wurzeln von den Pilzmycel behaftet findet'' (Hartig, 1888, p. 122). Hartig never did propose that infected roots were quite so abundant as Frank claimed, partly it seems because he believed the differences between long- and short-roots were differences brought about by the fungus rather than because of inheritance. His last discussion of the habit appeared in his ''Lehrbuch der Anatomie und Physiologie der Pflanzen'' in 1891. While he still did not favor the beneficial theory his chief criticism of it was that it had not been proved to be correct and that infection varied: "Der Umstand, dass immer ein grosser Theil der Wurzeln auch im humosen Boden frei von Pilzen ist, dass die Pflanzen auch ohne Mycorhizen vertrefflich gedeihen, zwingt uns, der Frank's chen Ernährungstheorie mit einer gewissen Reserve gegenüberzustehen." (*op. cit.*, p. 151.)

Although he originally supported the early views of R. Hartig, v. Tubeuf by 1903 had completely altered his opinion. He divided all plants into seven groups depending on the character and abundance of their mycorrhizae and included trees possessing ectotrophic mycorrhizae among those which are always mycorrhizal.

It seems quite probable, in view of this change of opinion by both Hartig and v. Tubeuf and especially since other participants in the original controversy never again questioned the commonness of the habit, that most of those participants found, on more careful examination, that fungus roots were abundant. Certainly it may be concluded from the foregoing analysis that the controversial aspects of the question in Frank's time centered chiefly in the functional nature of mycorrhizae rather than in their occurrence in nature. Failure in the past to recognize this quality of that controversy has done much to delay the general recognition of mycotrophy as a normal and widespread phenomenon.

SUMMARY OF MODERN LITERATURE

While the foregoing review supplies ample evidence of the commonness of the mycorrhizal habit in trees and shows that even the foremost advocates of the non-symbiotic theory eventually recognized this fact, a survey of other more recent work is not redundant. Especially is this true in view of the repeated assertion by W. B. McDougall (1914-1928) that mycorrhizae, although produced in many plants, are infrequent, abnormal, and pathological structures. During the last 40 years numerous investigators have directly or indirectly added information of value in establishing the fact of normal mycorrhiza-formation in trees; but to review their papers is unnecessary. Quotations from a few only, representing the more extensive and summary studies of this period and selected with the view of including observations from many parts of the world, should suffice.

Beginning with European studies, one finds that in addition to furnishing a scholarly review of literature George Sarauw (1893a) thoroughly explored the roots of all forest trees in Denmark as well as in parts of France and northern Germany (Sarauw 1893a, 1893b, 1903-1904). He writes of these observations as follows: ''Everywhere, forest trees from their first year to their greatest ages possess mycorrhizae with the same constancy. In nurseries and in botanical gardens fungus roots are less abundant than in forest thickets where very often all short-roots of trees become mycorrhizal'' (1893a, p. 242, my translation). For Germany Ernest Stahl (1900) made a thorough study of root conditions in the entire plant kingdom. He sought the explanation of the entire mycorrhizal habit, and from his observations on roots of hundreds of plants in many localities decided that relative poverty in mineral salts characterized the substrates in which mycorrhizae appeared. In the case of conifers and cupulifers, infection was so uniformly present in all habitats examined that other broad-leaved trees had to be used for this study, ''. . . bei welchen die Mykorrhizen weniger constant auftreten. . . .'' (*op. cit.,* p. 543), i.e., *Betula, Salix, Populus,* members of the Pomaceae, *Acer, Ulmus, Aesculus,* and other trees.

The most extensive and thorough studies of tree mycorrhizae in recent years have been those of Melin (1917-1927) in Sweden. In that country the majority of the forests are characterized by raw humus soils and in these virtually all short-roots are uniformily converted to mycorrhizae, ''I mossrika tall- och granskogar finner man nämligen praktiskt taget alla sugrötter omvandlade till mykorrhizor . . .'' (1924, p. 201). In the south of Sweden, on the other hand, mull soils are not infrequent and there infection is less constant, ''. . . sind die Nadelbaummykorrhizen auf diesen Bodentypus oft sehr schelcht entwickelt'' (1923, p. 109). After extended examinations in England Paulson (1923-1924) concluded that mycorrhizae occur ''. . . abundantly, almost without exception on the roots of *Quercus Robur, Fagus sylvatica* . . . *Carpinus Betulus* . . . *Betula alba, Castanea saliva* . . . *Pinus sylvestris* and *Taxus baccata* in woodlands of the southeastern countries, especially in those located on a light soil'' (1924, p. 231). In arctic regions the evidence assembled by Hesselman (1900) also substantiates the constancy of the habit; ''Die Mykorrhizabildung ist bei *Polygonum viviparium* L. eine konstante Erscheinung, . . .'' (p. 37); in *Dryas octopetala* L., ''Die Verpilzung ist ebenso konstant . . .'' (p. 38); ''. . . kommen bei den arktischen Salix-Arten allgemein Mykorrhizen . . .'' (p. 36).

Relative to the Orient, in recent years a series of papers by Japanese writers all indicate that the habit is general in Japan. While Masui (1926-1927) maintained that in most cases mycorrhizae are injurious, his descriptions in many cases give evidence of their abundance. For example, with reference to *Pinus* he states, ''In Autumn, when new roots become plentiful, they are infected by the mycelium of the old mycorrhizas and are all transformed into new mycorrhizas'' (1927, p. 159). Takamatso (1930, p. 614) notes that ''Die meisten Gewächse im solfataren-Gebiete auf dem Berg Hakkoda besitzen Mycorrhizae. . . .'' A study of the roots of *Pinus densiflora* and *P. Thunbergii* in many types of soil by Shimizu (1930) revealed an abundance of mycorrhizae in most situations. The most extensive survey of the habit throughout the plant kingdom since 1900 was recently completed by Asai (1934) who concluded as follows (p.

118-119): "Unter 148 gepruften höheren Pflanzenfamilian, ausser den 15 mykorrhizafreien Kräutern, 11 Wasserpflanzen . . . habe ich bei allen Fallen . . . Mykorrhiza festgestellt."

That they also abound in the tropics was shown long ago by Janse (1897) for the island of Java. Saprophytes and orchids of all kinds in the tropics are also known to possess constant fungal infection (Burgeff, 1932). In subtropical regions of the southern hemisphere [Australia and Africa (Samuels, 1926; Kessell, 1927; Anonymous, 1931)] and in the northern tropics [that is, in the Philippines (Oliveros, 1932)] the constant possession of mycorrhizae by species of introduced pine in all successful plantations is well known.

Returning finally to America, where McDougall's studies have been pursued, the extensive observations of several modern investigators furnish the concluding chapter of this history. A cross section of forest-growing plants of Iowa led Lohman (1927, p. 55) to conclude that "Mycorrhiza-like fungi are of common occurrence in the Iowa forest flora and are associated with higher plants of wide family relationships." Pessin (1928, p. 32) recorded profuse mycorrhizal development on nursery seedlings of southern pines, "Mycorrhizas were found in abundance on roots of seedlings of *Pinus palustris, P. echinata, P. caribaea,* and *P. taeda.* It seems inconceivable, at least to the writer, that seedlings which appear to be literally covered with mycorrhizas and yet seem perfectly vigorous should harbor a parasitic fungus." A study of the majority of the woody plants of the eastern and midwestern states which occupied Kelley (1932) for a number of years did not reveal a single species in which infection was not present. In the same year McArdle (1932) examined roots of trees growing in plantations in Michigan and concluded as follows: "At least 98 per cent of the rootlets on the better quality sites of the spruce plantations, and probably 75 per cent of the rootlets in the white pine plantations were estimated to bear mycorrhizae'' (p. 293). Doak (1927), and Hatch and Doak (1933) have been constantly engaged in a study of tree mycorrhizae in the Central Atlantic States and in parts of the Middle West and in Scandinavia over a period of several years. They report that they have ''rarely observed short-roots (on forest trees over one year of age, growing in good forest soils with pH less than 5.5) that were not mycorrhizal'' (1933, p. 87). The recent studies of Henry (1933-1934) have likewise extended considerably the known list of American plants possessing mycorrhizae.

Against such unanimity of evidence, McDougall's claims can hardly survive. Scrutinized with care they likewise fall short on other grounds. As pointed out by Hatch and Doak (1933), one error common to the work of several modern students of ectotrophic mycorrhizae is failure to recognize the fundamental and inherited differences between long- and short-roots. More serious in McDougall's studies has been his assumption that the abundance of fleshy fungi in the vicinity of tree roots serves as a measure of mycorrhizal abundance. A tree which possesses abundant mushrooms beneath it one year and none the next is believed by McDougall to possess no mycorrhizae in the latter year (1914, p. 64). This assumption led him (1928, p. 144) to assemble from ''forest rangers and other natives'' the information that during a prolonged drought in the south in 1925-1926 few toadstools had been observed. He concluded that ''very few if any mycorrhizae were produced in the Pisgah National Forest during this prolonged dry period, . . . on the other hand, there was no evidence that any of the trees had suffered any ill effects from the dry weather or the absence of mycorrhizae. . . .'' (*Ibid.*) Certainly this method of collecting ecological data has no very extensive background of reliability. The accuracy of McDougall's claims may therefore be questioned even without reference to the vast accumulation of contradictory observations the world over.

Thus, on the question of the general occurrence of the mycorrhizal habit in forest trees, the literature displays a degree of uniformity which is wholly unexpected in view of its more publicized controversies. It must surprise the reader as it has the reviewer to learn that among all students of ectotrophic mycorrhizae no one who has examined roots in nature over a period of years by reasonably accurate methods has persisted in regarding mycorrhizae as infrequent structures. The probable fact is that in all natural forest habitats 100 per cent of all short-roots of trees producing ectotrophic mycorrhizae are normally mycorrhizal. And short-roots numerically represent well over 95 per cent of all root-ends in most trees.

Factors Responsible for Variation in the Abundance of Mycorrhizae and the Theories of Mycotrophy

Although the conclusion may be justified that forest-grown trees normally have virtually all of their short-roots converted to mycorrhizae, the fact remains that in some soils these structures are infrequent or even absent (Hartig, 1886; Sarauw, 1893a; Melin, 1925, etc.). If the cause of these variations in the abundance of mycorrhizae could be discovered, considerable information on the character of the relationship between fungus and tree root might be revealed. Many investigators have recognized this possibility and have attempted to discover the causes of variations and to formulate theories to explain their findings. This literature is assembled in the present section.

Beginning with a number of papers in the early eighties (1880-1885) differences in the degree to which roots became mycorrhizal were recorded, but without reference to environment (Reess, 1880, 1885; Kamiensky, 1882, 1886; Gibelli, 1883). Even this early, such observations played an important part in shaping the opinions of these workers. Thus Reess, at first, found uninfected roots to be as plentiful as infected ones and consequently he could not arrive at a definite opinion as to their effect on plant growth. A primary reason for Kamiensky's belief that fungus roots in *Monotropa* facilitate nutrient absorption was the constancy with which infection occurred. Similarly, his ''parasitic'' interpretation of the situation in trees was prompted by his failure, for reasons still unknown, to find infected roots in great abundance. The conclusions reached by Gibelli (1883) that the fungus is tolerated only because it causes no damage was also a direct outcome of his finding a great many trees without evident infection (that is, those which were subsequently found to have endotrophic mycorrhizae).

THE CONCENTRATION OF MYCORRHIZAE IN HUMUS SOILS

Even in his earliest papers Frank (1885a, 1885b, 1888) recorded a direct correlation between the abundance of mycorrhizae and the humus content of soil. These observations led Frank (1888) to propose the humus theory and to carry out a number of experiments to support it. The experiments were rather crude and the results may be interpreted in several ways, but they constitute an important part of Frank's contributions. Seedlings of beech were grown in pot cultures of meadow soils, garden soils, humus, sterilized humus, and sand; and in some cases mycorrhizal seedlings were transplanted to these substrates. Mycorrhizal development was less profuse or wholly absent in all cultures except those with forest humus. Seedlings grown in sterilized humus grew more poorly than in unsterilized humus. These results were believed to support the theory that mycorrhizae are organs concerned with the utilization of forest humus, and that they disappear in soils lacking this material. The poor development of mycorrhizae in the cultures of meadow soil, for example, implied to Frank that the organic remains of plants other than trees are not readily utilized by tree mycorrhizae. It was on this basis that he explained Robert Hartig's (1886) disturbing report that mycorrhizae were wholly lacking on oaks and other trees growing in the gardens of the forest experiment station at Munich.

That mycorrhizae are more profusely developed in the humus layers of forest soils than in any, or nearly any, other soil layer or type has frequently been confirmed. Among Frank's contemporaries these differences were reported by Robert Hartig (1888, 1891), Vuillemin (1890, p. 327), Sarauw (1893a, p. 183), v. Tubeuf (1888-1903), and Reess (1887). Hartig's observations are the most notable, since it was his report of their absence in garden soils that precipitated much of the controversy about the prevalence and significance of fungus roots. Hartig's subsequent confirmation of the abundance of infection in humus soils has been quoted in an earlier section. The investigations of Sarauw (1893a, p. 183) in parts of France, Germany, and Denmark confirmed those of Frank on the question of distribution so exactly that he scarcely more than mentioned them: ". . . mine Iagttagelser til forskjellig Aarstid paa unge og gamle Skovfyr paa Bornholm, Sjaelland, Fyen, i Brandenburg, Paris og Fontainebleau have givet Resultater, der stemme overens med de ovennaevnte Forskeres, tillader jeg mig iøvrigt at henvise til disses Afhandlinger. . . ." The extensive observations of Stahl (1900), which will be dealt with in detail below, yielded similar confirmation of the concentration of mycorrhizae in humus soils: "Am reichlichsten vertreten sind sie auf humusreichem Substrate; mit abnehmenden Humusgehalt treten sie, jedoch nie bis zum völligen Schwinden, zuruck." (p. 544.) For species of *Monotropa,* Peklo (1908), Christoph (1921), and Francke (1934) have all confirmed the greater abundance of mycorrhizae in humus than in other soils. Indeed, Peklo (1908) even reported the complete absence of infection in specimens growing in soils rich in clay. Peklo's report stimulated Francke (1934, p. 5) to investigate the question of distribution with great care. While he never found infection completely absent, he confirmed the commonness of the relationship between the humus content of soils and the degree of infection in *Monotropa* roots: ". . . eine zuffallende Abhängigkeit vorhandes ist in der Stärke der Wurzelverpilzung, . . . von dem Gehalt des Bodens an organischen Stoffen."

For forest trees Melin (1917-1927) has firmly established the generality of this rule in Sweden. In the raw humus forests over most of Sweden, practically all short-roots become mycorrhizal; but in the south of Sweden where there are mull soils, infections are frequently much less numerous or even absent.

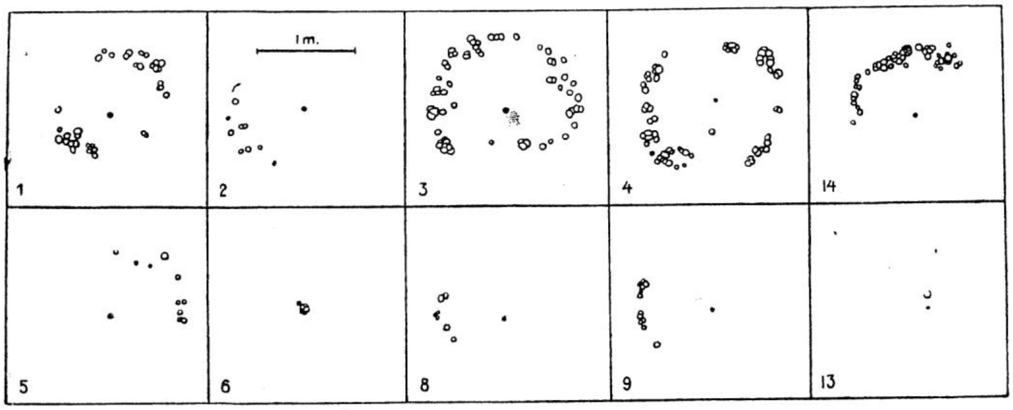
Many other references of a similar character are found in mycorrhizal literature, but further citation can scarcely strengthen the present picture. Attention may more profitably be directed to an examination of the possible cause of this observed relationship.

THEORIES EXPLAINING THE ABUNDANCE OF MYCORRHIZAE IN HUMUS

INFECTION THEORY

One of the commonest views, especially among investigators who regard mycorrhizae as injurious and among mycologists, is that these fungi are found in greatest abundance in humus soils for the reason that they find their optimum environment in forest habitats (Hartig, 1888; McDougall, 1914; Lange, 1934). This view is untenable, since fructifications of mycorrhizal fungi may become more abundant in open field soils, if the appropriate tree roots are present (Text Fig. 7), than they normally do in forests.

Under these circumstances mycorrhizae also abound (Romell, 1921; Palm, 1930; Horn, 1933). They are also very numerous and well developed in alpine and arctic regions where humus is scarce (Hesselman, 1900; Schröter, 1908; Wulff, 1902; Peyronel, 1930), and even in sandy soil where organic materials could not be detected by chemical means (Möller, 1903).



Text Fig. 7. FRUCTIFICATIONS OF *HEBELOMA CRUSTULINIFORME* OVER MYCORRHIZAL ROOTS OF TEN TREES OF *BETULA LENTA* PLANTED IN FIELD SOILS.

(After Horn, 1933).

The Mineral Salt Theory of Stahl (1900)

That plants which lack mycorrhizae in cultivated and fertilized soils frequently possess them in uncultivated soils was first recognized by Schlicht in 1889. A student of Frank, Schlicht interpreted his observations on the basis of the humus theory.

On a background of similar discoveries Ernest Stahl (1900) advanced the most comprehensive, intricate, and keenly perceptive hypothesis to explain mycotrophy that has ever appeared. He sought to discover the reason that among plants of different species, growing side by side in the same nutrient environment, some should possess mycorrhizae while others lacked them. The constant possession of mycorrhizae by some plants, regardless of the character of the soil in relation to nitrogen, suggested to

Stahl that the habit had a more general utility than acquisition of organic nitrogen. He found by independent examination that, despite the absence of demonstrable quantities of nitrates in some humus soils, autotrophic plants growing in them possessed nitrates in their leaves. The fruiting bodies of fungi also contained nitrates in quantity. That these nitrogen salts were not found in the leaves of mycorrhizal plants did not signify, in Stahl's opinion, that such plants obtained their nitrogen in organic form (*op. cit.,* p. 633).

These considerations and the observation that the abundance of mycorrhizae decreases with increase in soil fertility led Stahl eventually to the opinion that the basis of the habit, as he expressed it, "Der Sinn der Mycorhizenbildung," is poverty in mineral salts. He postulated that plants which are incapable of absorbing large quantities of salts, because of restricted root systems and sluggish transpiration currents, become mycotrophic. Mycorrhizae are, therefore, especially abundant in infertile soils because here the salt deficiencies of plants are aggravated.

While modern studies on selective permeability are generally believed to indicate that salt and water absorption are unrelated processes, biologists of earlier periods regarded them as interdependent. The bulk of Stahl's paper, accordingly, comprises a series of comparative studies of the rates of water movement in plants of widely removed systematic relationships, and correlation of these rates with the abundance of mycorrhizae and with the availability of mineral salts in different environments. Measurements of water movement in the hundreds of plants examined were of course impossible, and Stahl resorted to indirect methods. Comparative records of the presence or absence in plants of structural or physiological features conducive to a rapid transpiration stream were assembled [such, for example, as the extensiveness of the root systems, the character of the leaves in relation to the ease of water loss, the possession of hydathodes, the habit of the plants (whether sun or shade species), the storage of synthesized carbohydrates in the leaves as sugars or as starches (increasing or reducing sap concentrations respectively and therefore transpiration), the formation of calcium oxalate crystals reducing the effective concentration of calcium, etc.].

In general, Stahl found close agreement between the volume of the transpiration current as measured by these means and the production of mycorrhizae. When the root systems were extensive and the plants possessed features which tended to increase absorption or water loss, mycorrhizae were relatively infrequent. A sluggish water current, on the other hand, was typically correlated with the appearance of root infection. The nicety with which these observations dovetailed with those which originally suggested the mineral salt theory, namely, fluctuations in the degree to which plants became mycorrhizal with differences in soil fertility, satisfied Stahl that his theory was correct.

Stahl was confronted with the difficulty of explaining the concentration of mycorrhizae in forest soils where nutrients other than nitrates were not known to be deficient. Although the deficiencies of nitrates in forest soils were early recognized (Ebermeyer, 1888), the situation in regard to mineral salts is only now beginning to be appreciated (Gast, 1937; Mitchell, 1934). In Stahl's opinion this was the crux—''die Kernfrage''—of the whole mycorrhizal problem. Stahl noted, first of all, 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. It was supposed that those autotrophic plants which possess unusually deep and extensive root systems, supplemented by structural or physiological features for increasing the rapidity of the transpiration current, could live in such environments. Plants equipped with mycorrhizae, on the other hand, could compete with fungi on equal terms. Thus severe competition between vascular plants and the more efficient soil fungi accounted in Stahl's opinion for the increased abundance of mycorrhizae in humus soils.

Attempts were made to test the correctness of this view of the importance of fungus competition. Plants were grown in pots of sterilized and unsterilized humus, and growth of their roots and of the whole plants were compared. Roots in sterilized humus grew more slowly and became much less extensive than those in corresponding unsterilized soils. The whole plants, however, grew to be much larger. These results were believed to indicate that with elimination of fungus competition a much greater quantity of mineral salts became available so that a less extensive root system could support a much larger top. Another check on the correctness of the theory was obtained by comparative ash analyses of mycotrophic and autotrophic plants growing in the same environment. With due allowance for the effect of hydathodes on salt contents, Stahl found in a limited number of plants a rather substantial agreement between the quantity of ash they contained and their root condition. Plants with a paucity of ash possessed mycorrhizae and those with high ash content were autotrophic.

Finally Stahl examined a number of parasites and insectivorous plants and concluded that these in common with mycotrophic plants represent nature's solutions of the same problem, namely, life in infertile substrates. In Stahl's words (p. 661): ''Relative Nährsalzarmuth, welche nach unserer Ansicht die Ausbildung der Mycotrophie, wie auch der Insectivorie und des Parasitismus, veranlasst hat, charakterisirt auch die Standorte, an welchen die Mycotrophie in den Vordergrund tritt, während bie zunehmendem Vorrath an mineralischen Nahrstoffen die Wurzelverpilzung seltener wird.''

Today the mineral salt theory is thought of more often as a monument to an ingenious and philosophical mind than as a serious explanation of mycotrophy. Many factors have contributed to this result. Among these the most serious has been the non-acceptance by physiologists of the assumption that absorption of water and of the mineral salts dissolved in it are related processes.[1] The theory is also criticized on the basis of inaccuracies or assumed inaccuracies in Stahl's observations. Many plants which were listed as autotrophic in his paper have since been found to possess mycorrhizae. The question is not asked, however, whether these plants were growing in less fertile substrates when found with mycorrhizae than in those substrates from which Stahl described them. The criticism is made that Stahl failed to find mycorrhizae, although they were present, in some of his experimental plants (for example, *Vaccinium*), grown in sterilized soils where mineral salts were abundant. The criticism is based on the unfounded assumption that the fungi regularly associated with the seeds of these plants also produce mycorrhizae, an assumption which repeated experiments by the sponsor of this view have failed to confirm (Rayner, 1925).

The opinion of Melin in 1925 on Stahl's theory is less a criticism than it is a weighing, in favor of the organic nitrogen theory, of the possible factors in Sweden to which the habit may be a response. He expresses these views as follows (Melin, 1925, p. 106): ''Während jedoch Stahl meint, dass die Mykorrhizapilze anorganische Salze an die Wurzeln vermitteln, liegt die Bedeutung der Mykorrhizen nach meiner Ansicht in erster Linie darin, das sie auf Böden vom Rohhumustypus den für den höh-

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[1] In 1935 Hitchcock and Zimmerman provided clear-cut proof that the movement of water and of materials dissolved in it to the leaves of plants are intimately related. This criticism of the Stahlian hypothesis is therefore questionable. More recently, Freeland, R. O. (1937). Effect of transpiration upon the absorption of mineral salts. (Amer. J. Bot. 24:373-374), has provided data which are even more conclusive. (Author, August, 1937.)

eren Symbionten nötigen Stickstoff leichter assimilieren können als die Wurzeln allein. . . . Es ist zwar möglich, dass ausserdem auch gewisse andere Nährstoffe in der Humusdecke, wie Kalium-und Phosphorsalze, durch die Mykorrhizen leichter aufgenommen werden als durch die Wurzeln allein; da aber diese Salze auch in dem darunterligenden Mineralboden zu finden sind, drüften sie durch nicht verpilzte Wurzeln auch von hier aufgenommen werden können.''

THE NITROGEN THEORY

The more commonly accepted explanation of the function of tree mycorrhizae and of their prevalence in humus soils is centered in the nitrogen theory. In this view it is proposed 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. In forests of cool climates, where humus tends to accumulate, nitrogen and other elements are gradually concentrated in this layer. It is believed that under these conditions the benefits of symbiotic association with fungi capable of utilizing organic nitrogen are greater than the dangers of reciprocal parasitism.

In less accurate form this view was first expressed by Frank in 1894. He was influenced chiefly by two discoveries: (1) that trees possessing mycorrhizae exhibit no trace of nitrates in their tissues, and (2) that forest soils are similarly deficient in these sources of nitrogen (Ebermeyer, 1888). In contrast, both agricultural soils and the plants growing in them possess large quantities of nitrates. Therefore Frank believed that in forest soils trees obtain their nitrogen as ammonium salts or in organic form. The facility with which fungi utilized these compounds indicated to Frank a special significance of mycorrhizae in forest soils.

Subsequently this view has been accepted and elaborated by many investigators. Von Tubeuf (1903), who first favored the non-symbiotic view of his teacher, R. Hartig, later became one of the outstanding advocates of the organic nitrogen theory. It is supported by Falck (1923) and by Francke (1934), both of whom believe that the absorption of carbohydrates is also a function of ectotrophic mycorrhizae. The most extensive and best known work on the theory is that of Elias Melin (1917-1927).

In northern Sweden, where most of Melin's studies have been carried out, the forest soils are of the raw humus type. In the southern part of Sweden the soils are of the mull type. Examined from the viewpoint of nitrogen availability, the mull soils exhibit lively mobilization rates of both ammonium salts and nitrates, particularly the latter (Hesselman, 1927). Under these conditions uninfected roots are believed to be fully capable of satisfying the nitrogen requirements of forest trees, and Melin (and recently Lindquist, 1932) has observed that here mycorrhizae are not infrequently poorly developed. In raw humus, on the other hand, the mobilization rates of nitrates in most cases are quite negligible and in the northernmost forests even ammonium salts are evolved only slowly. Under such conditions it would appear that nitrogen requirements must come largely from organic sources of this element, and since mycorrhizae are here profusely developed the facts fit the theory. The discovery of a similar correlation between the nitrogen mobilization rates of newly drained bogs and the development of mycorrhizae on the trees growing in them supplied the original stimulus which interested Melin (1917) in the mycorrhizal habit. It was found that in the main part of the bogs, where nitrate mobilization rates were negligible, seedling growth was directly proportional to the abundance of mycorrhizae on their roots (Text Fig. 10). Along drainage ditches, however, where the banks were covered with subsoil, nitrates were abundant, and here seedlings grew vigorously in the absence of mycorrhizae.

Nearly all work on the physiology of tree mycorrhizae, recorded in the literature, has been carried out with the object of elucidating the organic nitrogen theory. In the next section of this paper the results of these experiments have been assembled, and when actual data were included in the original papers these have been reproduced in graphical or tabulated form. They demonstrate conclusively that the nitrogen theory is untenable as a full explanation of the mycorrhizal habit in trees. Möller (1902, 1903, 1908), Müller and Weiss (1907), and Melin (in his latest, 1927, published experiments), all failed to find the indirect relationship between nitrogen availability and the abundance of mycorrhizae which the nitrogen theory postulates. Indeed, the exact opposite relationship was discovered. For this reason Möller (1903) questioned the correctness of the whole theory that mycorrhizae are beneficial organs. Müller and Weiss (1907) merely questioned whether the significance of mycorrhizae is understood. Melin (1927, p. 492) concluded that the character of the humus itself determines the results, and that the development of mycorrhizae is favored by the factors which bring about an increase in the nitrogen mobilization: ''Es ist meines Erachtens wahrscheinlich, dass in erester Linie die Humusformen selbst direkt oder indirekt die Ursache für die verschiedene Ausbildung der Mykorrhizen gebildet haben'' (1927, p. 492).

In subsequent years McArdle (1932) and Rayner (1934) have carried out experiments on tree mycorrhizae that were planned from the viewpoint of the nitrogen theory. The method used in McArdle's study, namely, growth of seedlings on synthetic organic sources of nitrogen in open cultures, precluded from the start any hope of success. Rayner's experiments yielded positive results but, as she apparently recognizes, their character does not permit one to conclude that the utilization of nitrogen is the essential function performed by mycorrhizae.

The belief is still general that ectotrophic mycorrhizae are of significance chiefly in that they enable trees to utilize organic sources of nitrogen, but at least three investigators have been unable to confirm this possibility by comparative studies of the abundance of mycorrhizae in soils in which the factor of nitrogen availability was varied. It would therefore appear that this explanation cannot account for the observed facts of mycorrhizal distribution in nature.

A number of other less general explanations of observed peculiarities in the local distribution of mycorrhizae have been suggested by Melin and are worthy of note.

EFFECT OF HYDROGEN ION CONCENTRATION ON THE DISTRIBUTION OF MYCORRHIZAE

In pure culture, Melin (1925, p. 96-101) discovered that the mycorrhizal fungi characteristic of raw humus soils grow best at pH values of approximately 4.5. At values below 3.0 or above 6.0 growth is markedly inhibited. In the raw humus soils of Sweden the hydrogen ion concentration varies from a little above pH 3.0 to a little less than 6.0, showing a close correlation with the values obtained for normal growth of mycorrhizal fungi in culture. On the other hand, in the mull soils of southern Sweden, where the lime content is high, the pH value approaches neutrality. Melin concluded that the probable explanation of the poor development of mycorrhizae in these soils is the unfavorable hydrogen ion concentrations for growth of mycorrhizal fungi. Lindquist (1932) for the same region related the absence of mycorrhizae to high nitrification rates.

Although hydrogen ion concentration may explain the absence of mycorrhizae in some instances, it probably does not play a very large rôle in determining mycorrhizal distribution in general. In America, for example, Lohman (1927, p. 49) reports the presence of mycorrhizae in forest plants regardless of soil acidity. Nor do pH values approaching neutrality retard the development of mycorrhizae on pine in Australia (Cromer, 1935). Furthermore, the optimum range of pH values favorable for growth of mycorrhizal fungi in mull and mineral soils, respectively, may differ. Melin's studies were with fungi which grow naturally in acid soils only.

THE THEORY OF TOXIC PRODUCTS OF FUNGAL METABOLISM

In pure culture, the products of metabolism of many mycorrhizal fungi rapidly inhibit continued fungal growth (Melin, 1925; Hatch and Hatch, 1933). Melin has suggested, accordingly, that the poor growth of overmature and long stagnant stands of spruce growing in heavy accumulations of humus in northern Sweden may in part be caused by similar accumulations of toxic substances in the humus. The idea was mentioned merely as a speculation: "Über diese Fragen habe ich aber bisher keine direkten Untersuchungen ausgeführt." (1925, p. 101.)

INFLUENCE OF SOIL AERATION ON DISTRIBUTION OF MYCORRHIZAE

To Melin we are indebted for still another explanation of peculiarities in mycorrhizal distribution, namely, that in saturated bog soils the fungi producing mycorrhizae are incapable of growth (Melin, 1917). When such bogs are successfully drained tree growth is possible and mycorrhizal fungi also gradually become established. Of all explanations of mycorrhizal distribution this is the only one which has been definitely proved.

CONCLUSIONS

The mineral salt theory, while having much in its favor in the writer's judgment, has been generally discredited. Experiments to elucidate the nitrogen theory have shown that it is inadequate or at best that it can explain only a part of the picture. Other interpretations very clearly have but local application, are wholly speculative, or are obviously incorrect.

As to the passive view of mycorrhizae championed on theoretical grounds by many workers and expressed in the well known words of Gibelli (1883), ". . . che certe forme parassitarie potessero avere un indigenato tollerato e tollerabile sulle radici del castagno sano, senza suo sensibile detrimento," one can only say that it is seemingly not the way of nature to isolate from its source of nourishment well over 95 per cent of all absorbing surfaces of one organism by a parenchyma-like envelope of another, without effects arising therefrom. Nutritional symbioses are altogether too common and widespread in both plant and animal kingdoms to suppose that here in plant roots the most common and intimate associations known between fungi and plants should be tolerated only because they do no harm. Indeed, in orchids (Kusano, 1911; Beau, 1922; Knudson, 1929, although he interprets his results differently; Burgeff, 1932) with endotrophic mycorrhizae, and in *Monotropa* (Francke, 1934) with ectotrophic mycorrhizae, the fact of beneficial symbiosis has been experimentally proved. The anatomical and cytological similarities of the mycorrhizae of these plants and those of vast numbers of other plants, including trees, are too close to suppose that their physiological functions are entirely dissimilar. The conclusion is unavoidable that mycotrophy represents a nutritional symbiosis. The problem is to discover its nature and cause. The experimental approach is the only one that appears capable of yielding conclusive results.

III. EXPERIMENTAL

Introduction

No completely successful method for exploring the physiology of tree mycorrhizae is known, and the development of suitable experimental techniques is a necessary prerequisite to further progress.

Because the influence of other microörganisms is eliminated, pure culture is generally regarded as most suitable for studying the effects of mycorrhizae on growth. In its usual forms, however, pure culture has failed to yield conclusive results and considerable improvement in the physical environment and in the facility with which the composition of the substrate can be controlled must precede its further application to these studies. Unfortunately, the alternative course recently suggested by McArdle (1932), that of using open culture methods with synthetic organic substrates, has long been recognized as inapplicable to the problem.

In view of the knowledge which can be gained concerning the nature of mycorrhizae through comparative studies of the development of mycorrhizae in soils of different characters (see preceding section), the soil culture (pot culture) method recently improved by Hesselman (1927) and Melin (1927) seems especially promising.

From the beginnings of interest in mycorrhizae, cytological and anatomical studies have formed a conspicuous portion of the evidence on which conclusions with respect to the character of mycorrhizae were based. Difficulties in the utilization of these methods experimentally, as in the utilization of microchemical techniques, arise from the fact that localized conditions in the root tips must be interpreted on the basis of the physiology of the whole plant. Cytological studies have shown that the activity of cortical cells embedded in mycelium is normally stimulated by the fungus rather than decreased or stopped, and that hyphae penetrating into these cells are usually digested. One can conclude little more from this knowledge than that mycorrhizal fungi are not pathogenic in the ordinary meaning of this term and that there is evidence of an exchange of nutrients. From anatomical relationships it is known that all water and nutrients which enter tree roots (that is, those forming ectotrophic mycorrhizae) by way of short roots pass through fungal hyphae, but in what manner and to what extent this is of significance to the tree is largely conjectural.

Although microchemical studies have shown that mycorrhizal roots may possess more or less of a given nutrient element than a corresponding uninfected root, the interpretation of this evidence on the basis of seedling physiology is hazardous. When such studies are made on roots collected from soil where the nutritional conditions vary in every part of the soil, almost any positive conclusion is speculative. These methods may be useful, yet by themselves they are incapable of solving the problem. Attention may more profitably be focused on those experimental methods in which gross effects of mycorrhizae on total seedling growth can be accurately measured.

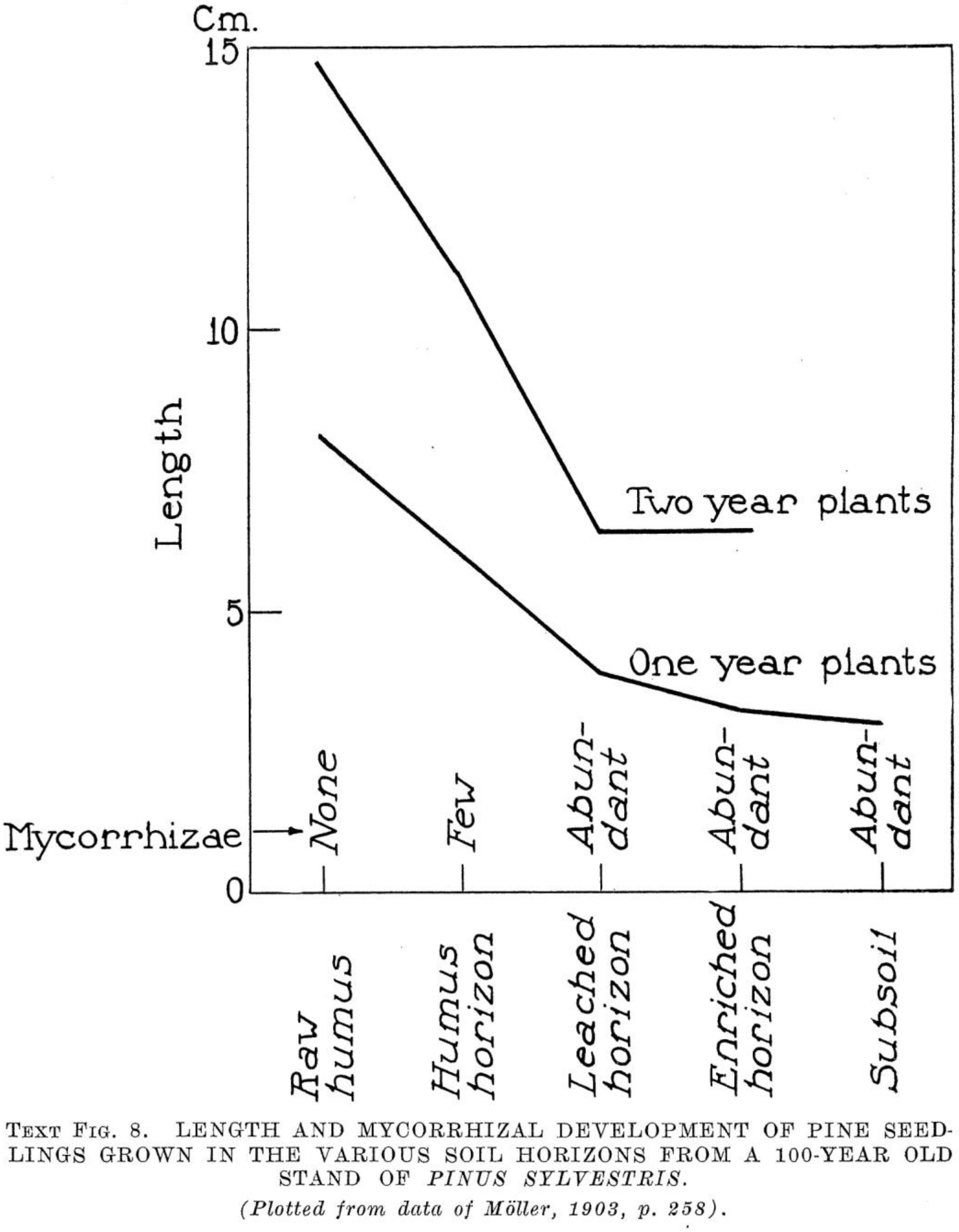
These considerations led the writer to select the pure and soil culture methods for exploring the habit. To yield conclusive results the accuracy of the methods had to be materially improved and much time was spent on this part of the investigation. It involved, among other things, the pursual of quantitative experiments and the use of statistical methods in interpreting results. With abundant pure culture and pot culture material available, a comparative study of the morphology and anatomy of mycorrhizal and non-mycorrhizal seedlings was also undertaken.

Soil Culture Experiments

PREVIOUS RESULTS

Studies of the comparative abundance of mycorrhizae on seedlings grown in pot cultures of different kinds of soil were first undertaken by Frank (1888). Mycorrhizae were found to be more plentiful on beech seedlings raised in humus than in meadow or garden soils. These results were at first thought to support the humus theory, but later they were fitted into the view that utilization of the organic nitrogen of humus is the specific function of mycorrhizae (Frank, 1894).

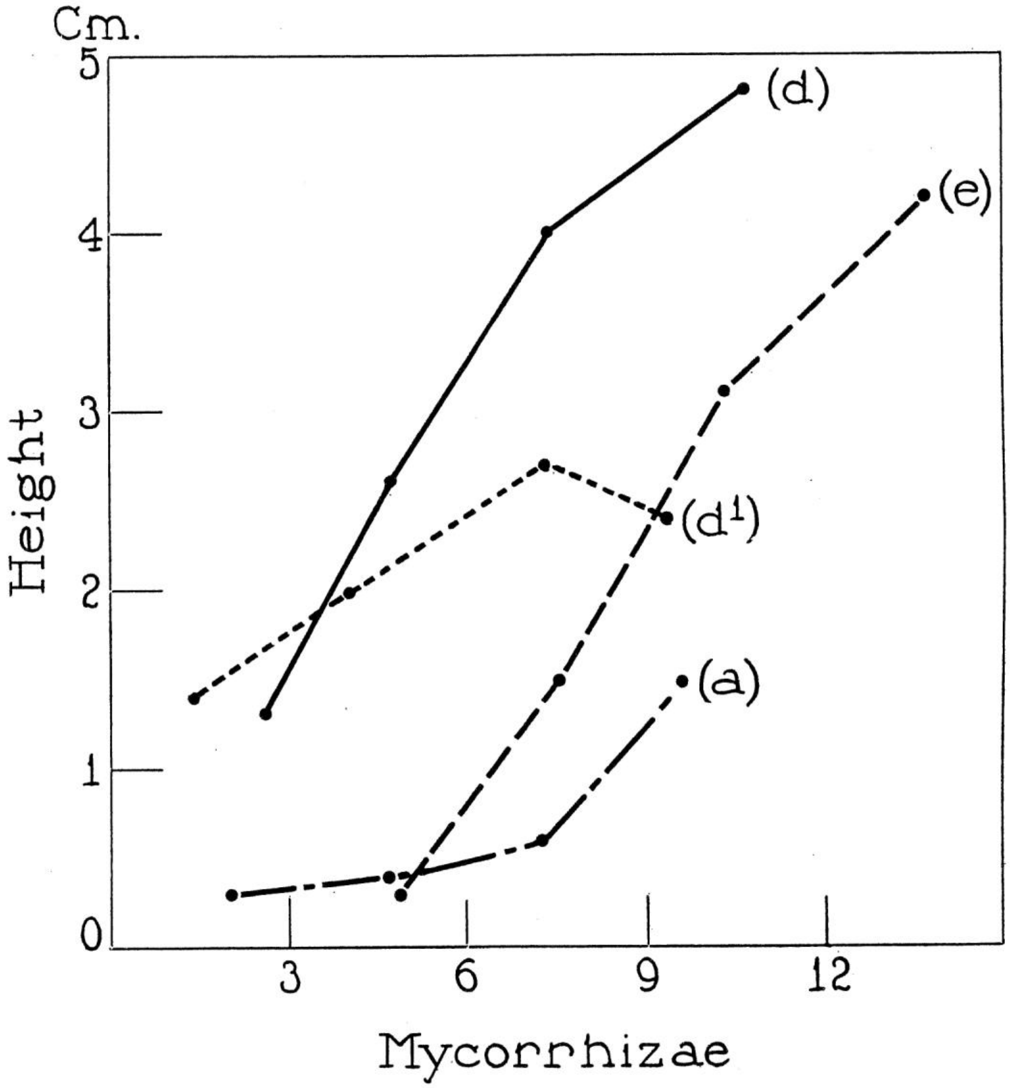
Results wholly contradictory to those of Frank were repeatedly obtained by Möller (1902, 1903, 1908) in a series of pot (or box) culture experiments. Seedlings of *Pinus sylvestris* were grown in the separate horizons of a 100-year old pine forest soil as well as in the nitrogen- and humus-free tertiary sand from the subsoil one and one-half meters below the surface. The height-growth and abundance of mycorrhizae on seedlings raised for one and two years in the various horizons are shown in Text Fig. 8. Included in Möller's papers are photographs of root systems and of whole plants which strikingly illustrate the differences shown in this figure. To learn whether mycorrhizae fix atmospheric nitrogen, Möller (1903) transplanted mycorrhizal seedlings to clean, almost pure sand ("Urboden") collected one and one-half meters below the soil surface (analyzed by Ramann and reported to be free of nitrogen and organic materials). Mineral salts were added to all pots, and nitrogen in the form of nitrates to half of them. After a year's growth the plants having access to nitrates had profuse mycorrhizae and were large and healthy, while those lacking nitrates had occasional mycorrhizae and were poorly developed. Although this experiment was designed to test the theory of nitrogen fixation, it demonstrates that mycorrhizae may be produced in inorganic substrates in the presence of abundant supplies of inorganic nitrogen. Möller (*op. cit.,* p. 333) stated of his results: "Die regelmässige Zufuhr des Salpetersäuren Natron hat, . . . die Mykorhizen in ihrer Entwicklung befördert."



TEXT FIG. 8. LENGTH AND MYCORRHIZAL DEVELOPMENT OF PINE SEEDLINGS GROWN IN THE VARIOUS SOIL HORIZONS FROM A 100-YEAR OLD STAND OF *PINUS SYLVESTRIS.*

(Plotted from data of Möller, 1903, p. 258).

In another experiment Möller (1908) transplanted small, mycorrhizal seedlings, raised in sand, to humus; and conversely, non-mycorrhizal seedlings, raised in humus, to sand. After a year's growth the mycorrhizal development of the plants was completely reversed. Plants grown in a mixture of the two soils exhibited intermediate growth and intermediate mycorrhizal development. Möller reported a large number of other



TEXT FIG. 9. RELATION OF MYCORRHIZAL DEVELOPMENT TO PLANT HEIGHT OF 182 BEECH SEEDLINGS GROWN IN SEED BEDS IN LIMED BEECH MOORLAND SOIL "(a)", HUMUS-RICH SANDY LOAM "(d)" AND "(d 1)", AND IN HUMUS-POOR LOAM "(e)". ON THE VERTICAL SCALE, "5" INDICATES PROFUSELY CORALLOID MYCORRHIZAE, "0" NO MYCORRHIZAE.

(Values from Müller & Weiss, 1907, p. 197).

experiments which demonstrated that addition of humus to infertile sandy soils invariably stimulated seedling growth, the degree of stimulation varying directly with the infertility of the soil. Möller concluded that the rôle of mycorrhizae in nitrogen utilization as postulated by Frank was not apparent and he questioned whether the true significance of mycorrhizae was known.

The development of mycorrhizae in relation to the size and vigor of beech seedlings was studied by Müller and Weiss (1907) in Denmark. The plants were grown for periods of one and two years in moorland and in nursery soils in the field, and in pot cultures of the moorland soil. Lime and inorganic nitrogen were added to the moorland soil in some treatments. Müller noted that under field conditions he had never found well developed coralloid mycorrhizae except on vigorous seedlings; and conversely, he had never found small unhealthy seedlings with coralloid mycorrhizae. The relation between the length of seedlings (average length of plants in each height class, 0-3 cm., 3-6 cm., 6-9 cm., etc.) and the mycorrhizal development of the beech seedlings raised by Müller and Weiss in the field is given in Text Fig. 9. These curves show precisely the opposite relationships from those of Möller (Text Fig. 8). It was impossible for Müller to decide whether the good growth of the seedlings resulted because of profuse development of mycorrhizae or whether the production of these mycorrhizae was in some way favored by vigorous seedling growth. That mycorrhizae are not always essential to healthy growth of seedlings was demonstrated as in Möller's experiments by the complete absence of mycorrhizae on seedlings raised in the moorland soil in pot cultures (Table I). It is now known that potting infertile soils greatly increases their fertility temporarily (Romell, 1934). It was also discovered that the availability of nitrates had no influence on root hair development, but the presence of nitrates was necessary for the formation of mycorrhizae. According to Frank's theory, on the other hand, mycorrhizae become useful to trees when nitrates are not available. These results led Müller, in common with Möller, to question the correctness of the current (1894-1906) concepts of the rôle of mycorrhizae in tree nutrition.

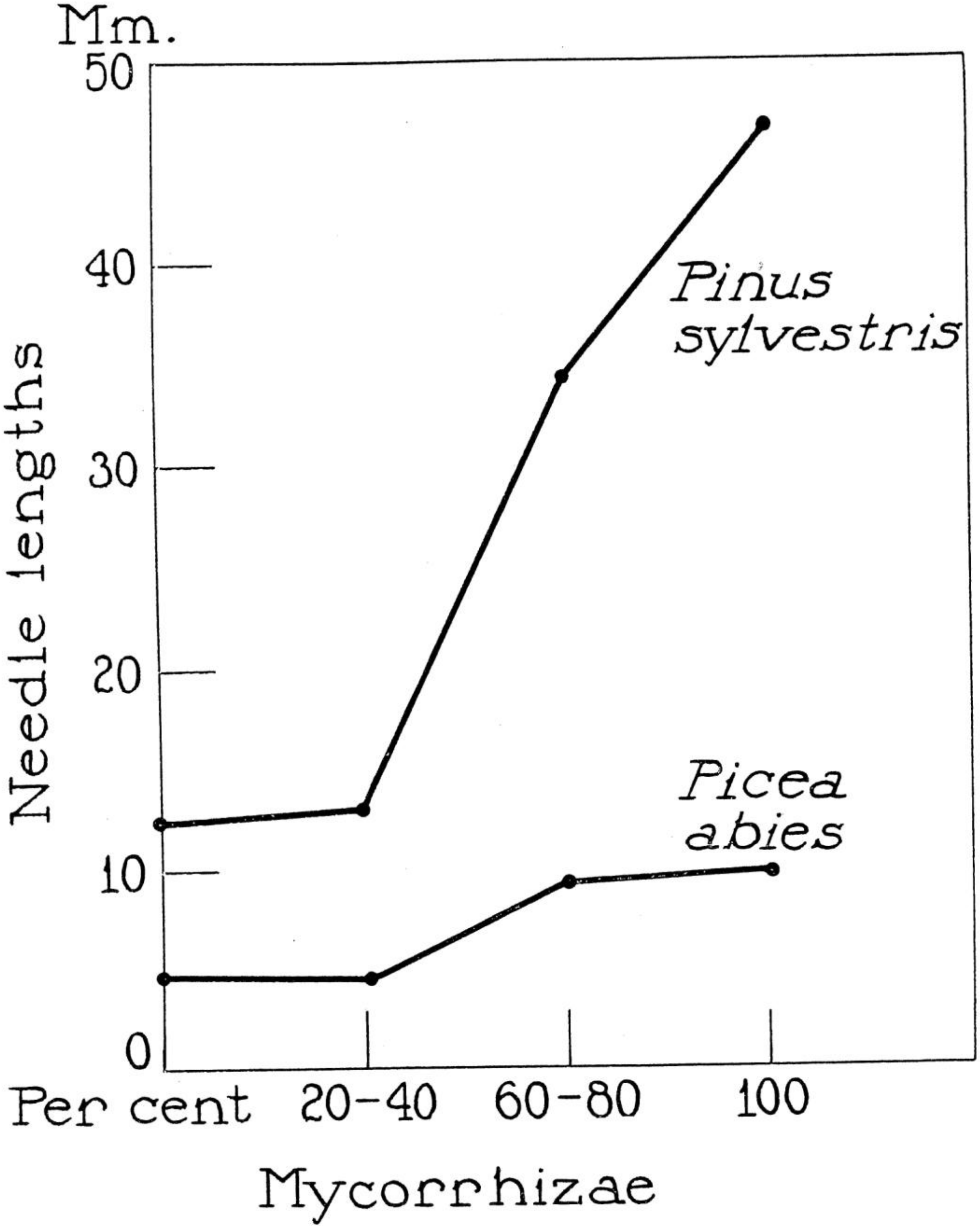
TABLE I

RELATION OF LIMING AND NITROGEN ADDITIONS TO AVERAGE LENGTH OF PLANTS AND MYCORRHIZAL DEVELOPMENT OF BEECH SEEDLINGS GROWN IN POT CULTURES OF SOIL FROM BEECH MOORLANDS IN DENMARK

(After Müller and Weiss, 1907)

Treatment	*No. plants*	*Av. length*	*Myc. develop.*
One year plants			
None	5	5.4	0.0
NO_3 salts	8	7.3	0.2
NH_3 "	6	7.2	0.8
Limed, no N.	5	9.1	0.0
" NO_3 salts	9	8.2	0.3
" NH_3 "	10	5.4	0.0
Two year plants			
Limed, no N.	4	12.2	1.8
" NO_3 salts	3	12.3	0.3
" NH_3 "	3	10.2	0.2

In one of the classical contributions to mycorrhizal literature Melin (1917) correlated seedling growth in newly drained peat bogs with the abundance of mycorrhizae on their roots. The case differed, apparently, from previous studies in that mycorrhizal fungi were initially absent from the soils in question and the production of mycorrhizae depended on chance inoculation of the seedlings by wind-blown spores. Healthy seedlings never were found in the bogs proper, except when infected. Seedlings showing poor growth increased their growth with infection, and such stimulated growth did not result from increases in the nitrification of the soil. All evidence pointed to the symbiotic rôle of mycorrhizae as the cause of normal growth. The relation found between the growth of 191 seedlings of pine and spruce, as measured by needle lengths, and mycorrhizal develop-



TEXT FIG. 10. RELATION OF NEEDLE-LENGTH TO PER CENT OF SHORT-ROOTS CONVERTED TO MYCORRHIZAE ON 174 SEEDLINGS GROWING IN NEWLY-DRAINED BOGS IN NORTHERN SWEDEN.

(Values from Melin, 1917, p. 398).

ment is shown in Text Fig. 10. As mentioned in an earlier section, seedlings grown along drainage ditches where nitrate mobilization was rapid lacked mycorrhizae, yet they grew vigorously. Melin favored the nitrogen theory of Frank as the most satisfactory explanation of the function of mycorrhizae and accordingly attributed the good growth of these ditch-grown plants (lacking mycorrhizae) to the abundance of nitrates along drainage ditches.

The influence of a variety of factors on growth and the mycorrhizal development of conifer seedlings in seedbeds and in peat was recently studied by Laing (1932). Additions of calcium phosphate and (or) ammonium sulphate to nursery beds of *P. sylvestris* increased the development of mycorrhizae. Ammonium chloride, on the other hand, had no effect on the character of the short-roots. None of these treatments influenced the formation of mycorrhizae on seedlings of *Picea Abies* (L.) Karst. The effect of a number of chemicals—lime, potash, magnesium carbonate, phosphates, and nitrates—on the formation of mycorrhizae in peat was also examined. Magnesium carbonate alone produced changes in the character of the short-roots, the number of mycorrhizae being increased. The usefulness of Laing's results in the present discussion is limited by the absence of chemical analyses and by detailed growth and mycorrhizal data.

The most spectacular results yet obtained are those reported by Oliveros (1932) in the Philippine Islands. Between 1909 and 1926 in a series of nine attempts to raise *Pinus insularis* at Los Baños, Laguna, from seeds collected at Baguio, Mt. Province, not a single plant survived. In 1919, 1922, 1927, and again in 1929 potted seedlings from Baguio were planted at Los Baños. These all survived and at the time of measurement in 1932 had grown in height an average of over a meter a year, nearly twice the growth made by the tree in its native range. A series of experiments was then commenced with sterilized and unsterilized soils, the latter collected from around the roots of these trees. Soil sterilization renders unsafe a direct comparison of the two sets of experiments as made by Oliveros. It is generally known, however, that sterilization usually produces an initial stimulation of growth because of increased availability of nutrients (Waksmann, 1932; Frank, 1894; Stahl, 1900; and many others). But in these experiments seedlings in sterilized soil grew an average of only 2.9 cm. in one series and 3.9 in another, while those in non-sterilized soils grew 10.4 and 12.0 cm. respectively in the same period (five months). Data on the development of mycorrhizae were not given.

In a recent publication Rayner (1934) has described the influence of soil inoculation and fertilization on the growth and mycorrhizal development of pine seedlings. The work was carried out in an infertile heath soil which had given poor results in earlier attempts to establish pine seedlings. Prepared nursery beds and pot cultures were inoculated with forest humus collected in Sweden, Corsica, and Ireland. Pure cultures of mycorrhizal fungi were also used for inoculating pot cultures of the heath soil. Finally, composted organic materials derived from sawdust, straw, and other organic wastes were added to the nursery beds and to the soil in pot cultures, and the growth and mycorrhizal development of the seedlings grown in them were observed. The results were presented only in the form of generalized statements and photographs. The latter show enormously stimulated growth of seedlings in some of the compost treatments and small but fairly consistent increases with soil and pure culture inoculations. While comparative data on the growth of seedlings in soils with and without the organic composts are not given, the differences shown in photographs appear to be of the same order reported by Möller (1908) for additions of humus to similar infertile sandy soils. Rayner (p. 116) believes that soil inoculations as well as compost additions stimulated the production of short-roots before any possible effect due to mycorrhizal activity could have been initiated. She (p. 122) concluded that a direct causal relationship exists between the development of mycorrhizae and thrifty development of various species of pine. Although this conclusion does not indicate the manner in which mycorrhizae are thought to benefit the seedlings, it is clear that Rayner associates stimulated growth with an increase in nitrogen acquisition.

The culture studies of Francke (1934) on *Monotropa hypopitys L.* have yielded results of unusual significance. While the tendency among modern students of the physiology of mycorrhizae is to pursue comparative studies on seedlings grown in wholly artificial substrates, Francke was unable to do this. *Monotropa* seeds could not be germinated under aseptic conditions and minute seedlings, resulting from seeds germinated in soil, had to be used. As expected, introduction of these into synthetic media was followed by profuse development of contaminating organisms, so that synthetic susbstrates were useless. When seedlings were grown in flasks of sterilized and washed humus, development of the contaminating organisms introduced with the seedlings was less profuse than on artificial substrates. These cultures were inoculated with various Hymenomycetes, especially species of *Boletus,* and with the specific ectophyte (apparently a *Boletus* sp.) previously isolated from *Monotropa* mycorrhizae. Plants supplied with their specific ectophyte survived and increased rapidly in size, while all others perished.

In these cultures a wide variety of microörganisms was present, but the specific mycorrhizal fungus of *Monotropa* alone was capable of influencing growth of the vascular plant. This, in the writer's opinion, is the factor in Francke's experiments which render them more significant than those of previous workers. The stimulated growth of inoculated plants on synthetic nutrients in pure culture has rarely, if ever, been shown to result from a specific exchange of nutrients between fungus and host as the result of the establishment of mycorrhizae. Rather, such growth can almost invariably be attributed to the effect of mycorrhizal fungi on the substrate, that is, reduction of complex chemicals, which presumably cannot be utilized by higher plants, to simpler utilizable forms. The inability of a multitude of microörganisms in Francke's cultures to promote the growth of *Monotropa* in this manner excludes the possibility that the specific mycorrhizal fungus stimulated growth in this way rather than through the mechanics of direct symbiotic exchange of nutrients with the seedlings. The cytological evidence of digestion of hyphae by host cells of the culture seedlings (Francke, 1934) renders this conclusion doubly secure. In specimens of *Monotropa* which are grown in humus soils all root surfaces are isolated from the soils by becoming mycorrhizal. The fact that the plants obtain all of their nutrients from the fungus is therefore singularly obvious.

Probably the most thorough attempt to relate the mycorrhizal development of tree seedlings to the availability of nitrogen in humus soils was that of Hesselman (1927) and Melin (1927). Since the pot culture experiments reported in the present paper were originally modeled after the experiments of these investigators, their methods and specific results are given in detail.

Seedlings of pine and spruce were grown by Hesselman in pot cultures of sand-mixed humus collected from various Swedish forests. The environmental conditions were uniform for all plants, so that soil differences alone were reflected in the results. The availability of nitrogen in the form of nitrates and ammonium salts was determined by storage of soil samples in flasks under optimum moisture and temperature conditions for three months followed by chemical analyses (Hesselman, 1927). Seedling growth was measured by the number and length of needles and by shoot lengths. At the time of harvesting Melin (1927) examined all root systems and preserved two or three typical seedlings from each treatment for detailed morphological and anatomical study. Additional pieces of roots bearing representative samples of all types of mycorrhizae were fixed, embedded, and sectioned for cytological details. Some of the results of this coöperative study are assembled in Table II.

The relation of N-mobilization of the soils to the per cent of short-roots converted to mycorrhizae is striking (Text Fig. 11). An increase of growth with increase in N-mobilization was found by Hesselman. These results are in agreement with those of Müller and Weiss (1907) and apparently are contradictory to the nitrogen theory of Frank (1894) and to Melin's earlier conclusions (1917, 1925).

Thus, of the several investigators who have used soil culture methods for elucidating the character of the ectotrophic mycorrhizal relationship, none has demonstrated that the specific rôle of mycorrhizae is the utilization or absorption of complex organic nitrogen. In fact, certain of the experiments definitely indicate that conservation of nitrogen is not the specific rôle of these organs. In the following account of the writer's experiments the nitrogen question is the first to be thoroughly tested. Thereafter attention is directed to other possibilities.

Investigations

STUDIES WITH RAW HUMUS SOILS FROM SWEDEN

Growth studies of the type pursued by Hesselman (1927) were initiated by Dr. P. R. Gast in 1930 at the Royal Institute of Experimental Forestry, Sweden. The experiments provided an excellent opportunity for studying the mycorrhizal relationships of pine seedlings and the writer gladly accepted the invitation of Dr. Gast to

TABLE II

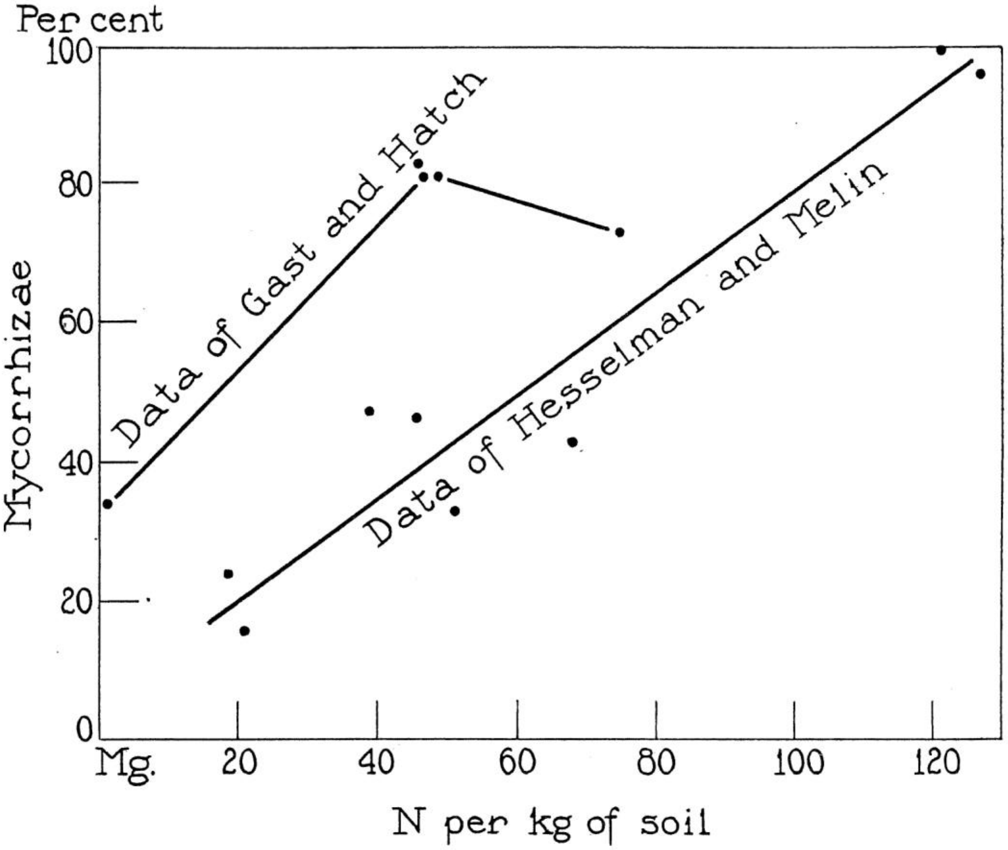
COMPARATIVE DATA ON GROWTH OF PINE SEEDLINGS, NUMBERS OF MYCORRHIZAE AND NITROGEN MOBILIZATION RATES OF HUMUS SOILS FROM FOUR SWEDISH FORESTS

(Assembled from Melin, 1927 and Hesselman, 1927)

Humus sample[1]	*Treatment Humus plus*	*Nitrogen mobilization of humus in mg. per kg.*		*Average shoot growth in cm.*	*Number root systems examined*	*Length of long-roots*	*Mycorrhizal short-roots*		*Total No. short-roots*	*SR*[2] / *LR*
		Nitrate	*Total*				*No.*	*%*		
I	Distilled water	12.1	21.0	4.00	2	434	40	15	259	60
	Humus extract	9.1	19.4	4.45	3	542	65	24	293	54
II	Distilled water	47.1	51.7	5.38	2	317	82	33	244	77
	Humus extract	41.1	46.1	6.20	2	328	85	46	187	57
III	Distilled water	56.5	68.8	5.73	2	293	68	42	144	49
	Humus extract	31.7	39.6	5.15	3	430	91	47	197	46
IV	Distilled water	113.7	127.8	10.28	3	1383	454	96	472	34
	Humus extract	106.8	120.6	11.56	2	1002	485	99	488	49

[1] As listed by Hesselman, 1927, p. 345. [2] SR short-roots, LR long-roots.

use this material. Coöperative studies have been continued in subsequent years with Dr. Gast at the Harvard Forest and with Mr. H. L. Mitchell at the Black Rock Forest. The specific objectives of the writer's part of these seedling studies have been to determine the influence on mycorrhizal development of the following variables: (1) different nitrogen mobilization rates in soils, (2) different radiation intensities and qualities, (3) artificial and natural variations in the availabilities of the mineral salts of soils.



TEXT FIG. 11. RELATION BETWEEN NITROGEN MADE AVAILABLE BY MOBILIZATION IN SWEDISH RAW HUMUS SOILS AND THE PER CENT OF SHORT-ROOTS CONVERTED TO MYCORRHIZAE.

(Based on short-root counts of 153 seedlings grown in 9 different soils).

In 1930 seedlings of *Pinus sylvestris* arising from seeds of known weights were grown by Gast in pot cultures of sand-mixed humus collected from four forests of Sweden. Two of the soils were collected from an overmature, slow-growing spruce stand in the Kulbäcksliden Experimental Forest in northern Sweden. They differed in that one—the Severely Burnt Bad Raw Humus—was collected from a clear-cut and severely burnt area [broadcast burning is regularly employed in this forest as a silvicultural measure for facilitating reforestation (Hesselman, 1917)] while the other—the Bad Raw Humus—was collected from an adjoining undisturbed part of the stand. The soil sample designated Good Raw Humus was collected on Brända Holmen, a small island in a large bog (in the same experimental forest) which supported a rapidly growing mixed stand of spruce, pine, and birch. The fourth soil was a humus of excellent quality from Jönaker, called Inoculation Soil by Hesselman (1927). These soils, the stands from which they were collected, and the arrangement and growth results of the experiments are fully described by Gast (1937). All root systems were examined by the writer at the time of harvesting; and for a representative number of plants from each treatment the number and kinds of short-roots were counted. One average seedling from each treatment was fixed for detailed anatomical study.

In general, the results (Table III) confirm those of Melin and Hesselman. Seedling growth (with one exception), nitrogen mobilization, and mycorrhizal development were all related in a parallel direction; that is, with increase in nitrogen mobilization of the soil, the number of mycorrhizae and the growth of seedlings also increased. The tendency for the percentage of the short-roots converted to mycorrhizae to increase with increase in nitrogen mobilization is shown in Fig. 11. In contrast to the results of Melin and Hesselman, as shown in the same figure, this increase was pronounced chiefly in the lower range of nitrogen availability.

The exception to this parallel relationship occurred in the Severely Burnt Bad Raw Humus. In this soil mycorrhizae were well developed (an average of 83 per cent of all roots in comparison with 34 per cent in the unburnt soil were mycorrhizal). Nitrogen mobilization was also comparatively large but growth was not correspondingly high (Table III). At the time that these results were first examined it was concluded that some other element besides nitrogen was available in such small quantities that the growth of these seedlings was limited. Eventually, however, it was found that, although potassium was somewhat low, the nitrogen content of the seedlings was proportionately even lower. The low growth is now attributed to a low internal content of nitrogen by Gast (1937). Just why absorption was markedly less than should have been expected from the supply in the soil is unknown.

With respect to radiation intensity, no consistently marked changes in the percentage of short-roots converted to mycorrhizae were discovered. Their number and development, however, in common with the total number of short-roots and with the extensiveness of the whole root system, was greatly reduced in the low radiation intensities. At 4 per cent radiation short-roots were wholly absent. This series of seedlings is not included in the data presented in this paper but it is fully described by Gast (1937).

Briefly, these experiments confirm for the fourth time (see Möller, 1902, 1903, 1908; Müller, 1907; Melin, 1927) the probability that ectotrophic mycorrhizae are not concerned primarily with the utilization of the organic nitrogen of humus. The possibility that they may be related to mineral salt deficiencies, as postulated by Stahl (1900), was suggested by abundant mycorrhizae but low weights of seedlings grown in the Severely Burnt Bad Raw Humus (see Table III). The following year at the Harvard Forest this possibility was examined using seedlings raised in humus with and without additions of mineral salts.

TABLE III

GROWTH, AS MEASURED BY DRY WEIGHTS AND N, K AND P CONTENTS, AND THE NUMBERS AND PER CENT OF SHORT-ROOTS CONVERTED TO MYCORRHIZAE ON THREE-MONTH SEEDLINGS OF *P. sylvestris* GROWN IN RAW HUMUS SOILS OF SWEDEN UNDER FOUR RADIATION INTENSITIES

(Data except that on mycorrhizae, from Gast, 1937)

Soil[1]	*Seedl. dry wts. mg.*	*Per cent contents*			*Shoot root ratio*	*No. seed-lings*	*Mycorrhizal data*		
		N	*P*	*K*			*No. short-roots*	*No. myc. short-roots per seedl.*	*Per cent myc. per seedl.*
				11 per cent radiation					
Bad raw humus	13.7	1.57	...	...	4.2	5	83	29	35
Sev. burnt b. r. h.	16.1	2.01	...	...	4.3	12	102	79	77
Mixture[2]	10.8	2.36	...	...	6.2	4	54	47	88
Inoculation soil	19.0	2.36	...	...	5.8	5	70	38	55
Good raw humus	18.4	2.68	...	...	5.7	13	73	47	64
				22 per cent radiation					
Bad raw humus	21.4	1.12	0.34	...	2.0	5	195	68	35
Sev. burnt b. r. h.	29.6	2.16	0.36	...	2.3	5	195	165	84
Mixture	27.2	2.01	...	...	2.2	3	192	151	78
Inoculation soil	48.2	2.52	...	...	2.7	5	293	258	88
Good raw humus	36.4	2.89	0.34	...	3.5	7	196	163	83
				27 per cent radiation (Infra-red removed)					
Bad raw humus	26.6	0.97	...	0.97	1.5	10	233	64	27
Sev. burnt b. r. h.	36.2	2.28	...	1.12	1.7	5	264	221	84
Mixture	38.0	2.24	...	...	2.0	3	243	186	76
Inoculation soil	48.5	2.54	...	...	2.1	9	323	293	91
Good raw humus	50.7	3.28	...	1.53	2.5	5	377	305	81
				50 per cent radiation					
Bad raw humus	29.9	1.12	0.32	...	1.4	6	290	110	38
Sev. burnt b. r. h.	42.4	2.47	0.34	...	1.8	10	377	326	86
Mixture	33.5	2.15	...	...	1.9	3	231	173	82
Inoculation soil	54.8	1.96	...	...	1.4	6	584	513	88
Good raw humus	85.6	2.93	0.30	...	3.4	10	406	256	63

[1] N mobilization rates in mg. per kg. soil = 1.3 in bad raw humus, 46.2 in severely burnt bad raw humus, 46.3 in mixture, 49.6 in inoculation soil, and 75.4 in good raw humus.

[2] Mixture = 5/6 inoculation soil and 1/6 bad raw humus.

HARVARD FOREST EXPERIMENTS, 1931

Scots pine seedlings were grown in humus collected from a mature stand of *P. Strobus* L. in the Harvard Forest. Except for nitrogen a balanced mineral solution (Gast, 1937) was added to half of the pots of seedlings.

The large number of seedlings in these experiments (711) made counting of the individual short-roots on each seedling impossible. A single seedling may have many hundred short-roots. Therefore, the number and kinds of short-roots were estimated as a percentage of the whole for each seedling. Data for the various treatments are assembled in Table IV.

TABLE IV

THE PER CENT OF SHORT-ROOTS CONVERTED INTO MYCORRHIZAE ON SEEDLINGS OF *P. sylvestris* GROWN IN HUMUS FROM THE HARVARD FOREST UNDER VARIED NUTRIENT AVAILABILITIES AND RADIATION INTENSITIES

Soil Treatment	*15 (?) Per cent (Radiation not determined)*		*24 Per cent*		*48 Per cent*		*55 Per cent (Infra red removed)*		*85 Per cent*		*Av. all treatments*
	No. Seedlings	*% myc.*	*No. Seedlings*	*% myc.*	*No. Seedlings*	*% myc.*	*No. Seedlings*	*% myc.*	*No. Seedlings*	*% myc.*	*% myc.*
None	58	38	112	20	114	38	86	76	113	20	38
Complete nut.—N	56	11	55	1	49	3	19	17	49	14	9

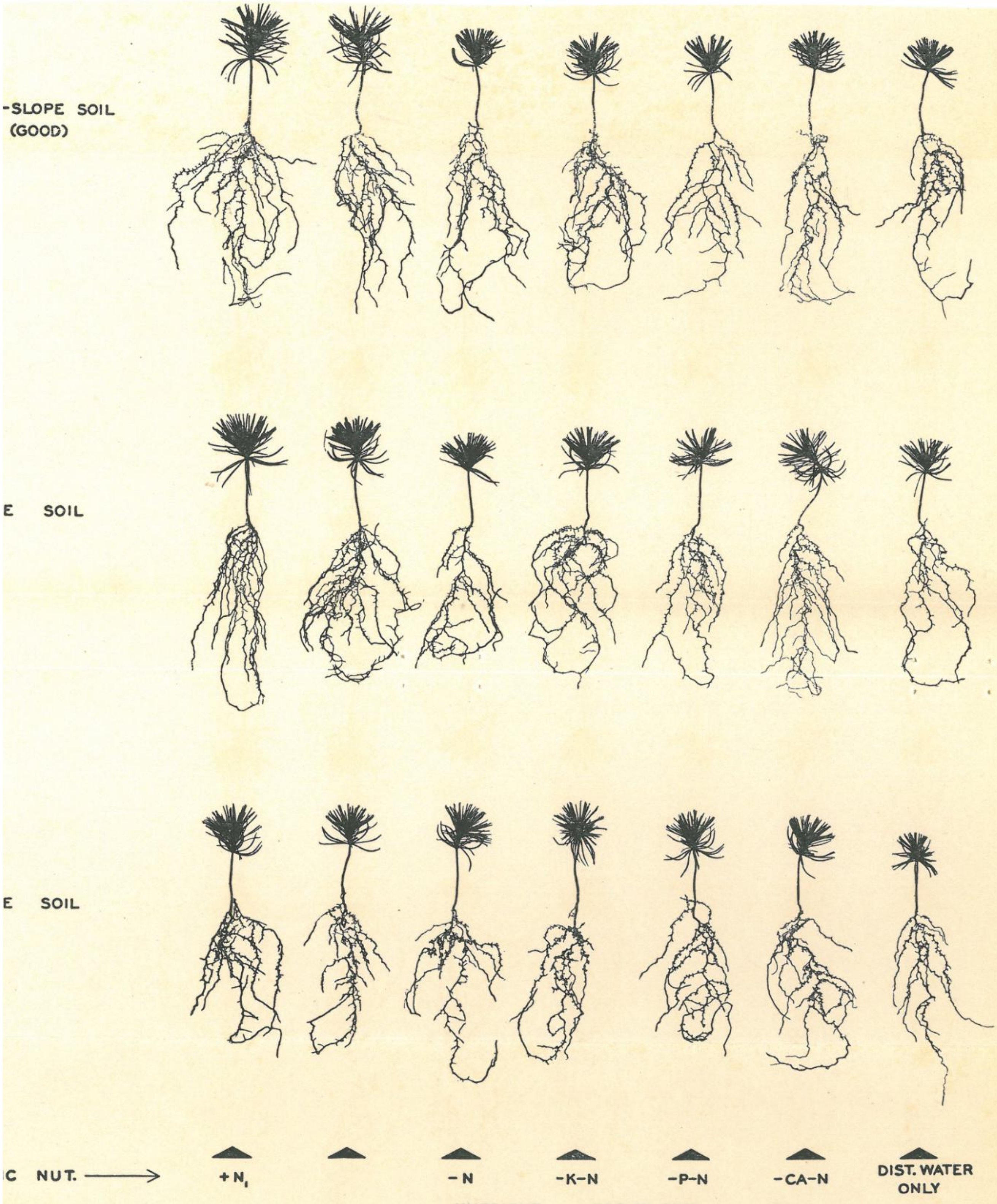
While the abundance of mycorrhizae on these seedlings was much less than in the relatively infertile Swedish soils, two results were outstanding: (1) addition of mineral salts in a balanced solution to soil cultures markedly depressed mycorrhizal production, and (2) differences in radiation intensity had no consistent effect on the formation of mycorrhizae *except when infra-red was removed.*

These results warranted the conclusion that mineral salts do have a profound influence on the development of mycorrhizae. The next step was to determine which elements are capable of influencing these organs; this was done in the Black Rock Forest experiments.

BLACK ROCK FOREST EXPERIMENTS, 1932

Seedlings of *Pinus Strobus* L., grown from seeds of known weights, as in the experiments with *P. sylvestris* described above, were raised in pot cultures of sand-mixed mineral soils from four localities in the Black Rock Forest. The soils were all collected from one slope; their designations as Ridge, Mid-Slope (good), Mid-Slope (poor), and Cove indicate their relative positions on this slope (and also the rates of tree growth in the mid-slope soils). On the basis of the growth of deciduous trees on the respective sites (Tryon, 1930), differences in soil fertility were suspected. Moisture relations were most favorable in the Cove. In the pot culture experiments moisture was uniform in all soils. It was found (Mitchell, 1934) that all soils were deficient in phosphorus (relatively insignificantly so in the Cove), and that the Ridge soil was low in nitrogen also. The Cove soil was especially rich in nitrogen.

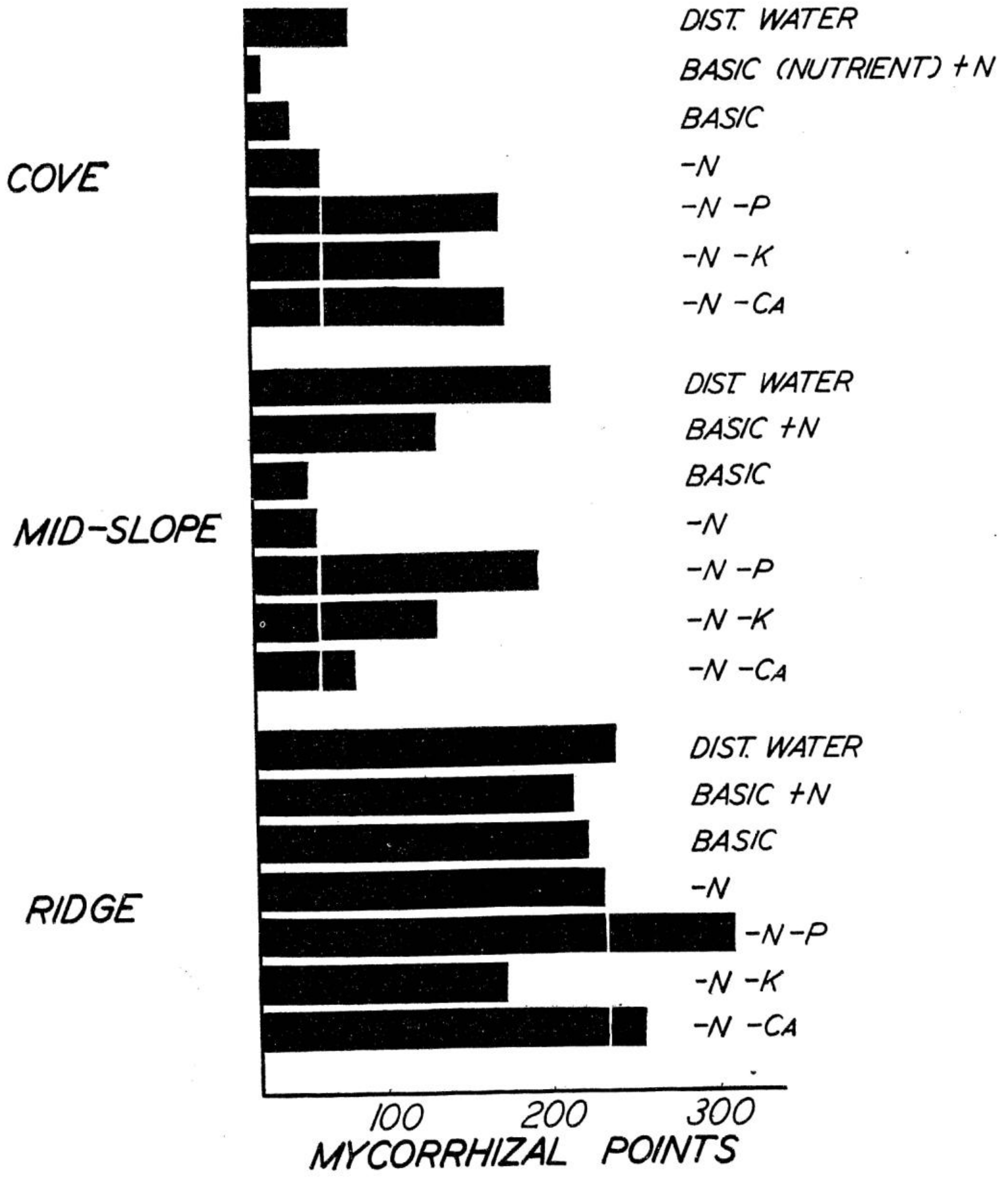
In addition to this natural range of fertility, the availabilities of four elements—nitrogen, phosphorous, potassium, and calcium—were varied by additions of nutrient solutions. The so-called ''basic nutrient'' contained all of these elements, together with magnesium, sulphur, and iron. In one treatment ($+N_1$) an extra amount of nitrogen was added, while in another ($-N$) nitrogen was omitted. In three other treatments ($-N-P$, $-N-K$, and $-N-Ca$) these elements were omitted respectively from the basic nutrient. Only distilled water was added to those pots in which natural soil fertility was tested. The quantities of nutrients added in all cases



ATE II. SILHOUETTES OF WHITE PINE SEEDLINGS GROWN IN SAMPLES OF THREE NATURAL SOILS SUPPLEMENTED WITH VARIOUS NUTRIENT COMBINATIONS.

Silhouettes by A. B. Hatch.

ent treatments in three soils—Ridge, Mid-Slope (good), and Cove—are presented in graphical form in Text Fig. 12. Complete data on mycorrhizal points, percentage of short-roots converted to mycorrhizae, dry weights, and nitrogen contents are assembled for all four soils in Table VI. Silhouette photographs of the root systems of average seedlings from each treatment are shown in Plate II. Enlargements of fairly representative long-



TEXT FIG. 12. RELATION OF NITROGEN ADDITIONS IN SOILS OF THE BLACK ROCK FOREST TO MYCORRHIZAL DEVELOPMENT.

TABLE VI

Dry Weights, Nitrogen Contents, Mycorrhizal Percentages, Mycorrhizal Points, and Correlation Coefficients Between Dry Weights and Mycorrhizal Developments of *P. Strobus* Seedlings Grown in Black Rock Forest Soils with Varied Nutrient Treatments

(Dry weights and N contents from Mitchell, 1934)

Treatment	*Per cent of short-roots mycorrhizal*		*Cor. coef. Dry Wts. & % mycor.*	*Mycorrhizal points*		*Cor. coef. Dry Wts. & myc. points*	*Ave. dry weights in mg.*	*Per cent nitrogen in seedlings*
		S.E.M.			*S.E.M.*			
				Cove Soil				
Dist. water	43	5.2	.411*	70	11.85	.331	241	1.65
	33	4.0	-.281	53	8.78	-.370	227	1.70
Basic nut.	1	0.3	-.346	1	. .	-.359	274	1.84
	26	5.6	-.434	36	9.50	-.244	281	1.90
Basic N_1	6	2.5	-.174	. .	. .	-.133	299	2.11
	8	3.0	.107	. .	. .	.072	283	2.05
Basic—N	6	2.4	.249	11	4.13	.330	268	1.67
	43	6.6	-.140	64	9.19	.025	271	1.48
Basic—N-P	66	5.0	-.525*	97	8.00	-.409	208	1.81
	93	2.5	.096	180	12.81	.330	201	1.83
Basic—N-K	95	1.9	. .	182	7.32	. .	286	1.61
	50	7.5	-.094	58	11.41	-.008	276	1.77
Basic—N-Ca	87	2.2	.308	144	8.30	.149	286	1.38

TABLE VI—*(Continued)*

Mid-Slope Soil (good)

Dist. water	92	1.6	-.222	193	14.10	-.095	226	1.23
	88	3.9	-.158	152	1.44	.080	229	1.29
Basic nut.	8	3.2	-.285	9	3.17	-.086	265	1.79
	25	4.9	-.135	38	8.53	-.005	292	1.67
Basic N_1	29	4.0	-.098	40	7.23	.003	304	1.78
	92	3.1	.019	171	1.08	.029	307	1.83
Basic—N	2	. .	. .	. .	. .	. .	. .	. .
	18	3.5	-.050	24	6.15	-.472*	271	1.22
Basic—N-P	94	3.1	-.329	149	8.97	-.117	220	1.32
	90	4.1	.079	172	13.20	.187	224	1.34
Basic—N-K	94	2.2	.069	129	5.07	-.004	249	1.30
	63	6.1	.206	76	9.59	.044	265	1.20
Basic—N-Ca	47	5.1	.098	66	8.99	.082	254	1.08
	39	5.3	-.029	49	7.58	-.034	257	1.08

TABLE VI—*(Continued)*

Dry Weights, Nitrogen Contents, Mycorrhizal Percentages, Mycorrhizal Points, and Correlation Coefficients Between Dry Weights and Mycorrhizal Developments of *P. Strobus* Seedlings Grown in Black Rock Forest Soils with Varied Nutrient Treatments

(Dry weights and N contents from Mitchell, 1934)

Treatment	*Per cent of short-roots mycorrhizal*		*Cor. coef. Dry Wts. & % mycor.*	*Mycorrhizal points*		*Cor. coef. Dry Wts. & myc. points*	*Ave. dry weights in mg.*	*Per cent nitrogen in seedlings*
		S.E.M.			*S.E.M.*			
			Mid-Slope Soil (poor)					
Dist. water	35	3.9	.256	58	. .	.307	235	1.45
	31	2.9	. .	. .	. .	. .	233	1.49
Basic nut.	19	3.8	–.325	32	7.81	.366	241	1.71
	97	3.1	. .	216	7.29	.543	302	1.72
Basic N_1	12	3.4	–.344	16	2.55	–.256	265	1.55
	11	2.8	–.033	11	. .	–.029	292	1.85
Basic—N	90	2.1	.411	137	8.24	.435	268	1.26
	15	. .	. .	. .	. .	. .	271	1.28
Basic—N-P	81	5.3	.586**	147	16.36	.534**	217	1.31
	74	5.4	–.398*	112	14.97	.196	230	1.32
Basic—N-K	56	6.3	.045	93	13.22	–.032	259	1.45
	87	. .	. .	. .	. .	. .	269	1.34
Basic—N-Ca	55	5.5	–.635**	64	8.64	–.489*	273	1.26
	28	3.9	.244	33	3.93	.446*	269	1.44

TABLE VI—*(Continued)*

Ridge Soil

Dist. water	95	..	..	210	..	..	228	1.20
	100	..	..	219	..	..	226	1.11
Basic nut.	90	..	..	190	..	..	231	1.41
	95	..	..	187	..	..	238	1.41
Basic N_1	95	..	..	205	..	..	263	1.55
	90	..	..	156	..	..	264	1.56
Basic—N	93	5.7	..	200	..	..	211	1.09
	99	..	..	..	..	..	227	1.08
Basic—N-P	95	..	..	237	..	..	189	1.20
	96	1.7	..	305	..	..	221	1.13
Basic—N-K	93	1.9	..	162	..	..	228	1.09
	85	1.2	..	112	..	..	207	1.07
Basic—N-Ca	90	..	..	218	..	..	246	1.21
	95	..	..	230	..	..	253	1.11

* Significant. ** Highly significant.

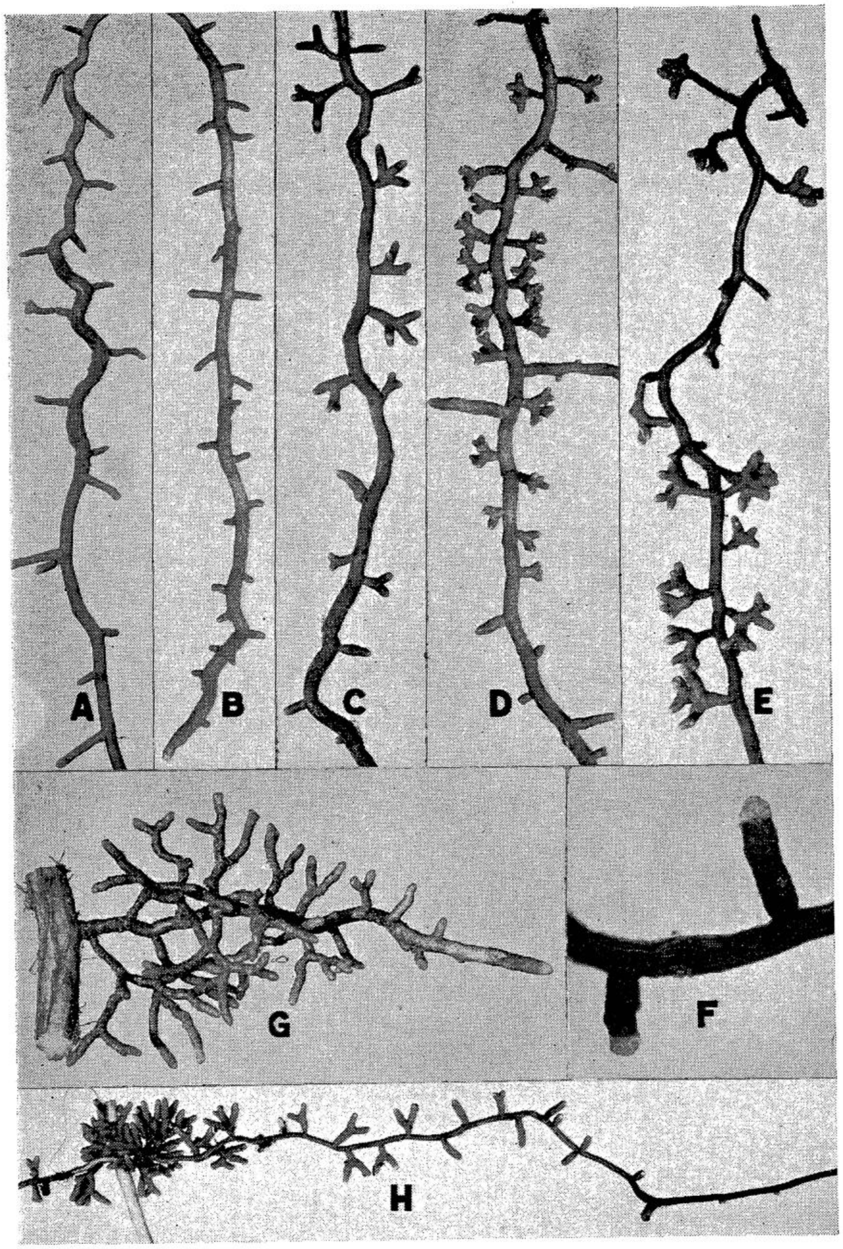


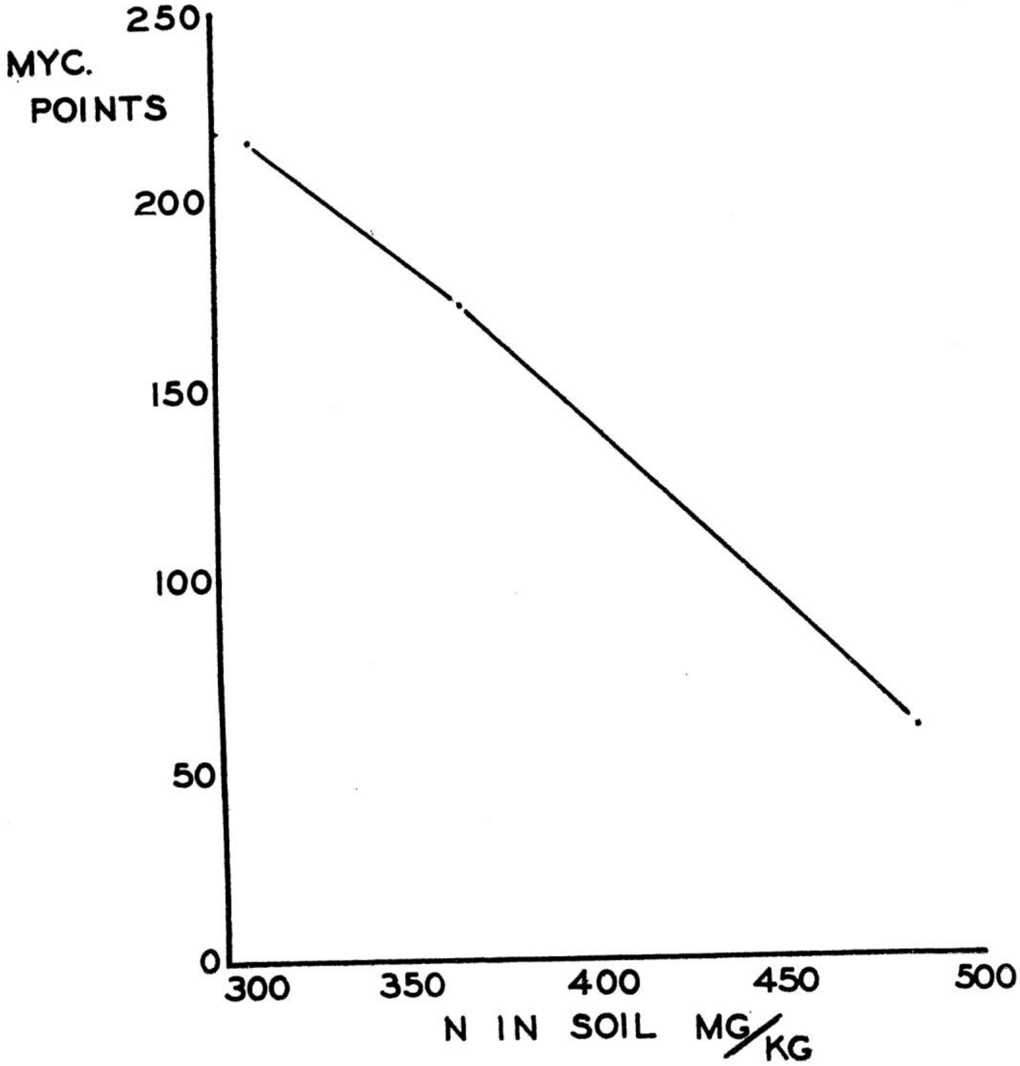
Plate III. AVERAGE SHORT-ROOT DEVELOPMENT IN VARIOUS ENVIRONMENTS.

A-E. Grown in fertile Cove Soil, Black Rock Forest with following treatments (x 2.5): A. No nutrients added; occasionally poorly developed mycorrhizae. B. Basic nutrient (Complete nutrient —N); no mycorrhizae. C. Basic nutrient minus calcium; mycorrhizae well developed. D. Basic nutrient minus potassium; mycorrhizae well developed. E. Basic nutrient minus phosphorous; mycorrhizae especially well developed. F. Non-mycorrhizal short-roots (that is, the pseudomycorrhizae in the sense of Melin, 1917, 1927, but not of Rayner, 1934) of *Pinus sylvestris* grown in sand showing the minute white nonsuberized absorbing tips (x 6). G. Dichotomous branching of uninfected short-roots of *Pinus taeda* when grown in pure culture with nucleic acid as source of nitrogen (descriptions on pp. 118, 137; x 2.5). H. Mycorrhizal short-roots of *Pinus sylvestris* showing increase in their lengths and dichotomous branching with age. The short-roots at the left end of the photograph are about 2 months old while those at the right have just emerged from the mother long-root.

Photo (H) by Professor E. Melin and the writer.

roots on seedlings in the Cove soil which illustrate their mycorrhizal equipment are shown in Plate III.

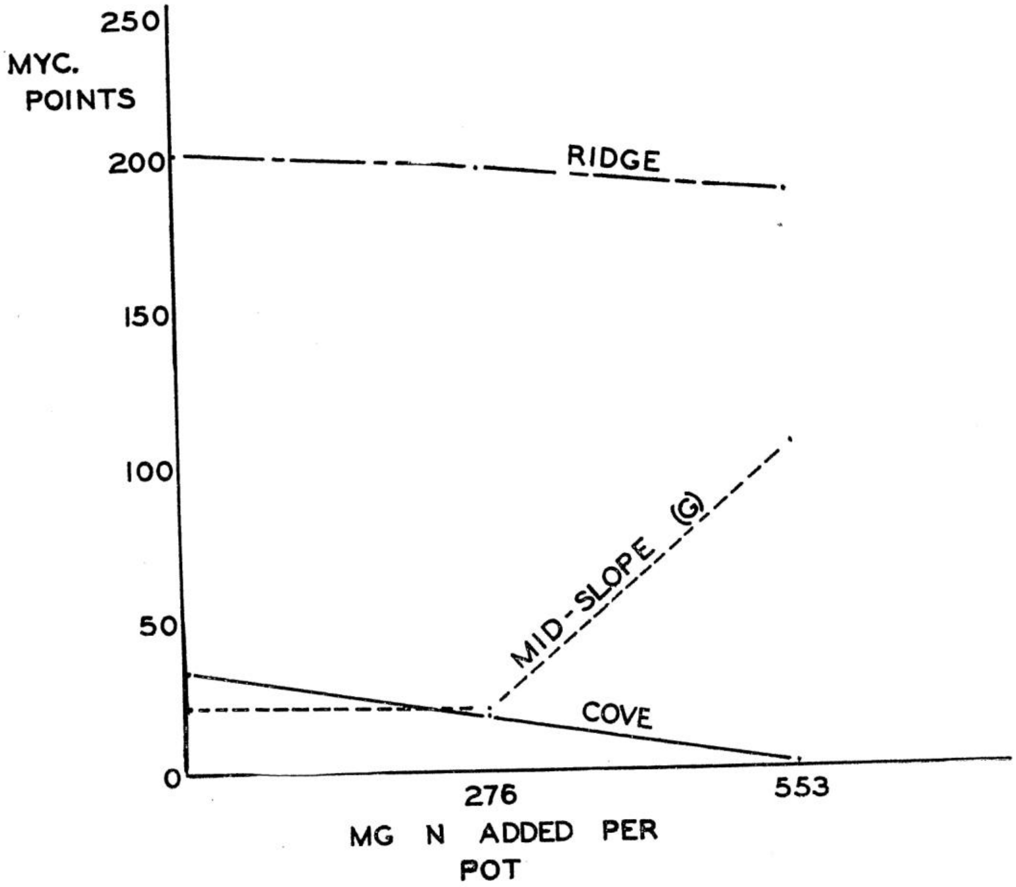
The relation of nitrogen availability to the development of mycorrhizae in the Ridge, Mid-Slope (good), and Cove soils are plotted in Text Figs. 14, a and b. With but one exception [Mid-Slope (good) soil, Text Fig. 14, b] these curves show the exact opposite relationship between nitrogen availability and mycorrhizal production from that reported by Möller (1902-1903), Müller and



Text Fig. 14, a. RELATION OF NITROGEN AVAILABILITY TO MYCORRHIZAL DEVELOPMENT IN THE BLACK ROCK FOREST SOILS.

(Nitrogen availabilities from Mitchell, 1934, Table 16; mycorrhizal points from cultures receiving distilled water only).

Weiss (1907), Melin (1927) and by the writer (for Swedish soils, Text Fig. 11). This reduction of mycorrhizae with increased availability of inorganic nitrogen was predicted by the nitrogen theory of Frank, Melin, and others. However, that nitrogen is not the sole or even the chief element influencing mycorrhizal formation in these experiments is demonstrated by the one exception to the rule, namely, the abundant development of mycorrhizae (Text Fig. 14, b) in the Mid-Slope (good) soil with very large supplies of inorganic nitrogen.



TEXT FIG. 14, b. RELATION OF NITROGEN ADDITIONS IN THREE BLACK ROCK FOREST SOILS TO THE DEVELOPMENT OF MYCORRHIZAE.

"Poverty" in mineral salts and in phosphorous, as anticipated by the Harvard Forest experiments, proved to be the chief factors responsible for variations in the production and development of mycorrhizae. The addition of a mineral salt solution, lacking nitrogen, to the three soils (Text Fig. 12) was followed by reduction in the development of mycorrhizae in every case. With omission of any one of the three elements, phosphorous, potassium, or calcium from this solution the root systems became profusely mycorrhizal. The magnitude of these changes in the three soils is represented by the portions of the columns to the right of the white lines in Fig. 12. On the basis of seedling growth in these experiments Mitchell (1934) determined that all Black Rock Forest soils are deficient in phosphorous. Mycorrhizae were especially numerous and well developed in all soils when this element was omitted from the basic nutrient. It is notable that in the relatively fertile Cove soil, which possessed few mycorrhizae in the untreated pots and even less with addition of a balanced salt solution (either with or without nitrogen), mycorrhizae were profusely developed when the nutrient environment was unbalanced by omission of one of the three elements, phosphorous, potassium, or calcium. This result rules out the possibility that the effect of the nutrient additions was to injure or to retard the growth of the mycorrhizal fungi. It also shows that even in fertile soils mycorrhizae appear, but in response to lack of balance rather than to actual poverty in mineral salts.

Thus individual deficiencies in phosphorous, potassium, calcium, and to some extent nitrogen, or lack of balance in the availabilities of these elements, gave rise in these experiments to the conversion of short-roots to mycorrhizae. The generality of these relationships is shown not only by the results obtained with artificial changes in fertility brought about by nutrient additions, but also by those obtained with natural differences in fertility as exemplified by (1) the profuse development of mycorrhizae in the infertile Ridge soil, (2) weak development in the fertile Cove soil, and (3) intermediate development in the Mid-Slope (good) soil of intermediate fertility.

The results obtained in the Mid-Slope (poor) soil constitute an exception to those just examined. However, the exclusion from the above tables and graphs of results obtained in this soil is supported by other considerations. By accident this soil was thoroughly air dried before it was mixed with sand for potting. It is well known that drying serves as an agent for the partial sterilization of soil (Waksman, 1932, p. 363). Partial sterilization increases the availability of all nutrient elements. Although the Mid-Slope (poor) soil, as its name implies, was collected from a slow-growing stand on the mid-slope, the pot culture tests place it well above the Mid-Slope (good) soil in fertility. Partial sterilization also influenced the development of mycorrhizae by killing most of the mycorrhizal fungi of the soil. It was found that nearly 95 per cent of all mycorrhizal short-roots in this soil (in the pot cultures) were produced by mycelium of the *M. r. nigrostrigosum* type (Plate IV). The presence of this fungus in abnormally large proportions is attributed to its ability to produce gemmae and bulbils and to its thickened cell walls (Hatch, 1934, C). These characteristics render *M. r. nigrostrigosum* unique among mycorrhizal fungi in its ability to resist desiccation. The mycorrhizal and growth data for seedlings in this soil series, together with those from all other soils, are included in Table VI. This soil was not used again in subsequent experiments.

BLACK ROCK FOREST EXPERIMENTS, 1933

For confirming and extending the 1932 experiments a second series of pot cultures with the Ridge, Mid-Slope (good), and Cove soils was set up in the summer of 1933 in collaboration with Mitchell. Glazed pots rather than porous pots were employed because it was noticed in

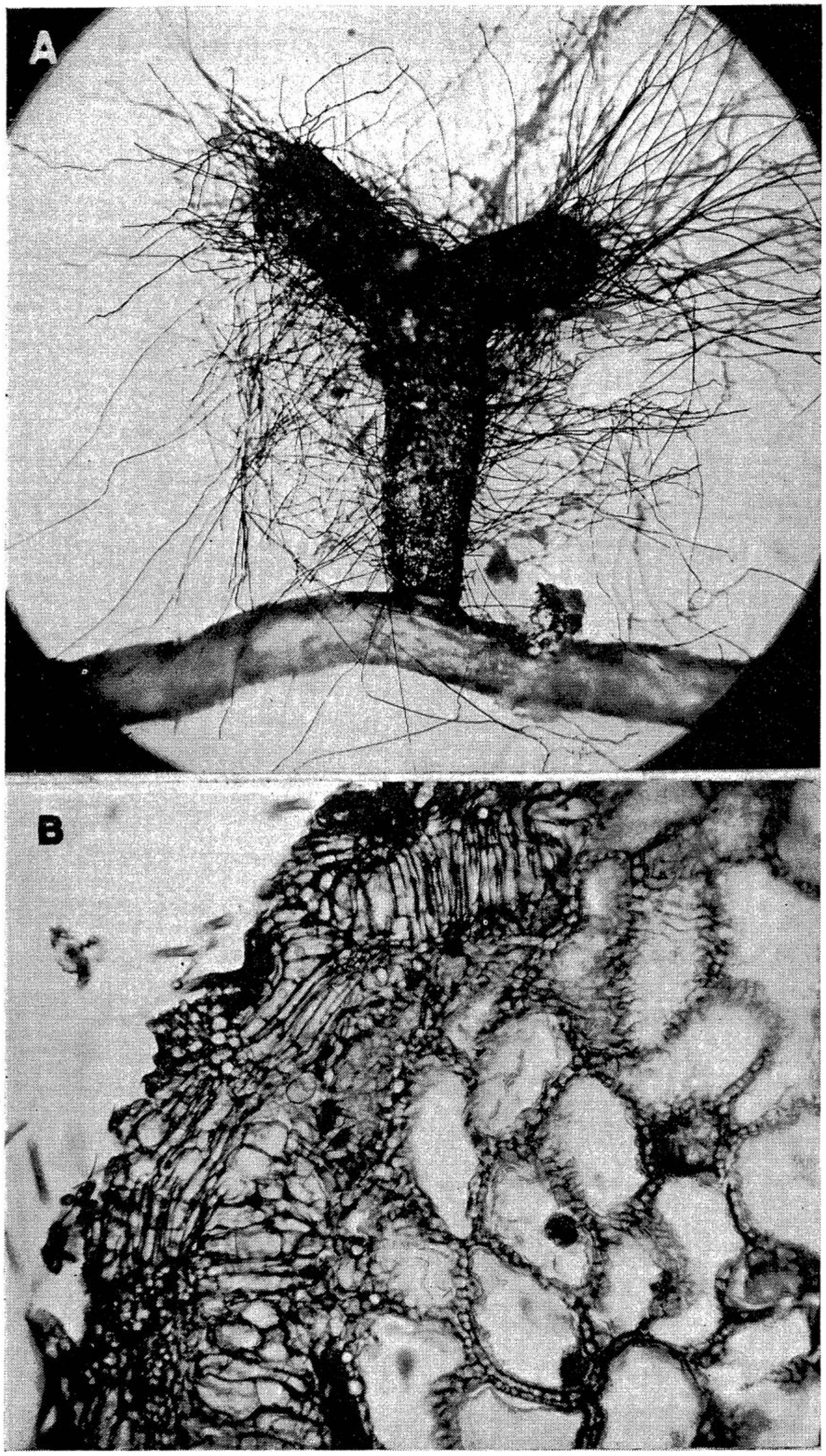


PLATE IV. APPEARANCE AND STRUCTURE OF MYCORRHIZAE PRODUCED BY MYCELIUM OF THE M. R. NIGROSTRIGOSUM TYPE CHARACTERISTIC OF 95 PER CENT OF ALL MYCORRHIZAE IN THE MID-SLOPE (POOR) SOIL AND OF ALL MYCORRHIZAE IN THE 1933 BLACK ROCK FOREST EXPERIMENTS.

A. External view showing individual hyphae radiating from the mantle (x 28). B. Tangential section showing character of the mantle and the intercellular net of mycelium (x 262).

1932 that evaporation from the porous surfaces tended to remove salts from the contained substrate. Since phosphorous was found to be deficient in the Black Rock Forest soils in 1932, the availability of this element was varied through wide limits. At the time of harvesting all plants were examined for mycorrhizae by the writer as in the preceding year.

The results of these experiments were quite erratic. In all treatments in the Mid-Slope soil, except those receiving no nutrient additions, mycorrhizae were almost wholly absent. The few infected roots which did appear were practically all of the *M. r. nigrostrigosum* type (Plate IV). In the Cove soil, only one pot yielded seedlings with mycorrhizae and this was one of three pots in which phosphorous was omittted. In the Ridge soil, mycorrhizae were fairly numerous in the — N and — K treatments but absent in the — P treatment. Therefore, while there was a tendency for mycorrhizae to become more abundant in the less fertile Ridge soil, the regularity with which they appeared and disappeared with deficiencies of individual elements in 1932 was not confirmed.

The glazing material of these pots contained copper and it was thought that leaching of this element, which is toxic to fungi, into the soil might in part explain the results. The glaze was also of a very poor quality and cracked profusely so that salts were leached from the substrates in almost the same quantities as in the earlier experiments with porous pots.

Two factors therefore required further investigation: (1) the influence of the type of container on mycorrhizal development, and (2) the relation of the availability of an individual mineral salt (phosphorous) to the development of mycorrhizae. The first of these was for checking the 1933 experiments, the second for checking the specificity of the mycorrhizal response which variation in an individual element (phosphorous) called forth in the 1932 experiments.

GREENHOUSE EXPERIMENTS, 1934

Soil from the Mid-Slope (good) plot of the Black Rock Forest was mixed with sand and potted in Cambridge two days after collection at Cornwall. Germinated *P. Strobus* seedlings (27 per pot) and one 3-months seedling of *P. insularis* were planted the following day (April 12). Four types of containers were employed: porous pots (as in the 1932 experiments), green glazed pots (as in the 1933 experiments), asphalt-painted pots (a coating used by Gast in 1931), and a special bottomless jug with chemical-proof glaze of high quality, designed for sand-nutrient culture studies by Ward (1935). Six containers of each type were used and these were divided into two groups, one receiving Mitchell's basic nutrient minus nitrogen, and the other receiving basic nutrient minus nitrogen and minus phosphorous.

Measurements of soil temperature in the four types of containers early in April (on other material) had revealed that during the warmest part of sunny days glazed containers ran consistently higher temperatures (5-10° C.) than the porous pots. Since the growth curve of mycorrhizal fungi reaches a maximum at 25° (Melin, 1925) and decreases rapidly at temperatures above 30°, the importance of maintaining moderate temperatures is evident. It seemed probable that the higher temperatures in the glazed pots may have contributed to the erratic results of the 1933 experiments. This factor was eliminated in the greenhouse experiments by installation of an outside sprinkling system, by shading during the middle of the day, and by packing sphagnum moss around the containers and moistening frequently.

By August 11, seedlings grown in the bottomless jugs were uniformly larger and greener than in all other containers. Those receiving phosphorous in the asphalt-painted pots came second with respect to vigor. The new needles on all plants except those in the bottomless jugs were yellowish green. Nutrients were added to all containers as follows:

Minus P Series

K_2CO_3	0.11 grams
$MgSO_4 \cdot 7H_2O$	0.65 ''
$CaCl_2 \cdot 2H_2O$	0.18 ''
NH_4NO_3	0.20 ''

Complete Nut. Series

KH_2PO_4	0.20 grams
$MgSO_4 \cdot 7H_2O$	0.65 ''
$CaCl_2 \cdot 2H_2O$	0.18 ''
P_2O_5	0.30 ''
NH_4NO_3	0.20 ''

The seedlings were harvested in part between September 13 and 26, and the remainder on November 26 and 27. Mycorrhizal points were estimated for each seedling individually and the total wet weights of white pine and benguet pine *(P. insularis)* per pot were determined. The results are assembled in Table VII.

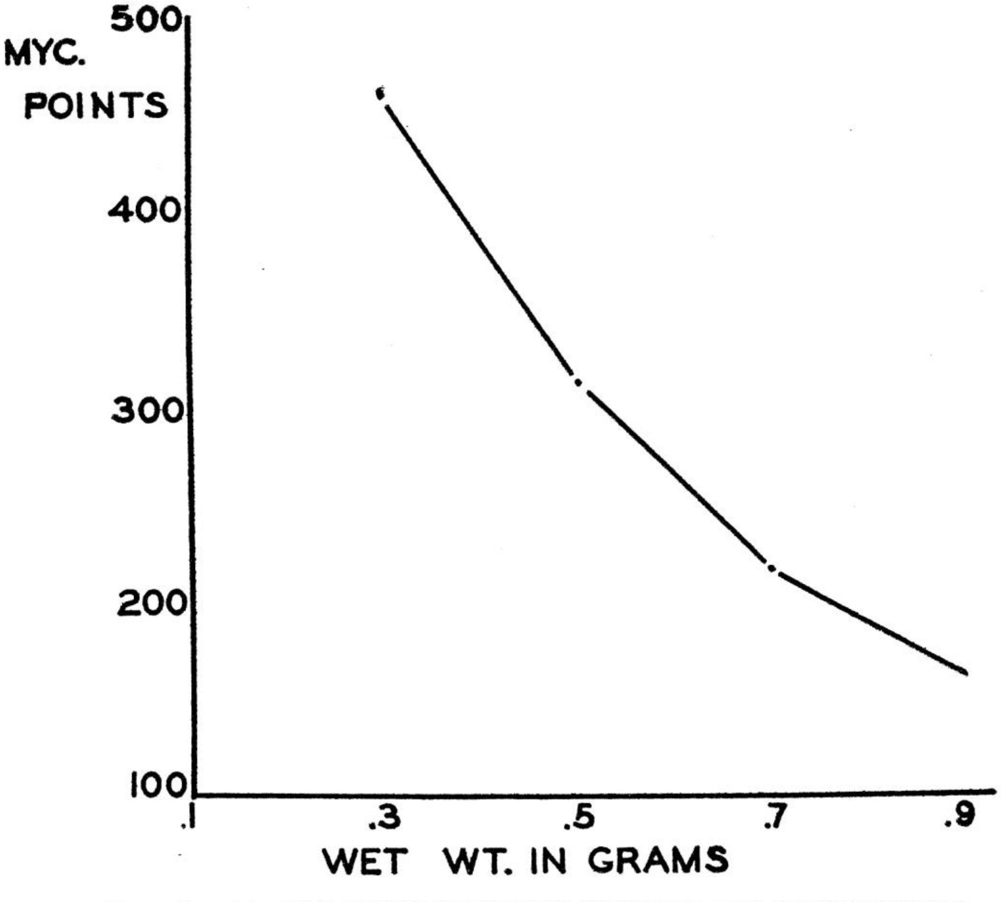
TABLE VII

RELATION OF TYPE OF CONTAINER AND NUTRIENT TREATMENT TO MYCORRHIZAL DEVELOPMENT AND WET WEIGHTS OF *P. Strobus* SEEDLINGS

(Greenhouse experiments, 1934)

Type of container	*Basic nut.*		*Basic nut. -P*	
	Wet Wt. per seedling	*Myc. points*	*Wet Wt. per seedling*	*Myc. points*
Porous pots	1.7	143	1.5	311
Green glazed	1.7	171	1.7	330
Asphalt paint	1.8	138	1.3	459
Bottomless Jugs	1.9	127	1.9	198

These results confirm the specificity of the relationship found in the 1932 experiments between the availability of an individual element (phosphorous) and the development of mycorrhizae. They show that the character of the green-glaze in the 1933 experiments was not the cause of the erratic results of those experiments. Rather it appears that high temperatures and some other unknown factor inhibited the production of mycorrhizae in 1933. The experiments do show that the type of container has a profound influence on the availability of mineral salts, as evidenced by lower seedling weights and by greater numbers of mycorrhizae in porous containers. Growth was greater in the chemical-proof glazed jugs than in any other type of container and mycorrhizal development was less profuse (Fig. 15).



TEXT FIG. 15. RELATION BETWEEN GROWTH AND MYCORRHIZAL DEVELOPMENT IN SEEDLINGS RAISED IN MID-SLOPE (GOOD) SOIL IN FOUR TYPES OF CONTAINERS.

Fiber Pot Experiments, 1934

In connection with a projected study of the influence of different species of mycorrhizal fungi on seedling growth following transplanting to different habitats in the field, a suitable method for introducing the appropriate fungi along with transplant seedlings is needed. For this purpose the planting of seedlings contained in a small quantity of soil, which in turn harbors the desired species of mycorrhizal fungi, seems most promising. The remarkable success obtained by Youden (unpublished until 1937) at the Boyce Thompson Institute in transplanting tomato plants contained in a special fiber pot, suggested the testing of such containers for this reforestation study. While the experiments with this pot, or cartridge (Plate V), were not pursued with the object of throwing light on the relationship between soil fertility and mycorrhizal abundance, they serve this purpose admirably and for that reason are included here.

The nutrient content of the fiber material (made by the Burt Co. Lt. who financed these experiments) was adjusted by Mr. Darrin (of the Mellen Institute for Industrial Research) to approximately that found by Mitchell (1934) to be optimum for growth of *P. Strobus*. The pH of the cartridges was likewise adjusted to approximately 5.2, a favorable hydrogen ion concentration for this pine. The fiber itself is a non-purified plant waste and the nutrient adjustments were made by additions of other natural plant wastes rich in organic nitrogen and by additions of relatively insoluble (rock) sources of phosphorous and potassium. A large share of the nutrient materials of the pot thus become available as the result of the activities of microörganisms only, and the cartridges serve as a fertilizer in addition to their function as containers.

Comparative growth studies of transplant-seedlings, and of seedlings of similar age raised in these cartridges and transplanted intact to large boxes of soil, were pursued in the Harvard University greenhouses in the spring and early summer of 1934. The soil used was a very infertile one from a stand of *Pinus rigida.* The seedlings *(P. Strobus)* were transplanted to this soil when one month old. After three months in the boxes they were removed (Plate VI) and the wet weights of the

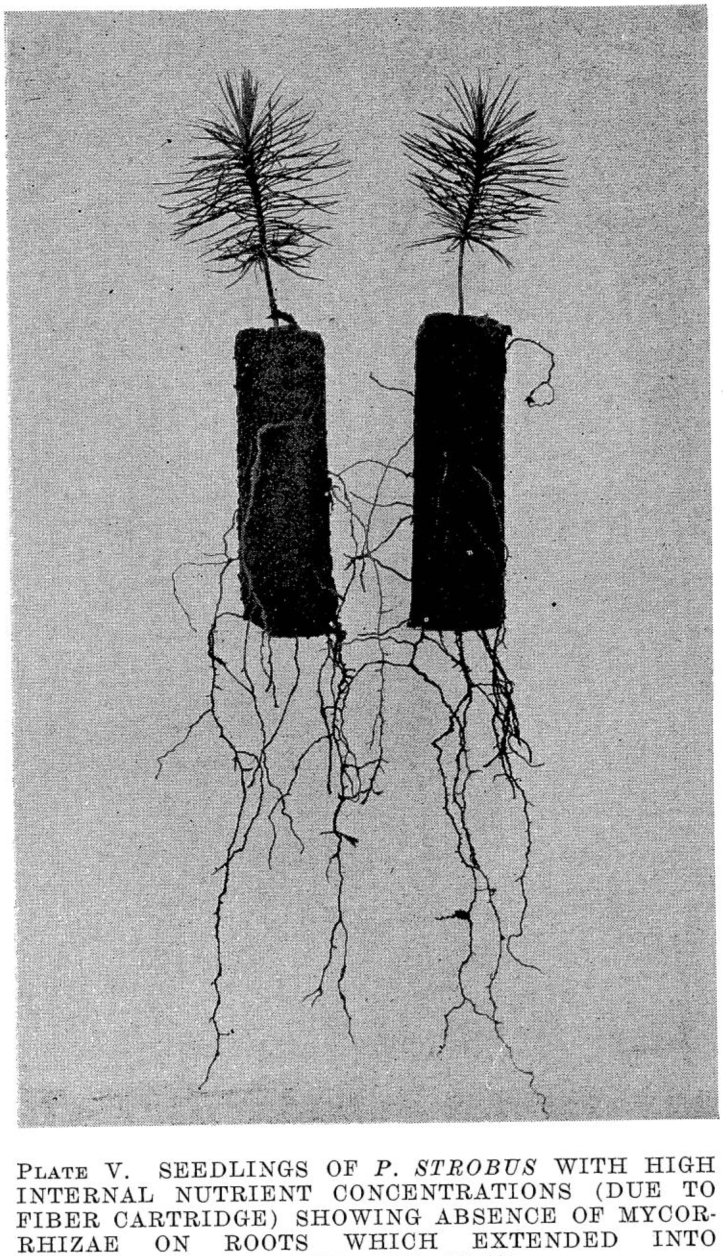


PLATE V. SEEDLINGS OF *P. STROBUS* WITH HIGH INTERNAL NUTRIENT CONCENTRATIONS (DUE TO FIBER CARTRIDGE) SHOWING ABSENCE OF MYCORRHIZAE ON ROOTS WHICH EXTENDED INTO INFERTILE SOIL (X ⅓).

individual seedlings determined, the tops and roots being weighed separately. These data and approximate figures on short-root numbers and mycorrhizal development are recorded in Table VIII.

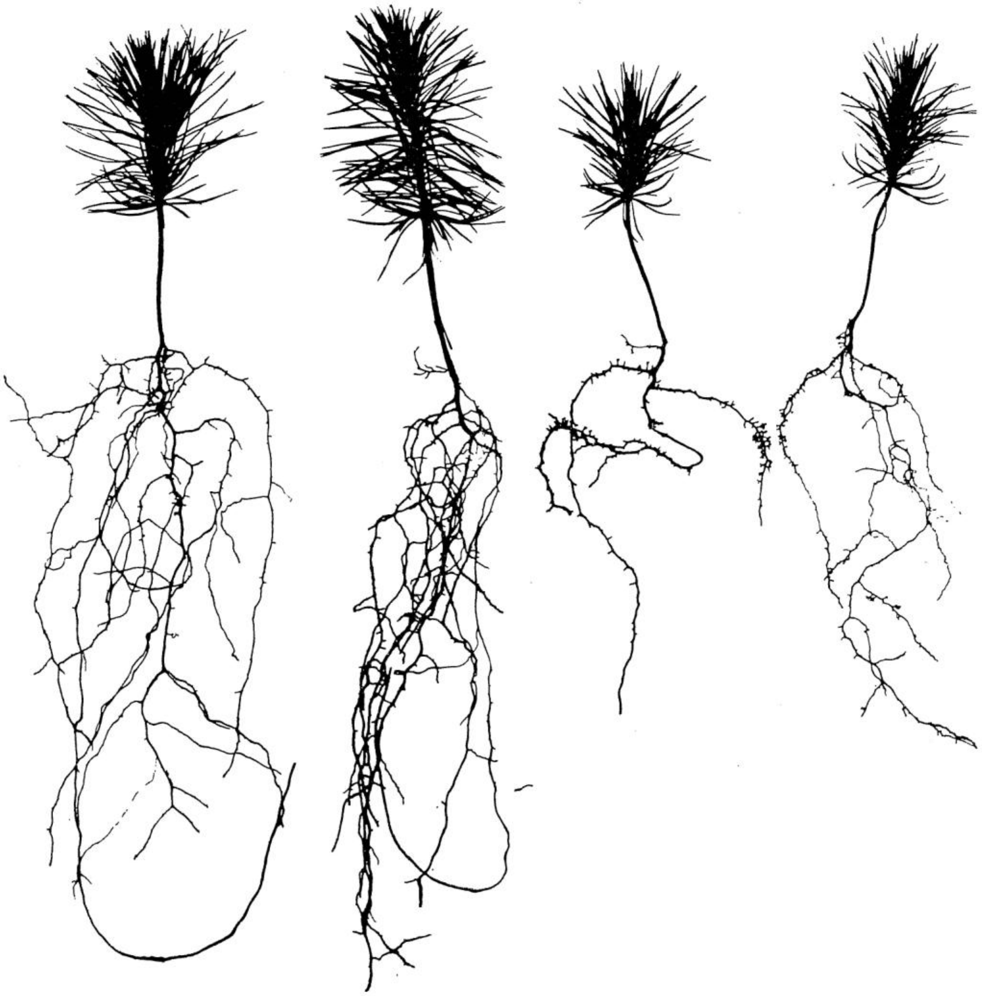


PLATE VI. GROWTH AND MYCORRHIZAL DEVELOPMENT OF 4-MONTHS *P. STROBUS* SEEDLINGS RAISED IN AN INFERTILE FOREST SOIL (A) WITH AND (B) WITHOUT FERTILIZER CARTILAGE.

The generality of the rule that mycorrhizae are produced under conditions of nutrient deficiency only, is again confirmed by these results. As shown in Plate V, even those roots which were far removed from the fiber containers were quite free of mycorrhizae, a fact which supports the belief that internal concentrations of nutrient elements control susceptibility to infection.

TABLE VIII

THE INFLUENCE OF FERTILIZER FIBER CARTRIDGES ON WET WEIGHTS, SHORT/ROOT RATIO, AND SHORT-ROOT DEVELOPMENT ON 4-MONTH TRANSPLANTS OF *P. Strobus* GROWN IN AN INFERTILE FOREST SOIL IN A GREENHOUSE

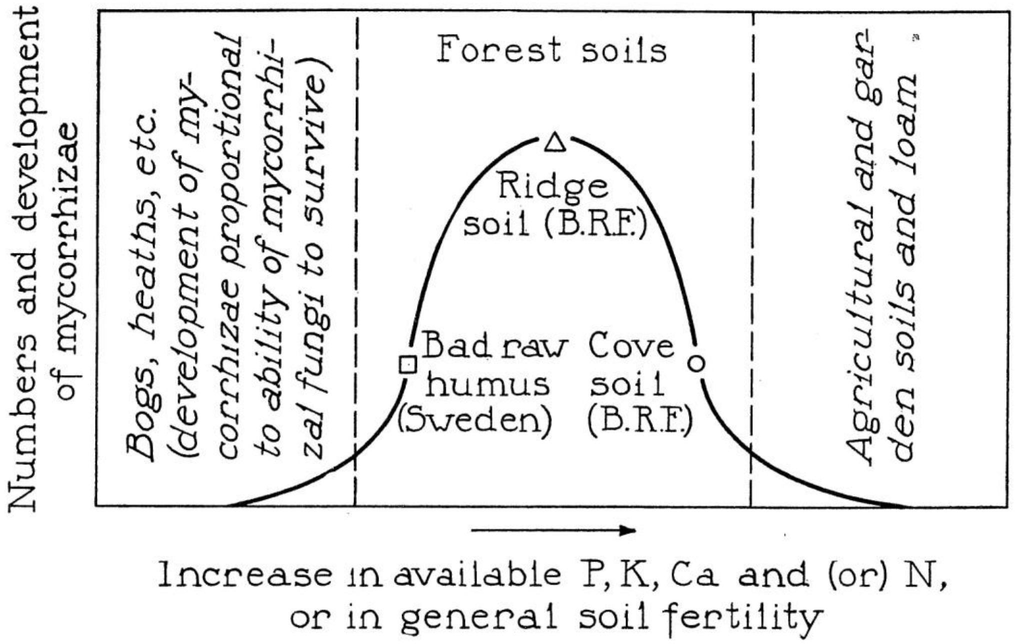
	No. seed-lings	*Av. wet weight*	*Shoot/ root ratio*	*No. short-roots per 10 cm. long-root*	*Myc. Points*
Transplant seedl.	126	0.773	2.37	50	150
Cartridge seedl.	104	1.347	1.83	18	1

DISCUSSION OF SOIL CULTURE EXPERIMENTS

RELATION BETWEEN MYCORRHIZAL DEVELOPMENT AND THE NUTRIENT ENVIRONMENT OF SOILS

That the abundance and distribution of ectotrophic mycorrhizae in trees are controlled by more factors than the availability of nitrogen is apparently established by these experiments. No evidence at all was obtained which supports the current belief that ectotrophic mycorrhizae are of significance chiefly in soils where nitrogen must be utilized in organic form. Instead, these studies confirm for *Pinus* that mycorrhizae appear under conditions of poverty in mineral salts, a relationship which Stahl in 1900 regarded as the cause of the mycotrophic habit throughout the entire plant kingdom.

In schematic and summarized form the relation of soil fertility to the development of mycorrhizae as revealed by these and the other experiments reviewed is plotted in Fig. 16. Progress to the right along the abscissa represents not only general increase in soil fertility but also increase in the four elements, nitrogen, phosphorous, potassium, and (or) calcium. The mineral soils studied by Möller (1903) and the humus soils studied by Müller and Weiss (1907, excepting their pot cultures), by Melin (1927), and by the writer in Sweden (p. 66), were all relatively infertile. On this graph they fall in the region



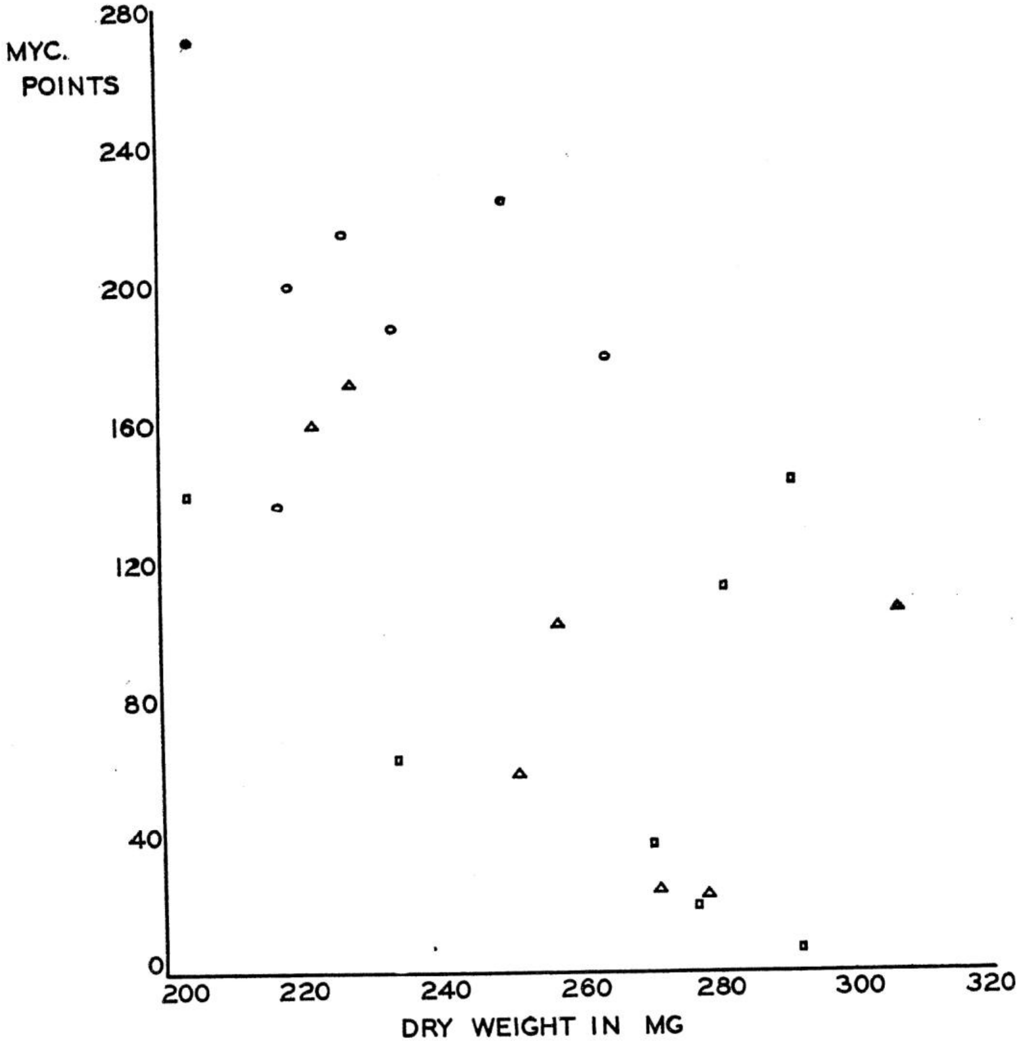
TEXT FIG. 16. SCHEMATIC DIAGRAM TO ILLUSTRATE THE RELATION BETWEEN THE DEVELOPMENT OF ECTOTROPHIC MYCORRHIZAE IN NATURE AND (1) HABITAT, (2) NUTRIENT ENVIRONMENT.

where the curve of mycorrhizal development is ascending. In this region increase in nitrogen, by stimulating the growth of mycorrhizal fungi, increases the number of short-roots which become infected on one-year seedlings. Conversely, the humus used in Möller's experiments, and in Müller and Weiss's pot cultures [since the potting of soils increases their fertility temporarily (Romell, 1934)], were rich in nutrients, as evidenced by excellent growth of seedlings; and the soils along the banks of drainage ditches where mineral-rich subsoil had been deposited, as reported by Melin (1917), possessed an abundance of all elements rather than of nitrogen alone. Garden soils, loamy soils, and agricultural soils, concerning which the literature of mycorrhizae is replete with examples of vigorously growing plants which lack mycorrhizae (R. Hartig, 1886; Frank, 1888; Sarauw, 1893a; Peklo, 1908; Nolle, 1910; Christolph, 1921; Francke, 1934; and many others), are similarly rich in mineral salts. All of these soils as well as those which the writer has investigated in America fall within the region of this graph where the curve of mycorrhizal abundance is descending. In this region decrease in the development of mycorrhizae is brought about in some unknown way by high internal (seedling) concentrations of nutrient elements, rather than by changes in the growth rates of mycorrhizal fungi.

The diagram (Text Fig. 16) also illustrates the relationships between the availabilities of individual elements and the development of mycorrhizae. The ascending region of the curve was explored only for differences in nitrogen availability. In this region an increase in nitrogen produced increases in mycorrhiza-development, presumably because extremely low nitrogen availabilities in the soils limited fungal growth. The opposite effect, namely, a decrease in the development of mycorrhizae with increase in the individual availabilities of four elements (phosphorous, potassium, calcium, and nitrogen), characterizes the descending region of the curve.

RELATION BETWEEN MYCORRHIZAL DEVELOPMENT AND DRY WEIGHTS AND NITROGEN CONTENTS OF SEEDLINGS

Despite the experimental demonstration of the existence of this clear-cut relationship between the nutrient environment of soils and the development of mycorrhizae, the question of the rôle of mycorrhizae in tree nutrition is by no means settled. One may, for example, regard the profuse production of mycorrhizae on seedlings growing in infertile soils as successful parasitism of these fungi on seedlings weakened by malnutrition. Indeed, the entire experimental work of the writer, if analysed by the usual method of comparing mycorrhizal developments in different soils with dry weights of seedlings indicates that a decrease in seedling weights is usually accompanied by an increase in mycorrhizal developments (Text Figs. 15, 17, Tables III-VI). In other words, it can be accurately stated that the most vigorous and rapidly growing seedlings lacked mycorrhizae in



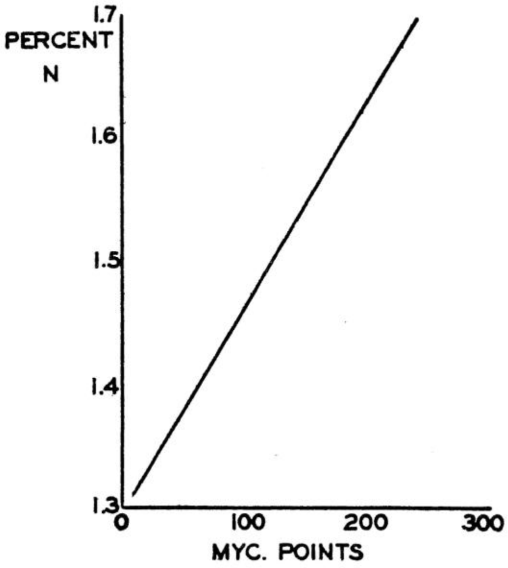
TEXT FIG. 17. RELATION BETWEEN DRY WEIGHTS OF SEEDLINGS AND MYCORRHIZAL DEVELOPMENT IN BLACK ROCK FOREST EXPERIMENTS, 1932. RIDGE ○, MID-SLOPE (GOOD) △, COVE □.

Values from Table VI, and Mitchell (1934) Tables 7, 9 and 10.

these experiments. A similar relationship was long ago recorded by Möller (1902-1903) for plants grown in the several horizons of one raw humus soil. For this reason Möller discarded the "beneficial" view of mycorrhizae. Still earlier, Henschel (1887) reported a similar relationship to be true of spruce seedlings.

But in the writer's experience it has been repeatedly demonstrated that such differences in the abundance of mycorrhizae are causally related to the nutrient contents of the soils. The growth studies on the seedlings of these experiments by Gast (1937) and Mitchell (1934) have likewise proved that the differences in seedling growth are the result, perhaps in all cases, of inadequate supplies of one or more of the elements essential to growth. It follows that *comparisons of the weights and mycorrhizal developments of seedlings raised in different nutrient environments are incapable of yielding direct information on whether mycorrhizae increase or decrease seedling growth. The problem can be approached only by comparing the growth and mycorrhizal developments of seedlings raised in one nutrient environment.*

In the writer's experiments the collection of data on the development of mycorrhizae on individual seedlings was for the purpose of enabling the correlation of mycorrhizal abundance with the individual weights and nutrient contents of seedlings. By this means it was possible to determine whether those seedlings in each pot which were above the average in weight or in nutrient content had greater or fewer numbers of mycorrhizae and *vice versa*. The ordinary correlation procedures outlined by Wallace and Snedecor (1931) were employed. A restricted correlation study was carried out between the nitrogen contents of individual seedlings and their mycorrhizal developments. A pot was chosen which had yielded an r value of approximately .0 in the correlations (see below) between dry weights and mycorrhizal developments [— N — K treatment, Mid-Slope (poor) soil]. Fifteen seedlings were selected which differed widely in their mycorrhizal developments. Determinations of the nitrogen contents and percentages (on basis of dry weights) were made by the micro-Kjedahl method of Pregl (1930). A direct relationship (Fig. 18) was found between the nitrogen percentages and mycorrhizal development, the value for *r* being .564 (significant). While this result bears out the belief that mycorrhizae facilitate nutrient absorption, in the writer's opinion the data are too few to be conclusive.



TEXT FIG. 18. RELATION OF THE PERCENTAGE OF NITROGEN IN SEEDLINGS TO THE DEVELOPMENT OF MYCORRHIZAE.

(nitrogen % = .00164 A × 1.31, in which A = myc. points).

Dry weights of seedlings (expressed as a percentage of the mean seedling of each pot) were also correlated both with the percentage of short-roots converted to mycorrhizae and with mycorrhizal points. The results (values of *r*) are included in Table VI. Of 36 pots of seedlings in the 1932 experiments which were analyzed for correlation (Table VI) 5 yielded significant or highly significant values of *r* when correlation of dry weights was with mycorrhizal points, and 6 when correlation was with mycorrhizal percentages. These were approximately evenly divided between positive and negative values of *r*. In one case, the — N — P treatment in the Mid-Slope (poor) soil, significant negative and positive values were obtained in check pots. The fact that "significant" values of *r* in these experiments were fortuitous is obvious. The data appear to signify that increase in mycorrhizal numbers has no influence on seedling dry weights.

While this result excludes the possibility that mycorrhizae were injurious, a prediction as to their possible beneficial rôle is not justified. The possibility that growth was increased by mycorrhizae in these experiments, but that this fact was not revealed by correlation analyses, is great. Internal concentrations of nutrient elements apparently determine whether mycorrhizae will or will not be formed. It follows that immediately after germination seedlings which for one reason or another contain low internal concentrations of elements, and which are therefore small, may tend to produce mycorrhizae more abundantly than larger seedlings with higher nutrient contents. But if mycorrhizae increase nutrient absorption (that is, are more efficient than unaided roots) the mycorrhizal seedlings, although at first small, may soon overtake the seedlings which initially were larger, but which produced few mycorrhizae. At the end of one season relatively uniform seedlings would be obtained and correlation studies would yield values approaching zero (the result obtained).

From a purely theoretical viewpoint, therefore, it may be predicted *that experiments carried out in soils where mycorrhizal fungi are uniformly present can never yield a conclusive answer to the mycorrhizal question.* Soils normally lacking mycorrhizal fungi, such as those in Australia, Rhodesia, or the Philippine Islands, where it is possible to vary the mycorrhizal development of seedlings by inoculating with pure cultures of mycorrhizal fungi, should alone be fruitful for this purpose.

Inoculation of Seedlings Raised in Prairie Soil with Pure Cultures of Mycorrhizal Fungi

In the experiments recorded so far every effort to determine whether or not mycorrhizae increase absorption, and therefore the growth of seedlings, has failed. It was just pointed out that any other outcome of experiments pursued with soil possessing a uniform mycorrhizal inoculum could not be expected. The need is for a natural or artificial substrate which normally lacks mycorrhizal fungi and into which these fungi may be introduced as pure cultures. Prairie soils, because of the normal absence of trees, seemed most likely to be free of the mycorrhizal fungi of pine. These fungi have been shown to be absent in several such soils by Kessell (1926), Anon. (1931), Oliveros (1932), and Roeloffs (1930) whose studies were reviewed in the Introduction. Soil was therefore imported from near Cheyenne, Wyoming for growth studies on pine with and without inoculation with pure cultures of mycorrhizal fungi. The details of the experiment are published elsewhere (Hatch, 1936) and only the results will be introduced here. They are assembled in Table IX, Plate VII, and Text Fig. 19.

Although these data are not as numerous as could be desired they provide (especially when considered in conjunction with the other information assembled in this paper) a convincing picture of the utility of mycorrhizae to forest trees. They demonstrate that the nutrient-acquiring activities of both long-roots and non-mycorrhizal short-roots of pine seedlings, in this soil, were *incapable of bringing any significant quantities of nitrogen, phosphorous, or potassium into the seedlings.* The content of nitrogen and phosphorous in the non-mycorrhizal seedlings is less than has ever been recorded for seedlings of

TABLE IX

Dry Weights, Root-shoot Ratios, and Nitrogen, Phosphorus, and Potassium Contents of Mycorrhizal and Non-Mycorrhizal White Pine Seedlings Raised in Prairie Soil, Including Comparisons With Lowest Percentages of N, P, and K Hitherto Recorded for This Pine in Soils and in Nutrient Sand-Cultures

Pot No.	Av. dry weights of seedl. in mg.	Root-shoot ratio	Nitrogen		Phosphorus		Potassium	
			mg. per seedl.	% of dry wt.	mg. per seedl.	% of dry wt.	mg. per seedl.	% of dry wt.
1	360.7 ± 8.7[1]	1.024	2.51	.695	.268	.0742	1.94	.539
2[2] (inoc.)	428.5 ± 19.2	.9176	3.01	.703	.566	.1320	1.93	.450
3 (inoc.)	448.4 ± 9.9	.672	5.39	1.202	.849	.1893	3.47	.775
4 (inoc.)	360.9 ± 4.8	.892	4.62	1.280	.729	.2021	2.57	.713
5	300.0 ± 7.0	1.365	3.16	1.056	.229	.0762	1.04	.347
6	301.4 ± 4.8	1.024	2.40	.795	.211	.0700	1.17	.390
Averages mycorrhizal seedl., pots 3, 4	**404.6**	**.782**	**5.00**	**1.241**	**.789**	**.1957**	**3.02**	**.744**
Averages non-mycorrhizal seedl., pots 1, 5, 6	**320.7**	**1.138**	**2.69**	**.849**	**.236**	**.0735**	**1.38**	**.425**
Lowest values hitherto recorded for 3-months white pine seedlings grown in any soil[3]				1.081		.0825		.335
Per cent of N and P in 3-months white pine seedlings grown in nutrient sand-cultures in which these elements were individually omitted; all other elements being optimal[4]				.720		.1040		None available

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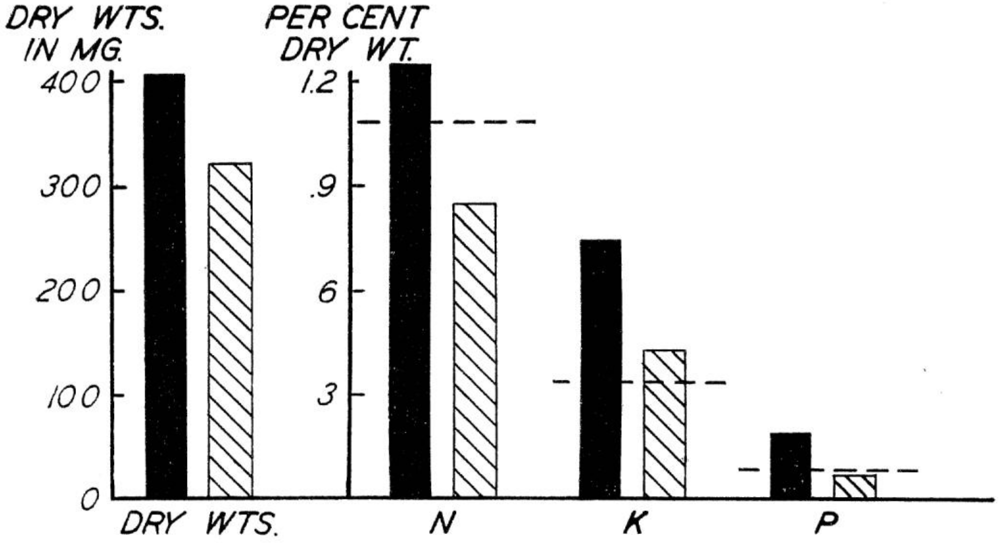
[1] Standard error of mean.

[2] In pot 2 mycorrhiazae were produced early in the season but at the time of pulling all were dead as evidenced by complete suberization of cortex. The values for seedlings in this pot are intermediate and they are excluded from the averages.

[3] N and P values from seedlings grown in Ridge soil, Black Rock Forest, Mitchell (13, Table 7; P values not reported); K values from seedlings grown in very infertile soil (Mitchell, unpublished).

[4] Described (Mitchell, 13, Tables 3, 4 and 14) but, except for N values, unpublished results of Mitchell.

pine grown under any soil conditions! The phosphorous content was even less than that of seedlings grown in the complete absence of phosphorous in nutrient sand culture! (A more convincing picture of seedling starvation would be difficult to find.) The less spectacular situation with regard to potassium is probably because this element is available in the soil solution in considerable quantities in prairie soils. Mycorrhizal seedlings, in contrast to those which were uninoculated, possessed normal quantities of these three elements. The seedlings in pot 2 (excluded from averages) on which the mycorrhizae died before the end of the experiment, possessed intermediate quantities of absorbed nutrients.



TEXT FIG. 19. COMPARISONS BETWEEN MYCHORRIZAL (BLACK COLUMNS) AND NON-MYCORRHIZAL SEEDLINGS RAISED IN PRAIRIE SOIL IN RESPECT TO DRY WEIGHTS, AND PERCENTAGE CONTENTS OF NITROGEN (N), POTASSIUM (K), AND PHOSPHOROUS (P).

Two months after these data were first published the fact that trees are incapable of growth in soils which lack mycorrhizal fungi was confirmed by Young (1936) in Australia. Pines inoculated with pure cultures of mycorrhizal fungi grew normally. All uninoculated seedlings died!

In press simultaneously with this Bulletin is a paper by Mitchell, Finn and Rosendahl (1937) which provides by far the most extensive and conclusive data yet obtained on nutrient absorption by mycorrhizal and non-mycorrhizal seedlings. Seeds of pine and spruce were planted in nursery beds in the spring of 1935 in a substrate consisting of infertile clay and well decomposed sawdust. Designed for checking under nursery conditions the results obtained in his pot-culture studies (Mitchell, 1934), the experiment included a fertilization

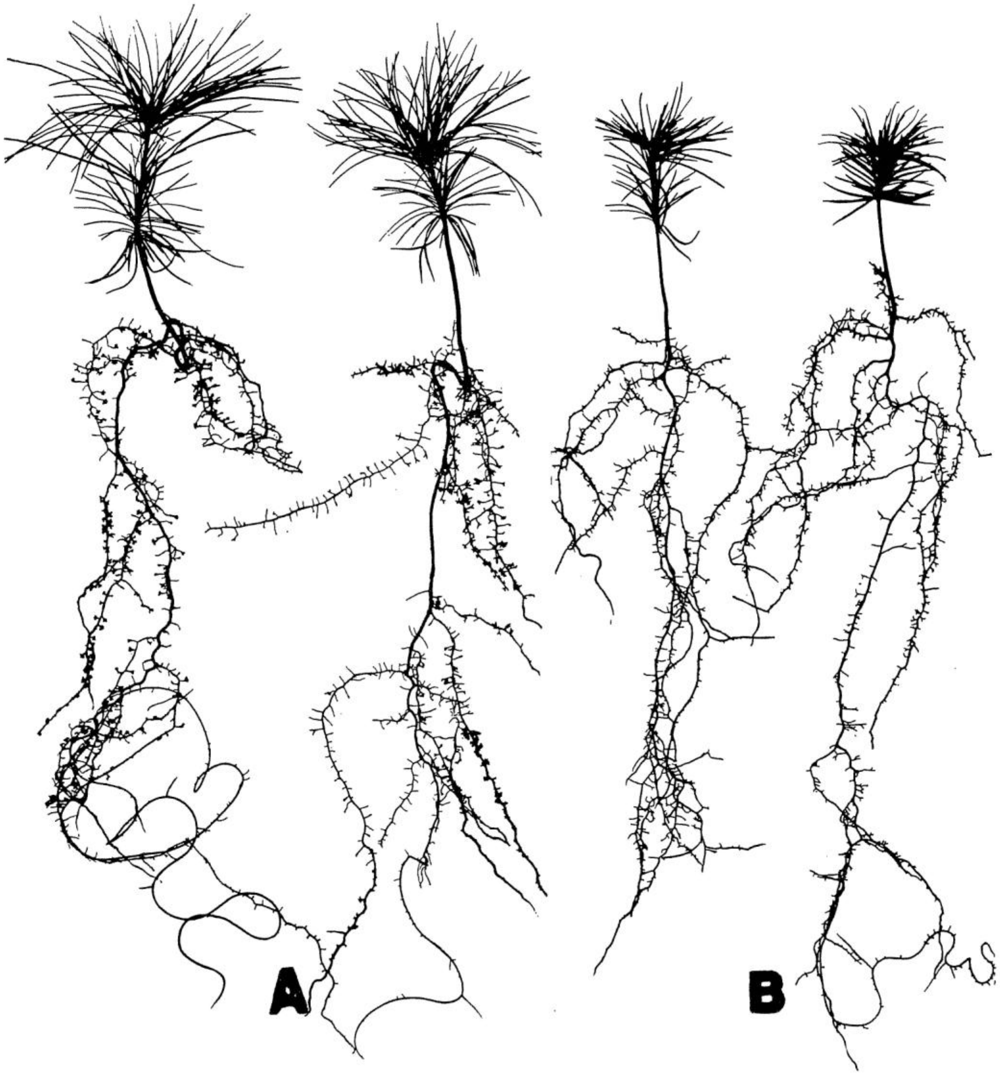


PLATE VII. AVERAGE SEEDLINGS OF *PINUS STROBUS* RAISED IN PRAIRIE SOIL.

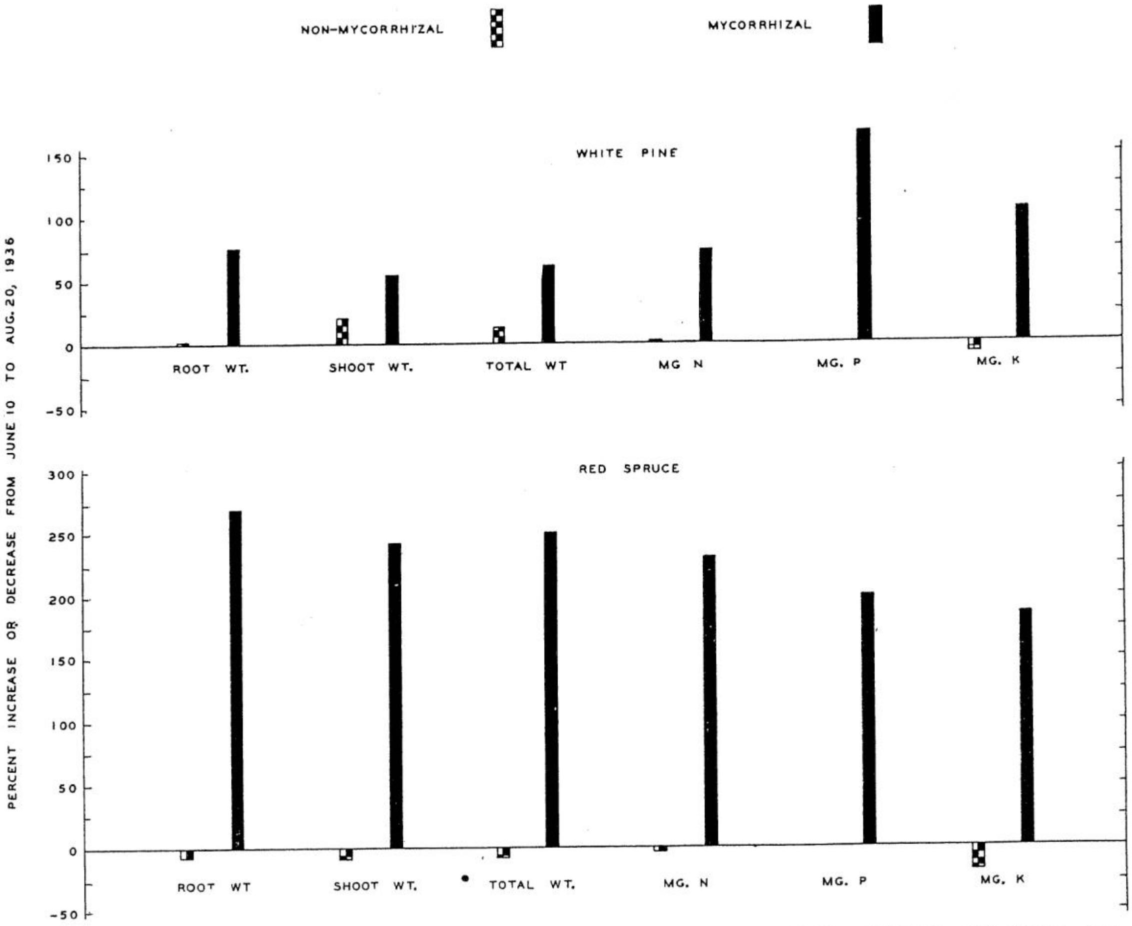
A, inoculated with mycorrhizal fungi; B, uninoculated.

series in which nitrogen, potassium, and phosphorous were independently varied. All beds except the checks, with no nutrient additions, were inoculated with soil containing mycorrhizal fungi.

The results confirm those reported in this paper. Mycorrhizae were well developed in the inoculated beds only when one of the three elements phosphorus, potassium, and nitrogen was deficient and they were lacking when all nutrients were readily available. The mycorrhizal seedlings in the control beds (those receiving no fertilizer and no artificial inoculation) grew poorly the first year. Early in their second season, however, certain groups of seedlings became markedly more vigorous than those throughout the remainder of the control beds. They were found to have acquired mycorrhizae (through natural agencies). It was noticed that the seedlings toward the center of these areas of more vigorous growth were progressively larger than those near the margins and the margins were extending.

Seedlings from the areas of vigorous growth (always mycorrhizal) and from the remainder of the beds (non-mycorrhizal) were sampled throughout the summer and analysed for phosphorus, potassium and nitrogen. The results are presented in Text Fig. 20. They conform to those presented in this paper and in addition provide clear-cut evidence that non-mycorrhizal seedlings were not only incapable of absorbing any mineral salts but actually lost salts to the soil. The data are quantitative and statistical examinations revealed that in all cases the probability was greater than 1000 to 1 that the differences found were significant.

It occurred to the present writer that Mitchell's experiment should provide an excellent opportunity for testing the correctness of the assumptions of Burges (1936) and Rayner (1934) that mycorrhizae benefit trees merely by converting non-available soil materials into readily available nutrients. It was suggested, accordingly, that chemical analyses be made of the soil from around the roots of the vigorous mycorrhizal seedlings and from around the non-vigorous, non-mycorrhizal plants. It has been shown elsewhere (Mitchell, 1934) that an increase in nutrient absorption by pine seedlings is reflected by an increase in available nutrients in the substrate. Analyses of the soil in this case, however, revealed no differences in available soil phosphorus, potassium, or nitrogen, despite large differences in the seedling contents of these elements (Mitchell, *et al,* 1937)!



TEXT FIG. 20. INFLUENCE OF INFECTION BY MYCORRHIZAL FUNGI ON THE DEVELOPMENT AND NUTRIENT CONTENTS OF 2-YEAR PINE AND SPRUCE SEEDLINGS GROWN IN NURSERY BEDS. GROWTH PERIOD REPRESENTED—70 DAYS.

From Mitchell (1937).

In my opinion the evidence is conclusive in showing (1) that in all but fertile agricultural soils pine and other trees which form ectotrophic mycorrhizae are incapable of existence without mycorrhizae, and (2) the utility of mycorrhizae centers in the absorption of at least four and probably all nutrient elements rather than nitrogen alone.

Although this series of soil culture experiments provides, by itself, a clear-cut picture of the utility of mycorrhizae to pine, it does not supply direct information on the manner in which mycotrophy operates. Significant indications of the actual mechanics of nutrient acquisition by mycorrhizae have been obtained in other experiments which were pursued simultaneously. Among these was a series of experiments on the utilization of organic and inorganic materials by seedlings growing under pure culture conditions.

Pure Culture Experiments

PREVIOUS STUDIES

PURE CULTURE STUDIES OF MYCOTROPHY IN TREES

Pure culture was first used in mycorrhizal studies by Noel Bernard (1904) for initiating the germination of orchid seeds. The more difficult task of culturing the fungi producing ectotrophic mycorrhizae and of synthesizing mycorrhizae on tree seedlings in pure culture was attempted by Fuchs (1911) and later by Peklo (1913). Success was first attained by Elias Melin in 1922.

Melin used Erlenmeyer flasks as culture chambers and sterilized humus or sand to which were added nutrient solutions, as the rooting media. He established the identify of fungi producing ectotrophic mycorrhizae with several species of trees (Melin, 1922, 1925). Similar syntheses have been made by Hammarlund (1923), Masui (1927), Hatch and Hatch (1933), and Doak (1935).

For physiological experiments on the organic nitrogen theory Melin (1925) designed a double flask chamber, one flask of which contained surplus nutrients and the other the sand substrate and seedlings. In these chambers, inoculated and uninoculated seedlings of *Pinus sylvestris* and *Picea Abies* were grown for three years with various supplies of nitrogen. While uninoculated seedlings utilized nucleic acid and peptone with difficulty, inoculated plants exhibited no evidence of nitrogen starvation. Unfortunately this result did not establish that mycorrhizal symbiosis is necessary or useful to trees, because ammonium salts were liberated into the substrate by the introduced fungi and these are readily utilized by trees in the absence of symbiosis.

Other factors which affect unfavorably the accuracy of experiments in the apparatus commonly employed include: (1) excessive humidities, which promote aerial growth of fungi which in nature are confined to roots only (Melin, 1925; Masui, 1927; Rayner, 1925, 1930; McArdle, 1932; Hatch and Hatch, 1933); (2) increased partial pressures of carbon dioxide in inoculated cultures (Bennet-Clark, 1933, p. 200, has recorded CO_2 concentrations of 25 per cent in cotton stoppered flasks containing *Aspergillus niger*) produce increases in plant growth but in a manner wholly unrelated to mycotrophy; (3) accumulation of products (in some cases toxic) of fungal metabolism and of unabsorbed ions in substrates in which the nutrients cannot be changed (Melin, 1925); (4) saturated substrates, when fine quartz sand is employed in the absence of aeration (Rayner, 1930; McArdle, 1932); (5) low radiation intensities resulting in low rates of carbohydrate synthesis.

It is generally believed that if mycorrhizae are beneficial, they must be accessory mechanisms only, and by most investigators they are thought to be concerned with acquisition of nitrogen from infertile substrates. In the closely allied field of nitrogen-fixing bacterial root-nodules, it is known that these accessory organs are produced under internal conditions of wide carbohydrate/nitrogen ratio only (Fred and Wilson, 1934). The necessity for maintaining rapid rates of carbohydrate synthesis by incerasing partial pressures of CO_2, by high radiation intensities, or by both, is therefore evident. To be conclusive the data should also be quantitative and this involves the growing of large numbers of plants—a task of large proportions in ordinary chambers (McArdle, 1932).

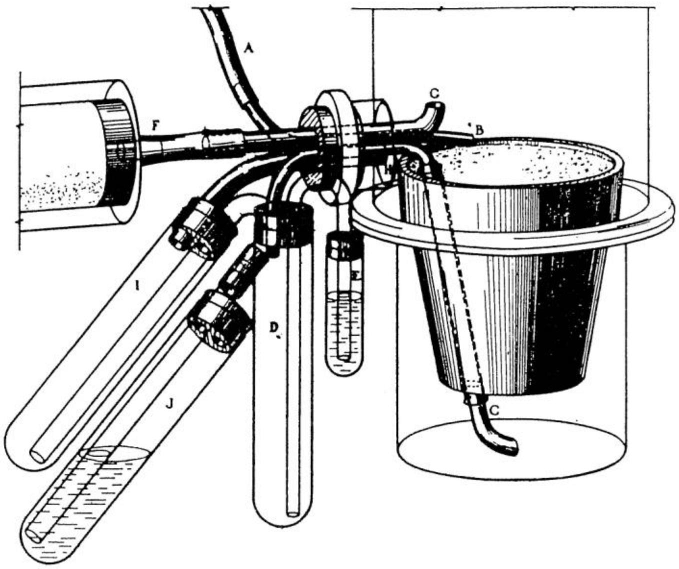
Despite these abnormalities of isolated environments, pure culture offers one of the best means for attacking the mycorrhizal problem. In the past it has yielded significant information. For continued usefulness, however, fundamental improvements in the technique of the method are essential. Many improvements in pure culture methods not employed by students of mycorrhizae have been described by workers in other fields. These methods were carefully reviewed in 1924 by Klein and Kisser in their monograph, ''Die sterile Kultur der höheren Pflanzen.'' Unfortunately, methods developed independently in America by Knudson (1919) are omitted. It appears, according to Klein and Kisser (1924), that Knudson's principle of separating the ''soil'' and aerial environments of pure culture seedlings were used in Russia early in the present century. The techniques for growing the roots of individual plants in pure culture and their tops in the atmosphere of a greenhouse had already been highly developed in Professor D. Prianischnikov's laboratory by 1911 (Schulow, 1911, 1913). Subsequently his students perfected methods for growing large numbers of plants in one container, their roots being in pure culture and their tops in the greenhouse atmosphere (Bobko, 1924). Since provision was also made in these experiments for changing nutrients at any time these methods are much superior to those used in mycorrhizal studies. The European methods culminated in that used by Klein and Kisser (1924). It differs only in minor details from the methods developed in Professor

Prianischnikov's laboratory. Reference is made to their monograph for all details.

Unfortunately, none of these methods is suitable for exploring mycotrophy in pine. They are capable of excluding contaminating organisms for only relatively short periods of time. Furthermore, the problem of handling large numbers of culture is prohibitive. The pure culture problem in mycotrophic studies is one of obtaining normal physical environments within wholly inclosed systems. A suitable culture chamber must permit: (a) the use of radiation intensities above 40 per cent of full sunlight; (b) the maintenance of normal temperatures during such exposures; (c) the maintenance of normal humidities and gas concentrations; (d) the changing of nutrient solutions and of substrate air at short intervals; and (e) the exclusion of contaminating organisms over long periods of time.

Experiments on pure culture methods were commenced by the writer in 1930 during his period of study under Professor Elias Melin at the Royal Academy of Forestry, Sweden. For the first experiments a chamber was assembled from standard glass parts (that is, bell-jar and glass base) which were fitted together by grinding them into a glass disk. The disk also supported a flower pot which served as the substrate container. Nutrients were stored in a two-liter suction flask attached to the chamber by means of glass and rubber tubing. The chamber was aerated by means of a small rotary pump, the air being passed through cotton-wool before reaching the chamber. Preliminary tests during the summer of 1930 demonstrated the feasibility of improving pure culture environments by these means.

When the experiments were renewed after the writer's return to the United States, glass chambers consisting of but two parts were employed (Text Fig. 21). The diameter of the lower chamber was reduced and the flower pot was supported by the walls of a lower chamber rather than by a glass disk. The entire apparatus as finally used is shown in Plate VIII, B. It consisted of the glass chamber (the parts cemented together with DeKhotinsky Cement) supporting the flower pot, a nutrient-storage flask (covered to exclude light), a Berkefeld grade V-filter for air filtrations, and outlet safety tubes for air and for used nutrients. Details of the air and nutrient connections are shown in Fig. 21. Nutrients were introduced through tube A-B and, after draining through the sand, removed through the system C-D-E. Air from a rotary blower was forced through the filter and passed into the chamber through tube F-G. It left the chamber through the system H-I-J. Mercury bichloride was placed in the second safety tubes of both the nutrient removal E and air exit J systems.



TEXT FIG. 21. DETAILS OF NUTRIENT AND AIR INLET AND EXIT TUBES OF THE ALL GLASS CHAMBERS USED AT THE BOYCE THOMPSON INSTITUTE.
(Courtesy U. S. Forest Service).

These chambers were placed in a glass compartment in a greenhouse at the Boyce Thompson Institute. The whole system was cooled by means of a fan which moved approximately 500 cu. ft. of air per minute (from a base-ment beneath the greenhouses) through the compartment. A continuous record of temperatures (1) in the greenhouse, (2) in the glass compartment, (3) in an unaerated chamber, and (4) in an aerated chamber was obtained by means of resistance thermometers. The maximum air temperatures (maintained for a few minutes only) on some of the warmest days of March, April, and May, 1932, as recorded at the four points, are given in Table X. From these data it is very evident that the cooling system was not adequate.

TABLE X

MAXIMUM AIR TEMPERATURES RECORDED IN AERATED AND UNAERATED PURE CULTURE CHAMBERS, IN THE GLASS CASE, AND IN THE GREENHOUSE ON WARM SUNNY DAYS AT THE BOYCE THOMPSON INSTITUTE DURING APRIL AND MAY, 1932

Date	*Pure culture chambers*			*Green-house*	*Glass case*	*Diff.*
	aerated	*unaerated*	*diff.*			
3-24	98	116	18	94	85	9
4-2	97	115	18	94	83	11
4-5	93	111	18	93	82	11
4-7	98	111	13	95	85	10
4-22	97	110	13	96	89	7
4-24	98	111	13	91	87	4
4-30	97	113	16	94	90	4
5-4	94	111	17	97	87	10
5-5	97	112	15	99	93	6
5-17	100	111	11	95	95	0
5-18	100	114	14	92	95	(3)

From the viewpoint of convenience in handling and of freedom from contamination the apparatus was also far from ideal. In the first experiments with the apparatus, four chambers were connected to one filter and to one nutrient flask. All became contaminated during the first month. The use of an individual nutrient flask and an individual filter for each chamber, as shown in Plate VIII, B, was a great improvement; but even then more than 65

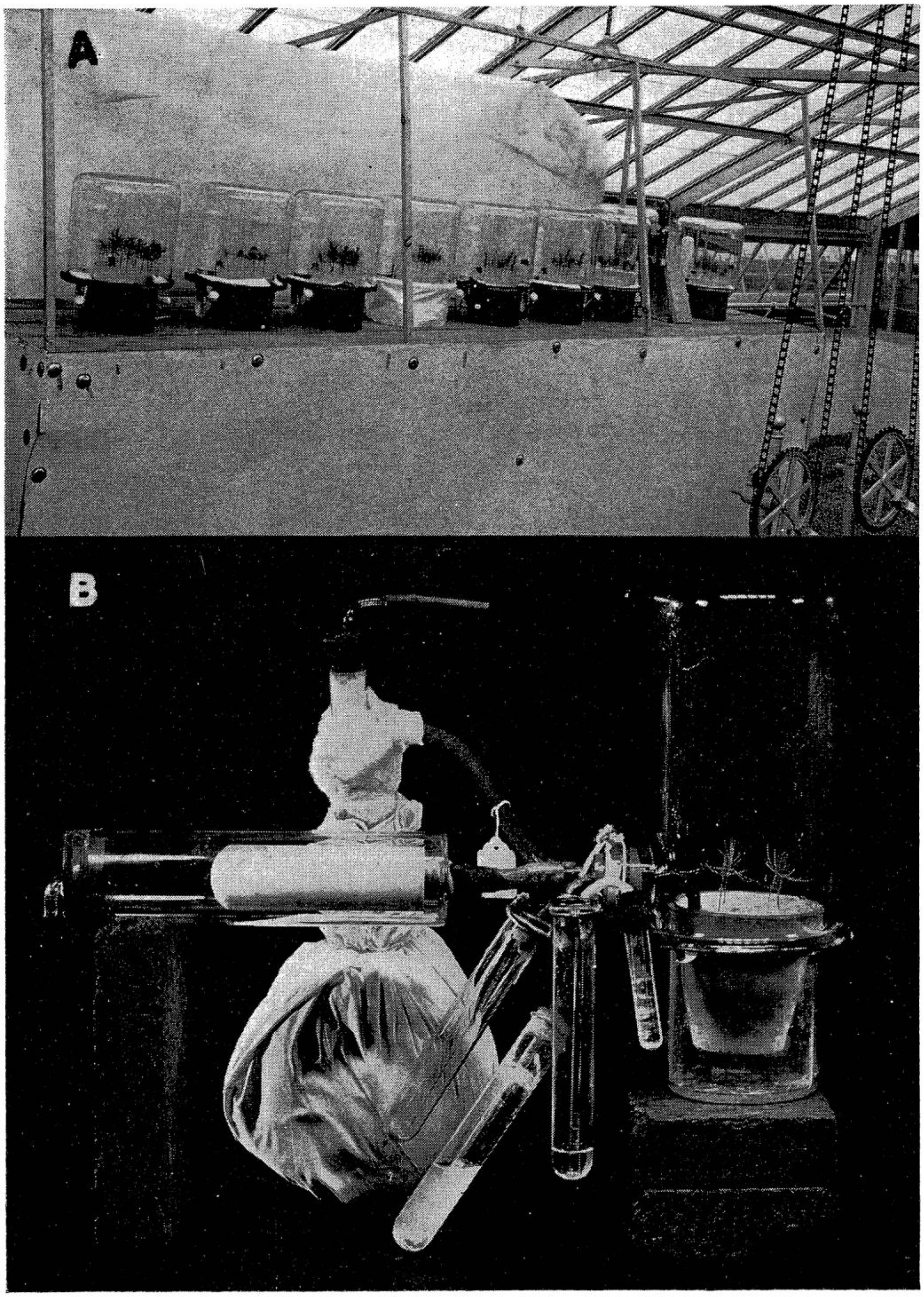


PLATE VIII. PURE CULTURE CHAMBERS USED IN (A) HARVARD AND (B) BOYCE THOMPSON INSTITUTE PURE CULTURE EXPERIMENTS.

per cent of all the chambers used became contaminated during a period of three months and twenty days. Contaminations were incurred during the planting and inoculating of seedlings and also during the course of the

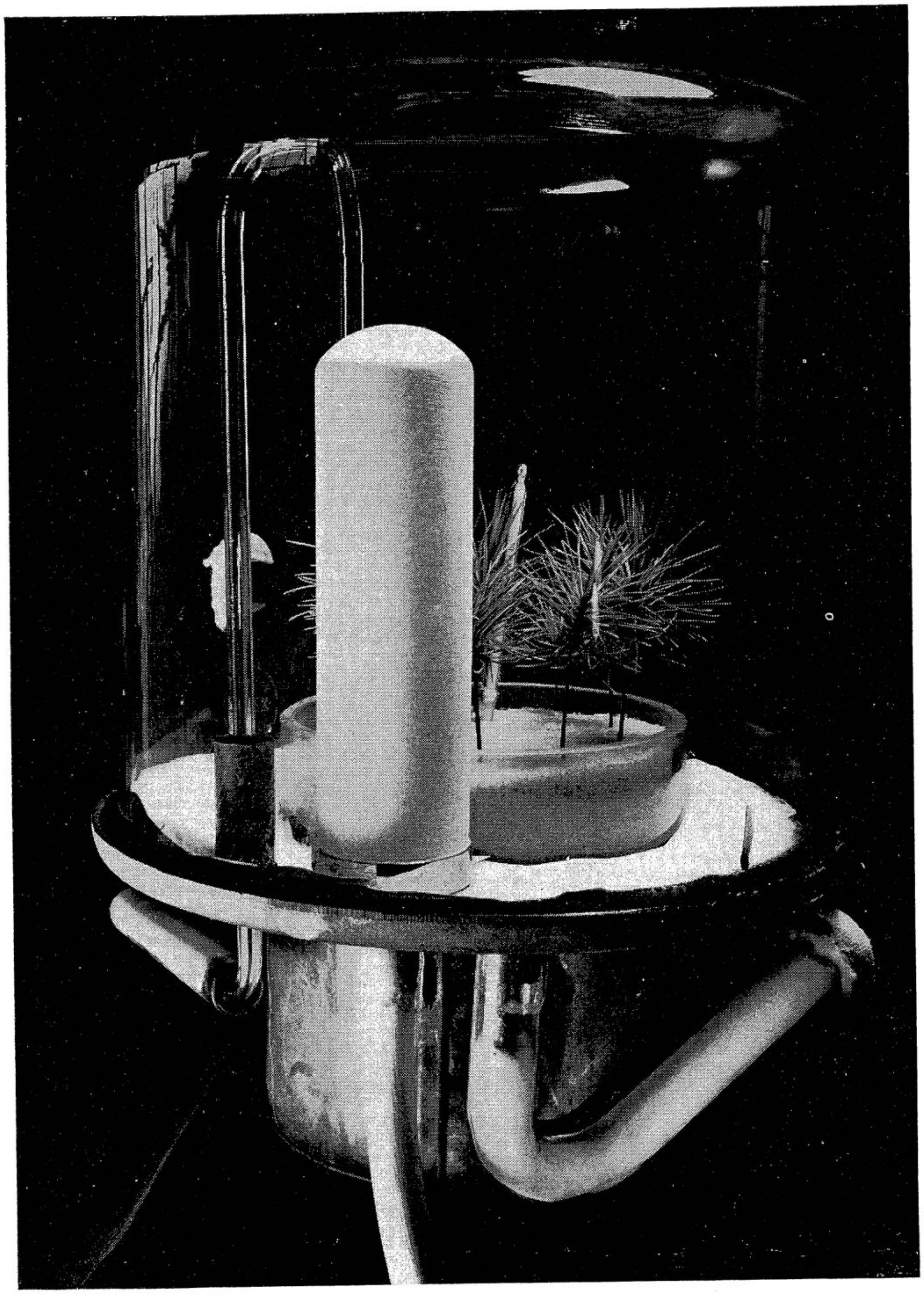


PLATE IX. DETAILS OF METAL BASE PURE CULTURE CHAMBER USED IN ALL HARVARD EXPERIMENTS.

experiments. The necessity for planting through the stopper-opening caused most of the difficulties since the glass chamber and the remainder of the apparatus had to be sterilized separately. Contaminations during the course of the experiment were usually through the nutrient removal system.[1]

To overcome the inconveniences of this apparatus various chambers were designed and tested during the spring and summer of 1932. All possessed metal bases for accommodating the several air and nutrient connections and all possessed an opening in the bell-jar for inoculating and introducing germinated seedlings. These features permit the sterilization of the entire apparatus as a unit and the introduction of all plant materials through an opening no larger than 17 mm. in diameter. The type of apparatus selected and successfully used in experiments conducted at Harvard University is shown in Plate IX. The bases of these chambers, during the second and third series of experiments, were submerged in individual water baths to hold the substrate at a suitable temperature. In still more recent attempts to improve the apparatus for general use the copper base was replaced by a glazed clay base and the whole chamber submerged in a water bath (Hatch, 1934, Fig. 1; 1936, Fig. 317). Details of construction and of the special techniques required in the use of this chamber have been recorded elsewhere (Hatch, 1934b, 1936).

With ordinary care the metal and porcelain base chambers are practically impregnable to contaminating organisms. Of 21 chambers which were not contaminated during the process of planting in two sets of experiments (1933 and 1934) not a single one became contaminated

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[1] These experiments were undertaken through coöperative agreement by the Boyce Thompson Institute and the Allegheny Forest Experiment Station, Philadelphia, Pa. The writer was a member of the latter Station and could spend only a part of his time at the Institute. The experiments were tended in his absence by Mr. Reno Marini, a Laboratory Assistant of the Institute, under the supervision of Dr. F. O. Holmes.

during the course of three and one-half months. In the planting process, however, 13 of a total of 34 chambers used in the two sets of experiments had foreign organisms introduced into them. Of these all but 4 were by one species of bacterium, and this organism was later found to be in the seeds. It follows that if adequate care is exercised in selecting seeds which are free of microörganisms, contamination of the chambers should be rare.

In the experiments here reported seeds were soaked over night in sterile water, treated for two minutes with 0.1 per cent mercury bichloride, rinsed in 10 to 15 changes of sterile water, and placed on agar slants. The slants were placed in a cold room at temperatures which varied from freezing to 8°C. After two months the tubes were removed from cold storage and the contaminated ones discarded. The seeds germinated about a week later and were immediately introduced into the culture chambers (carried out in a transfer chamber). In starting a third series of experiments (not reported here) in the summer of 1934 it was found that bacterial infection of the seeds could be demonstrated by pouring a small quantity of liquid sugar-nutrient into each test tube 3 or 4 days before they were ready for planting. Infected seeds were relatively few, since in the two series of experiments a total of 598 seeds were introduced into the 34 chambers and only 13 chambers became contaminated.

Investigations of Seedling Growth in Pure Culture

SYNTHESIS OF MYCORRHIZAE

Preliminary to the physiological experiments a number of Hymenomycetes were obtained in culture by the writer and Constance Talbott Hatch and tested for their ability to produce mycorrhizae with pine seedlings (at the Mycological Laboratories, Royal Academy of Forestry, Stockholm, Sweden). Of 18 fungi suspected of being mycorrhizal organisms, 12 produced mycorrhizae with

Pinus Strobus L. (Plates 69-71, Hatch and Hatch, 1933) and two with *P. resinosa* (Hatch, 1934c, Figs. 4-6; page 118 of this paper). In the several experiments of which descriptions follow some of these fungi, as well as two species obtained in culture and established as mycorrhizal organisms by Doak (1935), were used.

The most significant difference between these synthesis experiments, in which excellent mycorrhizae were obtained, and the physiological experiments, of which the descriptions follow, was that of nutrient availability. The sand used in the synthesis experiments was of fluvio-glacial origin. Professor Melin supplied the following analysis of this sand: 60 per cent feldspar, 30-35 per cent quartz, 5-10 per cent mica, amphibole pyroxene, and other materials. The base exchange capacity of this sand, as determined by Professor P. R. Gast at the Harvard Forest, is several hundred per cent greater than that of the quartz (over 99 per cent silica) sand used in the physiological experiments. That appreciable quantities of the nutrients added to the fluvio-glacial sand becomes adsorbed to the sand is indicated by the shift in the concentration of hydrogen ions in the solution from pH 6.57 before to pH 4.2 after autoclaving (Hatch and Hatch, 1933, p. 329). Such a shift does not occur when the same nutrient solution is autoclaved in the quartz sand of the physiological experiments. Thus, the nutrients in these synthesis experiments were apparently largely adsorbed and out of solution while those of the physiological experiments were in solution and readily available.

GROWTH OF PINE SEEDLINGS ON ORGANIC SOURCES OF NITROGEN

The initial studies carried out in the "all glass" (Plate VIII, B) and in the "copper base" (Plates VIII and IX) chambers were planned with the object of obtaining quantitative estimates of the utilization of peptone and nucleic acid by mycorrhizal and non-mycorrhizal seedlings. They were based on the earlier work of Melin (1925) which had yielded evidences of nitrogen starvation in non-mycorrhizal seedlings. But the liberation of ammonium by the mycorrhizal fungi introduced into the chambers rendered uncertain the conclusion that these fungi facilitated the utilization of organic nitrogen *by virtue of their symbiotic mode of life.*

To avoid these complications the writer (1) changed nutrients at frequent intervals, (2) introduced non-mycorrhizal fungi into a second set of control cultures, and (3) raised the carbon/nitrogen ratio of the culture medium. Addition of dextrose to the amount of 0.3 per cent was sufficient for the latter purpose. With this proportion of dextrose available, peptone and nucleic acid were utilized only for the nitrogen they contained rather than primarily as a source of energy for fungal metabolism. Under these conditions ammonium does not accumulate in the soils or in nutrient solutions (Waksman, 1932, p. 444-451).

Two series of experiments with nucleic acid as the source of nitrogen were carried out at the Boyce Thompson Institute in the glass chambers (Plate VIII, B). In one *Pinus resinosa* was grown with *Boletus luteus,* one of two organisms which yielded mycorrhizae with this pine in the synthesis experiments (not previously reported). In the other, *Pinus taeda* was associated with *Mycelium radicis nigrostrigosum* and with *Boletus subluteus* (cultured by Dr. K. D. Doak). In addition to the uninoculated cultures a third of the chambers were inoculated with three soil fungi, *Mucor rammannianus, Nortierella isabellina,* and *Penicillium divaricatum* (obtained from Dr. C. W. Watson). The nutrient employed contained the following quantities of salts and organic materials per liter of solution:

KCL	0.11900	grams
$MgSO_4 \cdot 7H_2O$	0.39400	"
$Ca\ (H_2PO_4)_2$	0.10100	"
$ZnSO_4 \cdot 7H_2O$	0.00017	"
$MnSO_4 \cdot 2H_2O$	0.00011	"
Dextrose	3.0	"
Nucleic acid (from yeast)	1.0	"
Iron citrate, 1 ml. of a 1% solution		

The solution was adjusted approximately to pH4 by addition of 5 ml. of 1/10 normal NaOH. During the experiment (three and one-half months) five liters of this solution passed through each chamber, additions being made two or three times weekly. Estimates of NH_3 in the solution removed from the chambers were made eleven times during the course of the experiments. Amounts larger than 0.34 mg. of NH_3 per 100 ml. solution were not found.

The sand substrate in these and all other physiological experiments, in contrast to that used in the synthesis studies, was silica sand (silica 99.89%). The grains were almost spherical and uniform in size (the portion retained between 20 and 30 mesh screens).

Mycorrhizae were produced in one case only. In cultures which were not contaminated or inoculated with the soil fungi growth was excellent (Plate X, B). The average weight of pure culture seedlings was 170.3 mg. and the shoot/root ratio 0.351. The average dry weights and shoot/root ratio of seedlings inoculated with the soil fungi were 57.0 mg. and 1.862 respectively. Stunted growth of the latter seedlings was caused by changes in the substrate which proved toxic to seedling roots (Plate X, A). The dichotomous forking of short-roots on seedlings grown on nucleic acid reported by Melin (1925) was confirmed in these experiments (Plate III, G).

The following year at Harvard University a third series of experiments was carried out in which peptone, the other source of organic nitrogen which was utilized only with difficulty by Melin's seedlings, was employed. Twelve germinating seeds (all of known weights, see Table XI) of *Pinus Strobus* were introduced into each of fifteen copper-base chambers (Plate IX). Five cham-

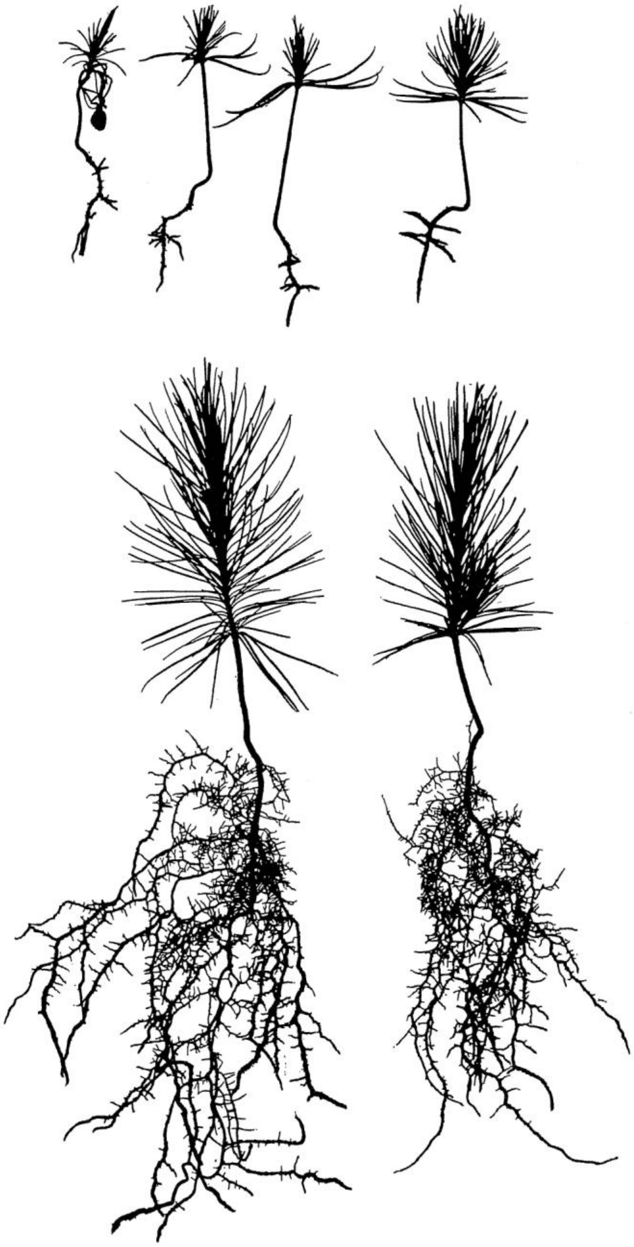

PLATE X. SEEDLINGS OF *PINUS TAEDA* GROWN ON NUCLEIC ACID AS NITROGEN SOURCE IN PURE CULTURE.

Upper: inoculated with soil fungi. Lower: pure culture.

TABLE XI

DATA ON 1933 PURE CULTURE EXPERIMENT WITH PEPTONE AS SOURCE OF NITROGEN

Pot No.	*Treatment*	*No. seed-lings*	*Av. wt. of seedlings in mg.*	*S. E. M.*	*Root/shoot ratio*	*Remarks*
12	No inoc.	10	.4551	.0013	1.006	
16	No inoc.	9*	.3484	.0025	0.862	Replacement seedling discarded
17	No inoc.	8*	.3159	.0038	0.965	Replacement seedling discarded, one seedling preserved
4	No inoc.	10	.3375	.0019	0.917	Contaminated with bacteria
11	No inoc.	9	.1491	.0010	0.357	Contaminated with Penicillium
1	B. bovinus	9*	.3784	.0063	0.905	Inoculum covered roots, seedlings 1-5 photographed, one seedling preserved
6	B. bovinus	8	.3150	.0039	0.886	Replacement seedlings discarded
15	B. bovinus	9	.3810	.0008	1.004	Inoculum covered parts of roots
28	B. bovinus	9	.4389	.0013	0.992	Inoculum covered parts of roots, one seedling preserved
13	B. bovinus	9*	.1300	.0006	0.666	Inoculum grew not at all, contaminated with bacterium; $HgCl_2$ injury from thermometer

TABLE XI—*(Continued)*

2	P. campestris	6*	.247	.0081	0.697	Inoculum grew, replacement seedlings discarded, one seedling preserved
23	P. campestris	10	.2770	.0026	1.017	Inoculum grew
30	P. campestris	8*	.3898	.0017	0.888	Inoculum grew, replacement seedlings discarded
19	P. campestris	8	.1224	.0010	0.329	Contaminated with Penicillium, seedlings 1-5 photographed, one seedling preserved
21	P. campestris	8	.3109	.0019	0.970	Contaminated with bacteria, replacement seedlings discarded

* Fresh weight of seeds averaged 21.5 mg. all other seedlings averaged 22.5 mg.

bers were inoculated with *Boletus bovinus,* five with *Psalliota campestris* (L. ex Fr.) Quelet, and five were not inoculated. *P. campestris* replaced the three soil fungi in these experiments because the latter produced changes in the nutrients of the previous experiments which inhibited seedling growth (Plate X, A). Judging from the culture studies of Styer (1930) and Waksman and Nissen (1932), *P. campestris* is very similar in its physiology to that of mycorrhizal fungi (Melin, 1925; Falck, 1923) and therefore should have nearly the same effect on the nutrient solution as these fungi. The full quota of ten seedlings desired in each chamber was not realized, so replacements (to render the cultures comparable to one another) were made. These were excluded from the plants analyzed for growth at the close of the experiment (Table XI). Seed coats were removed from the chambers at the time of making replacements since they constitute a possible source of delayed contamination. The nutrient solution contained the following organic and inorganic materials per liter:

KH_2PO_4	0.11160	grams
$MgSO_4 \cdot 7H_2O$	0.17700	''
$CaCl_2$	0.06204	''
$ZnSO_4 \cdot 2H_2O$	0.00017	''
$MnSO_4 \cdot 7H_2O$	0.00011	''
Bacto peptone	1.0	gram
Dextrose	3.0	grams
Iron citrate, 1 ml. of a 1% solution		

The solution was adjusted to pH 5.6 by addition of hydrochloric acid. During the experiment the substrate was flooded seven times with freshly prepared nutrient solutions of this composition (except that zinc and manganese were omitted the last three times).

Complete and summarized results are assembled respectively in Tables XI and XII. The appearance of five seedlings from each of two chambers, one contaminated with *Penicillium* and the other without microörganisms, is shown in silhouette photographs in Plate XI. Micro analyses of the first three seedlings from the left, Plate XI, B, revealed an average nitrogen content of 2.83 per cent, potassium content of 1.7 per cent, and phosphorous content of .436 per cent (averages of 9 separate determinations in each case; made by Mr. E. Snow, in Professor Gast's laboratory at Petersham). The average dry weights of 27 uninoculated seedlings (429 mg.) and of 34 seedlings inoculated with *Boletus bovinus* (428.4 mg.) exceeds by 122 mg. the highest weights of seedlings of comparable age and seed weights raised by Mitchell (1934, Table IX) in soils of the Black Rock Forest. [These seedlings were raised in the Mid-Slope (good) soil and received the basic nutrient plus an additional quan-

TABLE XII

SUMMARY OF DATA ON GROWTH OF *P. Strobus* SEEDLINGS IN PURE CULTURE WITH PEPTONE AS THE SOURCE OF NITROGEN. ALL SEEDLING WEIGHTS CORRECTED AS OF SEED CLASS 5

(See Mitchell, 1934; Table XVII)

Treatment	*Av. of seedlings in all pots*		
	No. Seedl.	*Dry Wt. in mg.*	*Av. root/shoot ratio*
No. inoculation	27	429.0	.944
B. bovinus	34	428.4	.929
A. campestris	24	351.8	.876
Contaminations			
Bacteria	18	363.1	.943
Penicillium	17	152.0	.343
Bacteria + poisoning by $HgCl_2$	9	152.1	.666

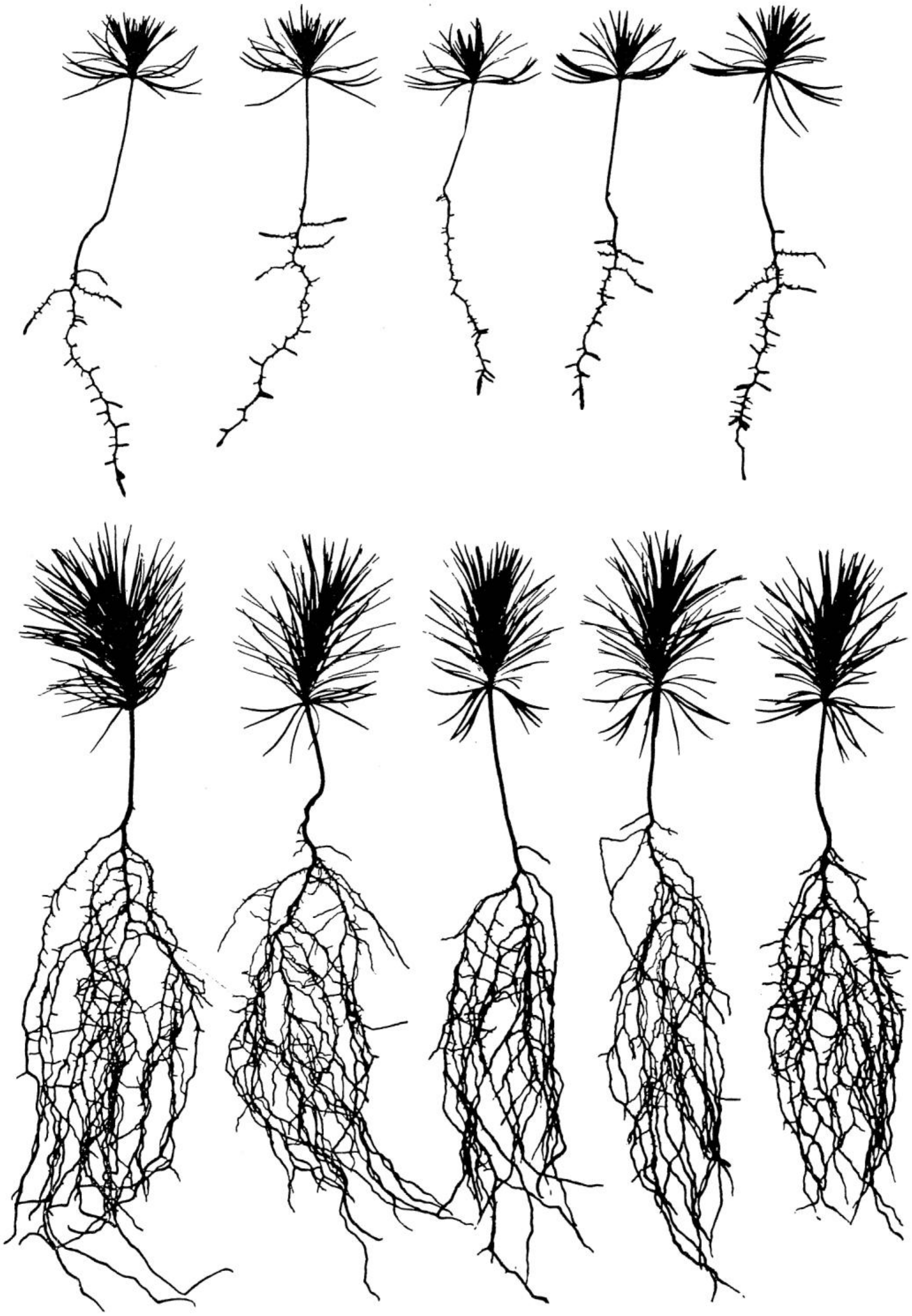

PLATE XI. SEEDLINGS OF PINUS STROBUS GROWN ON PEPTONE AS THE SOURCE OF NITROGEN IN PURE CULTURE.

Upper (A): contaminated with *Penicillium* sp. Lower (B): pure culture, no inoculation.

tity of nitrogen, in all 553 mg. of NH_4NO_3.] The average weight of 429 mg. also exceeds by 37.5 mg. the maximum yield obtained by Mitchell in the best of his sand cultures. The maximum yield in Mitchell's cultures was 381.5 mg., obtained with 300 ppm. of nitrogen in the nutrient solution.

DISCUSSION

Nitrogen starvation of the kind found by Melin (1925) was not found either in the nucleic acid or peptone experiments. The ready utilization of organic nitrogen resulted in high internal concentrations of nitrogen; and since other elements were also present in abundance, mycorrhizae were not produced (See soil culture section). A wholly reasonable explanation of the differences in the facility with which seedlings in these experiments and in those of Melin utilized peptone and nucleic acid, is not apparent. It may be pointed out, however, that the two sets of experiments were carried out under quite different environments. Also, the quantity of dextrose used in my cultures was six times as great as those of Melin (0.3 and 0.05 per cent, respectively). To what extent these differences influenced the utilization of organic nitrogen is unknown. Since the use of lower quantities of dextrose renders comparative studies of symbiosis fruitless because of the liberation of ammonium into the substrate, the value of exploring the cause of these differences would probably be small.

The question of the form in which nitrogen was absorbed is an important one which can not be settled except by further experimentation. Since quantitative estimates for ammonium in the used solutions were made after each flooding and this form of nitrogen never appeared, the indications are that nitrogen was absorbed in organic form. In view of Knudson's (1919) experiments, which indicate that plant roots do not secrete enzymes, it is improbable that organic nitrogen was decomposed at the surfaces of absorbing roots and so did not appear in the substrate solution. Furthermore, if decomposition of organic nitrogen as a result of enzymes liberated by autolysis of sloughed-off root-cap cells had been appreciable, ammonium would have appeared in the used nutrient solutions. The evidence points to absorption of nitrogen in organic forms.

While it is generally accepted that amino acids are absorbed by plant roots, opinion is equally general that larger molecules can not pass through absorbing membranes. As long ago as 1907, however, Kraus, Portheim, and Yamanouchi reported that animal antigens were absorbed by plant roots. They obtained a positive test for horse serum (by the precipitation technique) in the leaves of plants growing in soil to which the serum had been added. Using the Schultz-Dale serological technique, Moritz (1932, 1934) has demonstrated the presence of egg albumin in plant leaves 48 hours after addition of the albumin to soil. Subsequently these results have been extended by Dr. K. S. Chester at the Rockefeller Institute for Medical Research. While Dr. Chester's work is not yet published, I have his permission to quote from a letter as follows: ''I have repeated Moritz's experiments, with . . . variations, and my results are in perfect conformity with his. I have used both beans and *Vicia,* watering with egg albumin, and unwatered controls, and in both cases was able to pick up egg albumin in the leaves. The serological tests were confirmed by using precipitin tests, complement-fixation tests, and anaphylaxis tests, in the latter case using the bean and *Vicia* materials both to sensitize and, in addition, to intoxicate.

''We can feel reasonably sure from this that the egg albumin is able to get into the circulatory system of these plants through the normal, sand-culture root system. This leaves the question open, however, whether the protein penetrates the root hairs or whether it enters through injuries, large or small, which might result from . . . the action of microörganisms.''

More recently Hitchcock and Zimmerman (1936) have demonstrated absorption by intact roots of alpha-naphthaleneacetic acid, indoleacetic acid, indolebutyric acid, indolepropionic acid, phenylacetic acid, and the methyl and ethyl esters of these acids.

It appears likely, therefore, that much larger molecules pass into plant roots than physiologists are accustomed to think is possible. The results obtained in these experiments support the hypothesis that nucleic acid and peptone were absorbed as such by the seedling roots. It is possible, however, that only the smaller moleculed fractions (impurities) of these chemicals were actually absorbed. It is believed that the serological technique used in combination with pure culture techniques offer the best possibilities for solving this important question.

GROWTH OF PINE SEEDLINGS ON INORGANIC SALTS

Because the pot culture experiments had demonstrated that mineral salt deficiencies call forth the mycorrhizal response, this possibility was examined in pure culture. For the mycorrhizal fungi the following species were employed: *Boletus bovinus, Boletinus pictus* (synthesis experiments reported by Doak, 1934), *Lactarius deliciosus* (L) Fr. (Plate I, A) and *L. indigo* (cultured by Doak). For the non-mycorrhizal fungi *Mutinus Curtissi, Ithyphallus Ravenelii* (cultured by Doak), and *Calvatia craniformis* (cultured by Doak) were employed. A total of 22 seeds, all of known weights, were introduced into each chamber. The nutrient solution contained the following quantities of organic and inorganic materials per liter:

KH_2PO_4	0.100 grams
$MgSO_4 \cdot 7H_2O$	0.169 ”
$CaCl_2$	0.054 ”
NH_4NO_3	0.154 ”
Dextrose	0.500 ”

Iron citrate, 1 ml. of a 1% solution

All chambers were provided with this nutrient at the beginning of the experiment (December 2-3, 1933). The seedlings grew well during the first month. To create starvation conditions the nutrients were replaced after the first 20 days with dextrose solution (0.5 grams per liter). A month later this solution was replaced by another having p. 154 grams of ammonium nitrate in addition to the 0.5 grams of dextrose. Thereafter the regular nutrient solution was used. All seedlings showed uniform evidences of nutrient deficiencies during February, 1934, and became dormant. Dormancy could not be broken by cold treatment, so on March 28 aeration of the chambers was suspended for a day and 0.2 ml. of ethylene chlorhydrin was allowed to evaporate into the chambers from the air filter. By April 20 some seedlings recommenced growth but others remained dormant and still others showed signs of injury. The experiment was discontinued because the accuracy of comparative growth studies at a later time would have been impaired by these inequalities. Although mycorrhizal fungi had covered the roots of some seedlings in each inoculated chamber, mycorrhizae were not produced.

The lack of mycorrhizal roots on these seedlings, despite their abundant covering of mycorrhizal fungi, was at first confusing. Gradually an explanation unfolded which centers in the availability of nutrient salts. Water plants, when growing in a water environment where nutrients although available may be exceedingly scarce, never possess mycorrhizae (Stahl, 1900; Asai, 1934). These same plants may possess mycorrhizae when growing in soil (Asai, 1934) where the problem of obtaining nutrients involves their removal from soil colloids and other base-exchange materials. Similarly, the occurrence of mycorrhizae in soils is determined by the availability of mineral salts (See the earlier sections for a review of this literature). When nutrients are readily available in the soil solution mycorrhizae are few or absent. When they must be obtained from base-exchange materials mycorrhizae are abundant.

One of the chief differences between this experiment and the synthesis experiments, in which profuse development of mycorrhizae was obtained, is in nutrient availability. In the present experiment the nutrients, although scarce, were dissolved in the water of the solution. In the synthesis experiments they were largely adsorbed to the surfaces of fluvio-glacial sand, and fungi possessed an advantage over roots in obtaining these adsorbed nutrients because of their relatively much greater absorptive surfaces.

The question now arises just why mycorrhizae should be more efficient in extracting nutrients from base-exchange materials than are tree roots.

INFLUENCE OF MYCORRHIZAL FUNGI ON ROOT DEVELOPMENT IN *Pinus*

CURRENT CONCEPTS

Most students of tree roots maintain that infection by mycorrhizal fungi leads to retarded root growth, to cessation of elongation, and eventually to the destruction of the infected roots. Those students who contend that mycorrhizae are injurious to the vascular plant logically cite such opinions in support of their views (R. Hartig, 1888, 1891; v. Tubeuf, 1888; McDougall, 1914; etc.). To those who are uncertain as to the nature of mycorrhizae these opinions constitute a serious obstacle to acceptance of the "beneficial" theory (Laing, 1932; McArdle, 1932). Supporters of the "beneficial" theory, however, advance the assumption that reduction in surface area is surely more than compensated for by the increased efficiency of mycorrhizae in absorbing one or more elements essential to growth (Frank, 1888; Stahl, 1900; v. Tubeuf, 1903; Melin, 1925).

The belief that the growth of mycorrhizal roots is retarded or inhibited as a result of infection has sometimes been based on comparative lineal measurements of mycorrhizal and non-mycorrhizal roots, apparently always with the assumption that in the absence of infection all tertiary roots would tend to grow to approximately equal lengths. In modern times Melin (1923) is much cited as having confirmed this as fact by pure culture experiments. But the longest non-mycorrhizal short-roots which Melin has ever found, even on two-year plants (in pure culture), were only 20 mm. long (Melin, 1925, p. 60).

A number of other features of mycorrhizal roots are sometimes thought to result from attack by mycorrhizal fungi. Among these are the absence in mycorrhizal roots of root hairs and root caps, fewer conduction elements (monarchy), and reduced numbers of cortical cell layers (Laing, 1932).

Until recently the correctness of these concepts had not been questioned despite many evidences of the distinctive character of long- and short-roots on seedlings raised in the absence of mycorrhizal organisms (Möller, 1902-1903; Nolle, 1910; Melin, 1925, 1927). First to determine whether or not attack by mycorrhizal fungi is the primary cause of slow growth of some mycorrhizal roots was Aldrich-Blake (1930). In discussing the fate of lateral root initials (diarch roots) on seedlings of Corsican Pine he (*op. cit.*, p. 23) wrote as follows:

> "Those lateral roots which are not arrested at an early stage may behave in one of two different ways. They may become infected with mycorrhizal fungus at a short distance from their point of origin, and dichotomize into two monarch roots, which may in turn further dichotomize, but never grow to any considerable length. Alternatively they may grow on as mother roots, and eventually be converted into pioneer roots of unlimited growth.
>
> "The question naturally arises whether lateral roots are predestined to develop in one or the other of the above mentioned ways, or whether their subsequent development is controlled by the conditions they encounter in the soil through which they grow. Sections through the diarch basal portions of roots which had developed as mother roots,

> and of those which had subsequently dichotomized, showed no general qualitative differences; though it was only in sections from the base of dichotomized roots that well marked resin canals were occasionally absent. However, when quantitative differences were taken into account a real difference became apparent. . . . The mean protoxylem diameter of the mother roots was 117 ± 6, while that of the basal diarch portion of the dichotomized roots was 73 ± 6, and the probability of significance of the difference between these two measurements was over 100 to 1. Thus it is clear that the roots which subsequently dichotomize, and those which develop, as mother roots, fall into two significantly different size classes . . . we are probably justified in concluding that the dichotomy of lateral roots of Corsican Pine is the response of a feeble root to strong fungal infection."

These facts indicate that the primary cause of slow growth in those diarch root initials which become mycorrhizal is not fungus invasion but the vigor or physiological state of the roots themselves. That tertiary roots vary within wide limits in their growth rates or vigor is amply evidenced by the form of the root systems which are produced in pure culture, where internal factors alone control elongation rates (Melin, 1925, Fig. 23-27). The limited growth and marked uniformity of short-roots produced on *Pinus Strobus* in my synthesis experiments (Hatch and Doak, 1933, Plate 58, B) first attracted the writer's attention to this question. Appearance in 1932 of papers by Laing and by McArdle, in which the character of mycorrhizal root systems were badly confused, provoked a review of the subject by the writer and Doak (1933) which included a report on part of the data from the writer's pure and soil culture experiments in Sweden.

The study revealed that the distinctive features of short-roots result from inherited or physiological qualities quite unrelated to infection by mycorrhizal fungi. In pure culture short-roots of *Pinus Strobus* were monarch in vascular structure, they lacked a root cap and possessed only four layers of cells in the cortex, their elongation rates were slow, and secondary growth did not occur (Hatch and Doak, 1933, Plates 57 and 58). The short-roots which became mycorrhizal in pure culture grew at equal rates; but in addition they dichotomized and thereby increased the number of tips per short-root (*op. cit.,* Plates 57, C and 58, C). By causing hypertrophy of the cortical cells mycorrhizal fungi also increased the diameter of the short-roots. In soils or sand cultures non-mycorrhizal short-roots (*op. cit.,* Plate 58, A) were not found which were greater than 2-3 mm. in length; but mycorrhizal short-roots grew to lengths of 10-12 mm., and in addition in nature were found with more than 80 individual tips (*op. cit.,* Plate 59, C). These facts were believed to support a view which is the precise opposite of that held by all previous investigators, namely, that mycorrhizal fungi stimulate root growth and thereby increase absorbing surface areas (*op. cit.,* p. 96).

NEW EVIDENCE ON THE INFLUENCE OF MYCORRHIZAE ON ROOT DEVELOPMENT

Data which show that mycorrhizal fungi increase the surface area of the root systems of *Pinus* are now abundant. To understand the exact manner in which increase occurs, and the way in which the increase may be of significance in seedling nutrition, examination of a number of generalities about absorption is essential.

It has long been known that plant roots tend to increase in extensiveness (that is, the root/shoot ratio increases) with decrease in soil fertility. In pine seedlings this relationship is specific for individual deficiencies of nitrogen, phosphorous, potassium, and calcium (Mitchell, 1934). The character and magnitude of changes in the root/shoot ratio of seedlings of *P. Strobus* with changes in the availability of a nutrient element is shown for a representative case, nitrogen, in Plate XII (Mitchell, 1934, Plate IX). When the concentrations of nitrogen or of the other elements are low the roots are extensive in relation to the tops, and when they are high

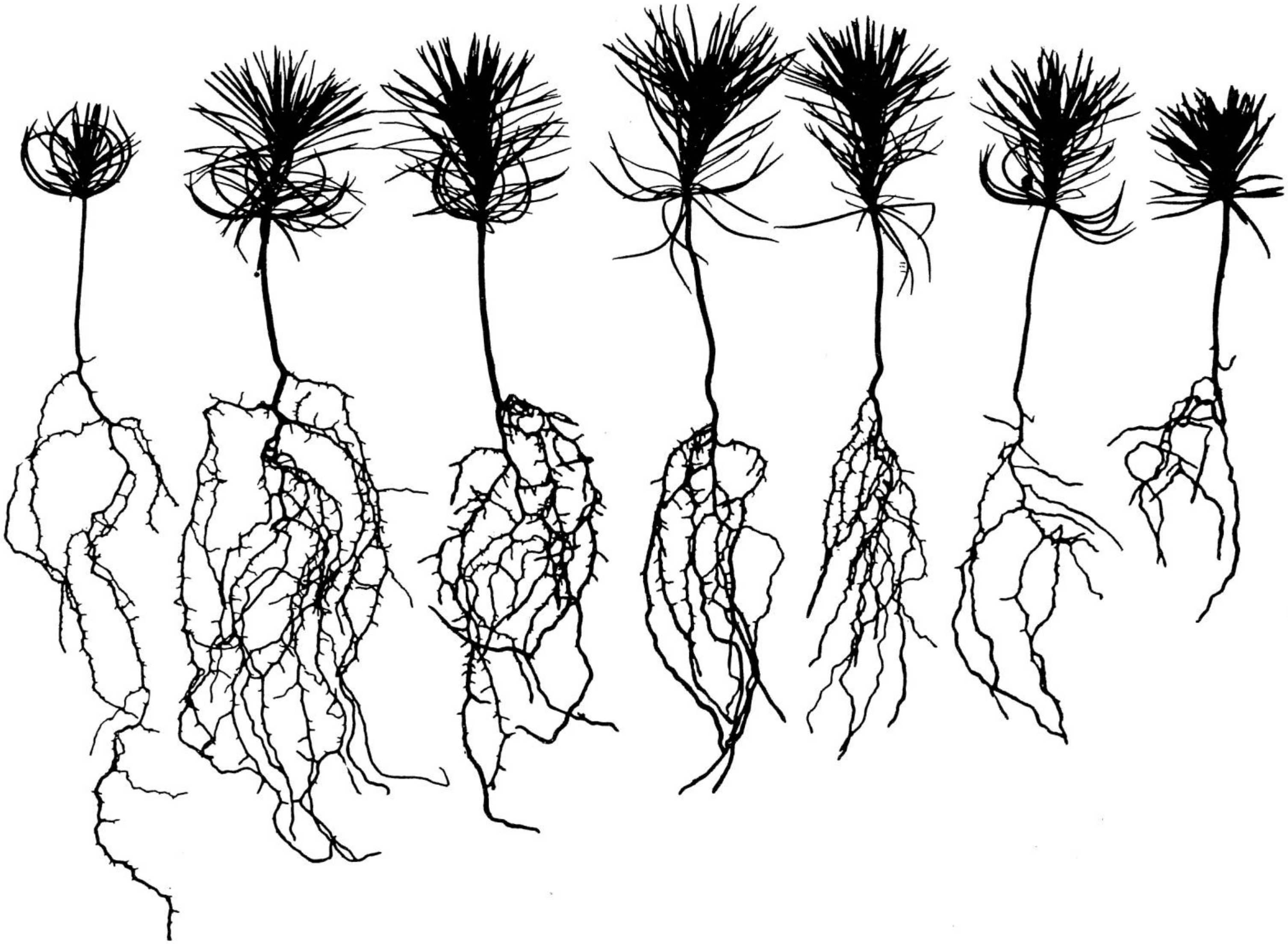

Plate XII. SILHOUETTES OF WHITE PINE SEEDLINGS GROWN IN SAND CULTURE WITH VARIED NITROGEN SUPPLY. LEFT TO RIGHT, p.p.m. N: 0, 88.3, 425.9, 838.9, 1239.0, 2077.9, 2490.8.

Photo by A. B. Hatch.

the root systems are proportionately small. Thus one mechanism which serves to increase or decrease absorption, depending on whether internal nutrient concentrations are low or high respectively, is variation in the absorbing surface area of root systems.

The question now arises as to whether short-roots respond, as do long-roots, to changes in nutrient environments by increase or decrease of surface area. In Plate XII, a progressive decrease in the number of short-roots per unit length of long-root is apparent in seedlings grown on the higher concentrations of nitrogen. A similar relationship was found for the elements, phosphorous, potassium, and calcium (Mitchell, 1934). At the highest concentrations (Plate XIII, A) in some cases not a single short-root appears (a fact which removes all doubt about the ability of long-roots to function in nutrient absorption, a question raised by Hatch and Doak in 1933). Decrease in the number of short-roots produced per unit length of long-root also occurs in soil culture seedlings. Analysis of Melin's (1927) data, for example, reveals a marked, although variable, decrease of these roots with increase of nitrogen mobilization of the soil (see column SR/LR, Table II). A far more striking demonstration of this relationship is obtained when the differences in the nutrient environment are large. In the fertilizer-cartridge experiments, for example, the number of short-roots per 10 cm. of long-roots decreased from 50 to 18 with increase in available nutrients (Table VII). The effect of these changes are the same as those which occur in long-roots, namely, reduction of ''absorbing'' surfaces under conditions of high nutrient availability.

Of much greater magnitude are the changes in the surface areas of short-roots, which are directly related to the character of fungus infection. In very fertile soils where mycorrhizae are not produced, or in soils or sand cultures where mycorrhizal fungi are absent or their normal growth inhibited, the type of short-root illus-

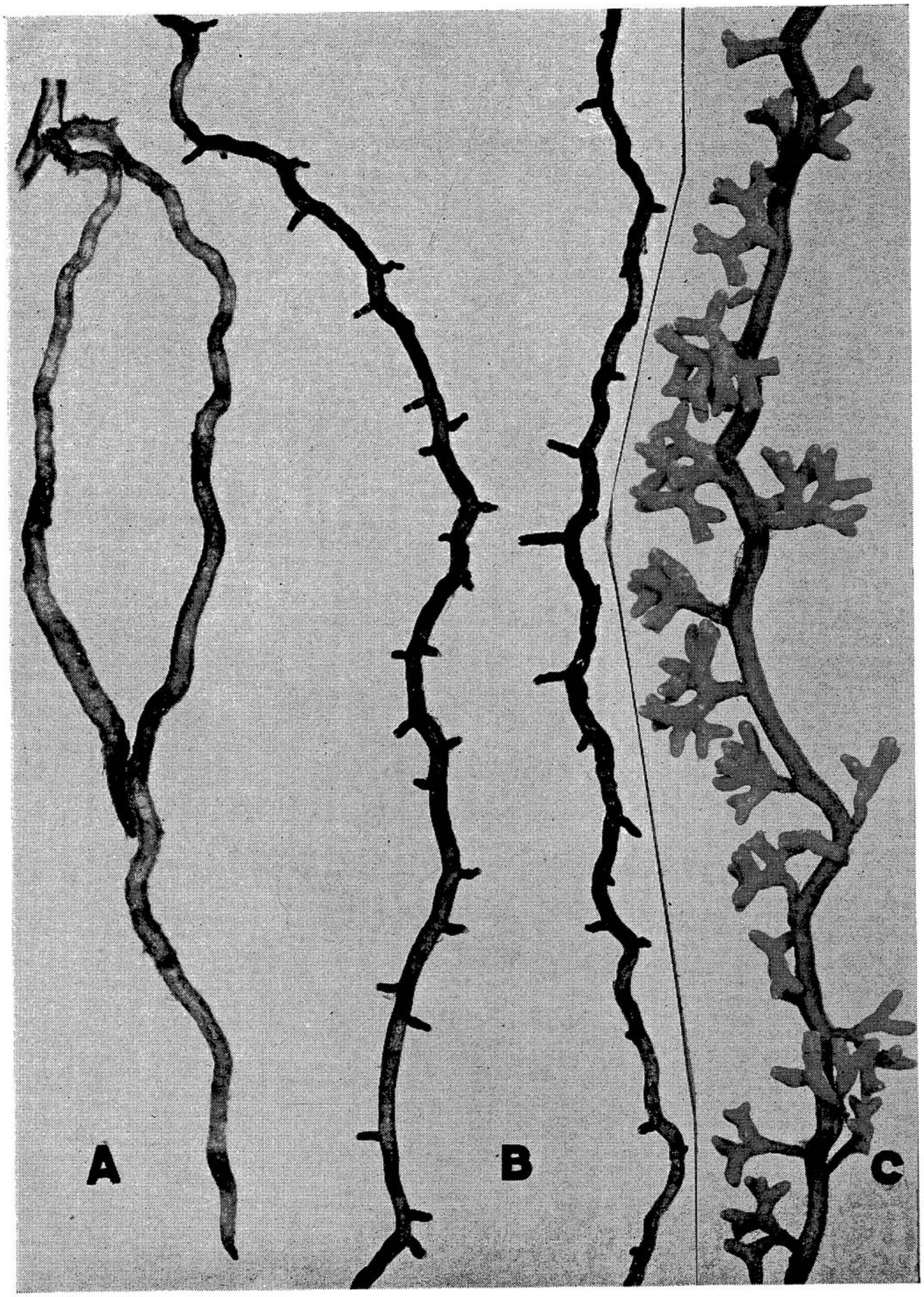

PLATE XIII. ILLUSTRATING THE RELATION BETWEEN THE ROOTING ENVIRONMENT AND SHORT-ROOT DEVELOPMENT IN SEEDLINGS RAISED IN BLACK ROCK FOREST EXPERIMENTS (X 4).

A. Long-roots lacking short-roots under conditions of high nitrogen availability (Sand culture experiments of Mitchell, 1934). B. Average development of short-roots in low nitrogen availabilities (Sand culture experiments of Mitchell, 1934). C. Short-root development in Ridge soil, Black Rock Forest, when invaded by mycorrhizal fungi. Non-suberized surface area many times as great as in B.

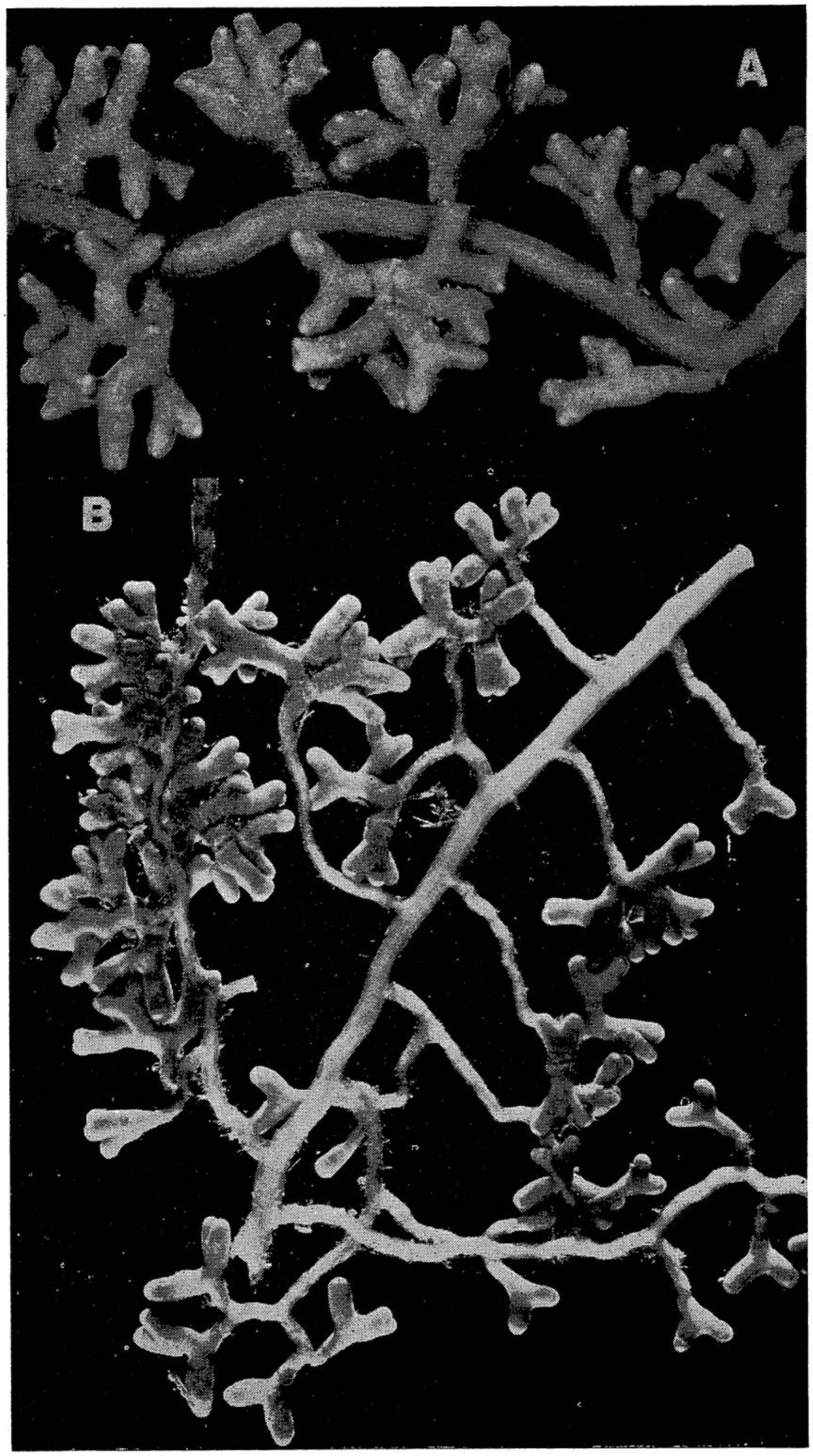

PLATE XIV. SHORT-ROOT DEVELOPMENT IN PRESENCE OF MYCORRHIZAL FUNGI IN SOIL.

A. Infected during emergence from mother long-roots; Ridge soil, Black Rock Forest (x 9). B. Infected after short-roots had attained an extraordinary length; Harvard Forest soil (x 5).

trated in Plate III, A, B, and F, and Plate XIII, B is produced. The total lengths of these non-mycorrhizal roots seldom exceed 2-3 mm. They are likewise suberized to within a few microns of their tips (Plate III, F). Their effective absorbing surfaces are therefore wholly negligible. Very probably the early termination of growth in these non-mycorrhizal short-roots is brought about in part by infection by casual soil organisms (Melin, 1925). In pure culture, where the influence of such infecting organisms is lacking, short-roots may grow to considerable lengths. Their continued growth with age is shown for pure culture materials in Plate III, G. This Plate III, G, also shows dichotomous branching, a process which occurs in the absence of infection only under special nutrient conditions, such, for example, as where nucleic acid is a source of nitrogen (see also Melin, 1925). In extremely rare cases the writer has encountered uninfected and even more rarely dichotomized short-roots in soil cultures. An example of such roots which, however, were attacked by a mycorrhizal fungus after they had attained an extraordinary length, is shown in Plate XIV, B. But these examples are found only in pure culture, rarely in soil cultures, and probably never in nature. Absorption by non-mycorrhizal short-roots, therefore, can not play a very significant rôle, if any, in nutrient acquisition.

When short-roots become mycorrhizal very large increases in their surface areas occur as follows:

(1) by continued elongation with increased age (Plate III, H) during one growing season (usually but not always);

(2) by multiple tip development resulting from dichotomous branching (Plates XIII, C; XIV, A and B; XV, A and B);

(3) by increase in the diameter of the root (Plate XV,

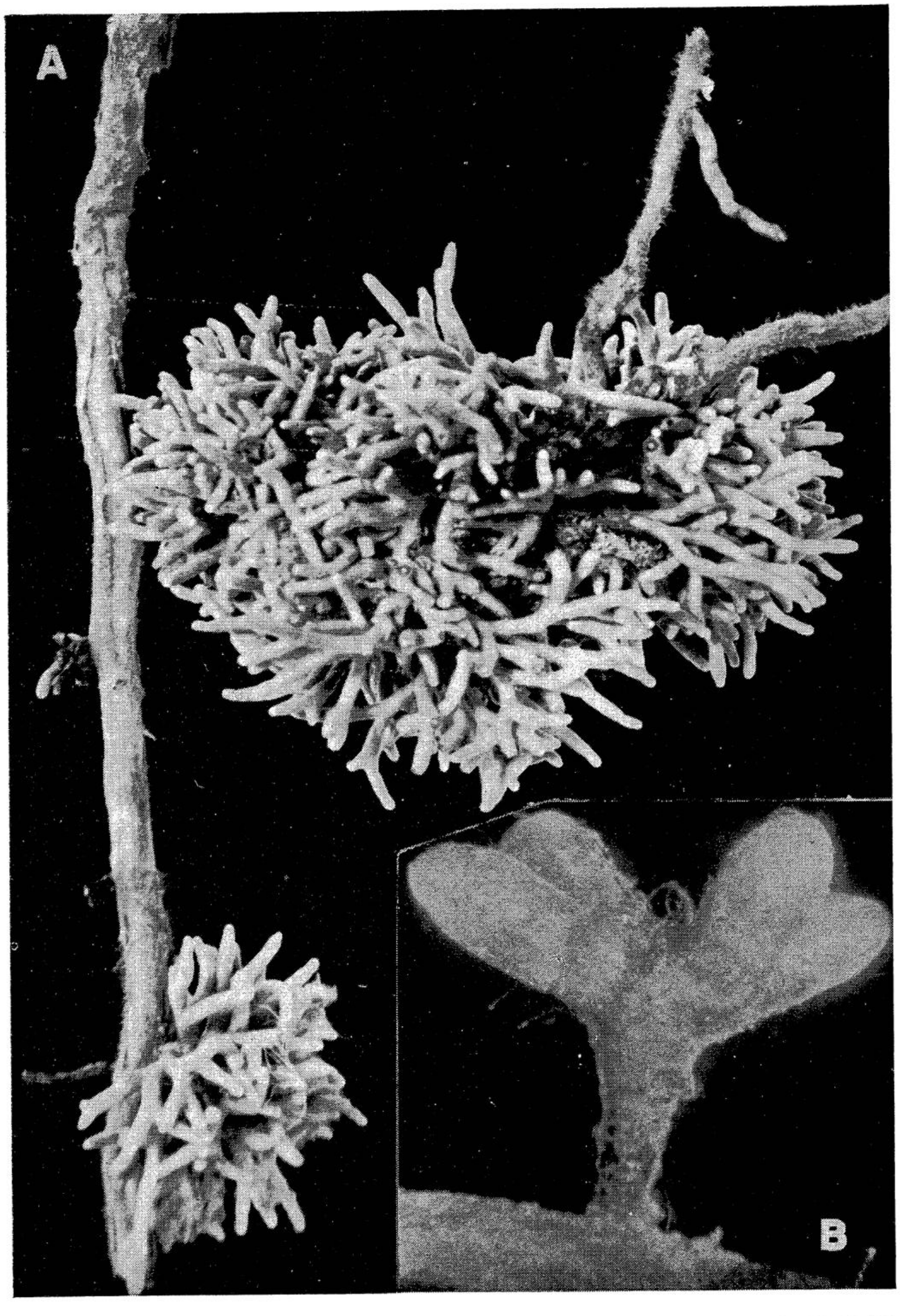

PLATE XV. INFLUENCE OF MYCORRHIZAL FUNGI ON SHORT-ROOT DEVELOPMENT OF PINE.

A. Larger clump consists of but 4 short-roots; lower clump of but one short-root (x 3.3). Compare for surface area with non-mycorrhizal short-roots in Plates III and XIII. Collected by P. R. Gast, Harvard Forest. *Photo by A. W. Nelson and the writer.* B. One short-root (x 29) which became infected some time after emergence from the mother long-root, showing increase in diameter of root and dichotomous forking which follows infection. (*Pinus sylvestris,* Sweden).

B), and therefore in its surface area (Lecomte, 1887; Hatch and Doak, 1933);

(4) by acquisition of the surfaces of fungal hyphae either
  (a) as a parenchyma-like mantle (Plate I, B),
  (b) as individual hyphae which pass directly into the soil from the mantle (Plate IV), or
  (c) as absorbing hyphae located at long distances from the short-roots but connected to them by rhizomorphs (Plate I, A); and

(5) by the action of the fungus in delaying suberization of the endodermis and cortex, thereby keeping the cortex of the entire root alive and active often throughout an entire growing season (Aldrich-Blake, 1930; Laing, 1932).

Attainment of the secondary condition in mycorrhizae, as in long-roots (Aldrich-Blake, 1930, p. 27), may be readily recognized from external appearances. Deposition of suberin in the endodermis and cortical cells cuts off the cortical cells from the conduction tissues and leads to their death. When this occurs they turn brown or reddish, (Plate III, F, shows this for non-mycorrhizal short-roots). The cortex of mycorrhizae frequently remains alive and active over the entire length of the short-root for a period, in some cases, of at least 4 months. Such an extreme active age is not attained by the cortex of any other root.

In diagrammatic form these relationships are shown in Plate XVI. All areas which are solid black represent cortical regions which are still active (in the primary condition) because of the absence of complete suberization of the endodermis and cortex. Inactive suberized cortex (in the secondary condition) and cortex cut off by the formation of the cambium (in the long-root only) are represented by ordinary lines. On the lower surface of the mother root the development of non-mycorrhizal short-

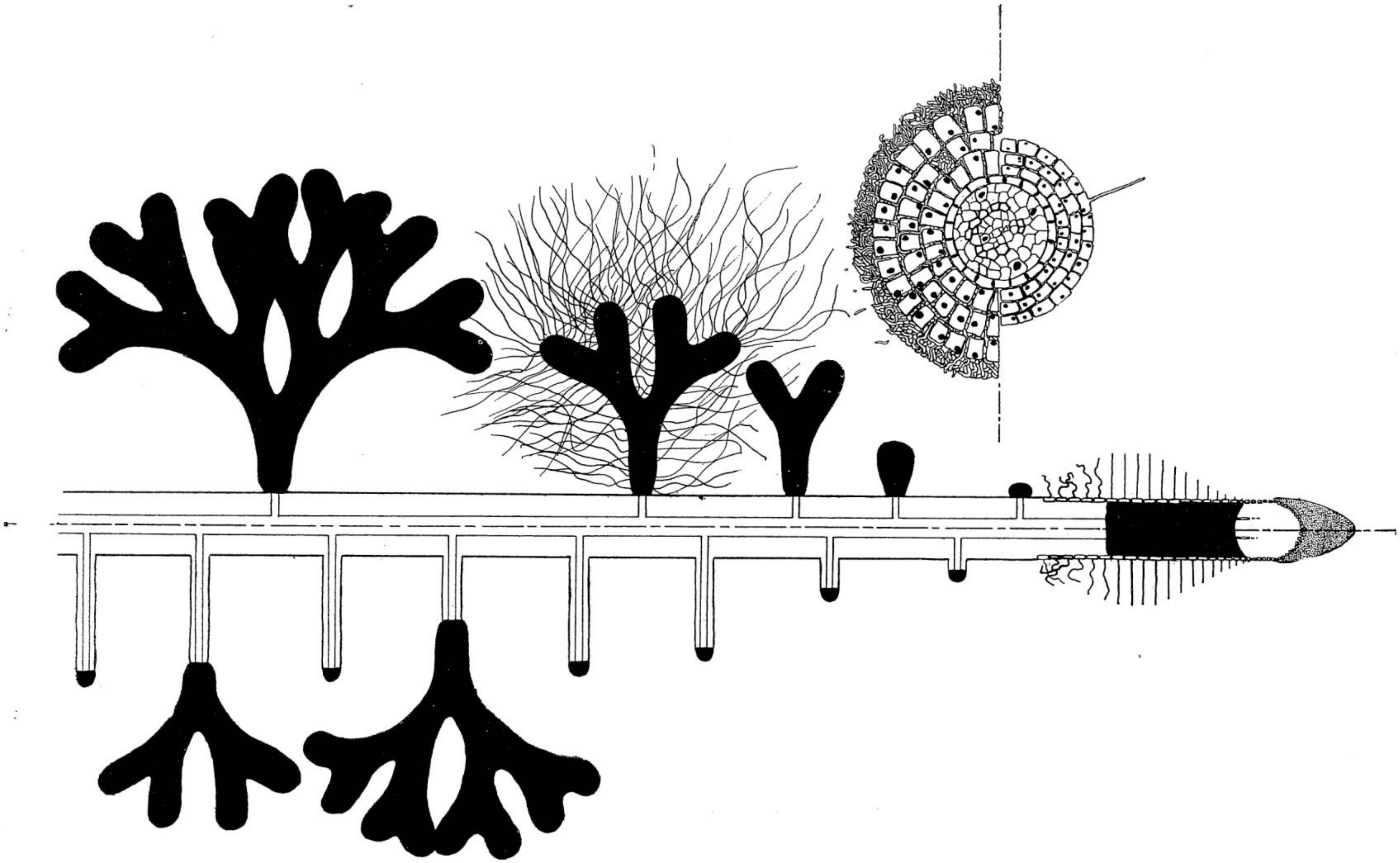

Plate XVI. DIAGRAMMATIC REPRESENTATION OF THE INFLUENCE ON SHORT-ROOT GROWTH OF INFECTION BY MYCORRHIZAL FUNGI.

Solid black represents absorbing surfaces. Explanation in text.

roots is indicated. Only their tips with relatively insignificant surface areas are in the primary condition (Plate III, F), and their growth terminates early. In two cases the stimulated development of such short-roots following infection by a mycorrhizal fungus is represented (see also Plate XV, B). On the upper surface of the mother root the development of short-roots which become mycorrhizal as they emerge from the cortex of the mother root is represented. A diagrammatic cross-section through monarch short-roots, half of which is represented as mycorrhizal and half as uninfected (pure culture), is also shown. The increase in diameter is the result of hypertrophy of cortical cells and of the presence of the mycelium. The hyphae extending out into the soil from one of the mycorrhizae are typical of those produced by *M. r. nigrostrigosum* (Plate IV; Rayner, Plate XII, Figs. 1, 2, and 5). Every feature represented in the diagram can be found in the actual photographs of root systems reproduced in this paper and in the papers of Hatch and Doak (1933), and Hatch and Hatch (1933).

Still another factor which is undoubtedly of significance in determining the efficiency of mycorrhizal and non-mycorrhizal roots is the vastly different surface/volume ratios of roots and of fungus hyphae. It is well known that the concentration of nutrients within plants is closely related to the magnitude of their absorbing surfaces (Åslander, 1931, 1933). It follows that surface/volume ratios determine in large part the concentration of cell contents, and this should be true of fungi as well as of vascular plants. The surface/volume ratio of a 2 micron hypha is approximately a thousand times greater than that of a 2 mm. root, and this comparison does not take into account the volume of any part of the vascular plant except absorbing roots! Fungus mycelium should be far richer in nutrients per unit volume than plant roots, and actual measurements confirm this suggestion

(Stahl, 1900). To the writer this is the crux of the mycorrhizal problem.

Thus, while actual comparative data on the rates of absorption by individual mycorrhizal and non-mycorhizal short-roots are lacking, it is clear that in soils short-roots acquire an appreciable surface area only by becoming mycorrhizal. Viewed from the vantage of this knowledge, which is contrary to all previous ideas on the influence of mycorrhhizal fungi on root development, mycotrophy in *Pinus* assumes a new significance; a significance which is based primarily on physical relationships.

IV. DISCUSSION

IN THE ancient Indian fable of the blind men's description of an elephant, the futility of basing conclusions on isolated observations is vividly portrayed. Few fields of biology today offer more striking examples of this ancient proverb than that of mycorrhizae. Scrutinized by themselves, few of the numerous data assembled in this paper yield any clue to the true nature of the mycorrhizal habit. Together they provide the bold outline for a picture of mycotrophy which is apparently capable of explaining all known peculiarities of the habit.

There can no longer be any doubt that mycotrophy in pine, and all other trees which form ectotrophic mycorrhizae, is widespread and habitual. It represents the normal destiny of *all* short-roots of trees growing in forest and many other soils; and short-roots represent over 95 per cent of all root tips in pine. It is inconceivable that a parasitic relationship of such general occurrence, which isolates from its source of nutrients such a high percentage of root tips, should be without significance in nutrient acquisition.

Such a belief is the basis of many theories. The problem of plant life not infrequently centers in deficiencies of nitrogen, and symbiotic solutions (bacterial root-nodules) are well known (Rayner, 1927). Students of mycorrhizae, casting about for an obvious need of plants for accessory nutrient-acquiring organs, have naturally focused their attention upon this element. But the evidence assembled in this paper proves conclusively that mycotrophy, in plants with ectotrophic mycorrhizae, is not a special adaptation for acquiring nitrogen. I am by no means the first to obtain experimental evidence contrary to the organic nitrogen theory. But lacking the wealth of data which are at my disposal, or in the absence of this data the extraordinary perception of a Stahl, students have been at a loss to account for conflicting experiments and observations.

The possibility that nutrient acquisition by plant roots may be far more of a problem than we are want to suspect, indeed that this problem may become so difficult in infertile soils that plants are wholly dependent upon symbiotic soil fungi, has escaped nearly all students but Ernst Stahl (1900).

The Stahlian theory has as its foundation an extensive correlation study of the influence of soil fertility upon the mycorrhizal development of plant roots. It included plants in almost all sections of the vegetable kingdom. As a result Stahl and many others have demonstrated that mycorrhizal development is inversely proportional to soil fertility. I have confirmed the existence of this relationship in pine by repeated quantitative experiments and extended it to include availabilities of individual elements (phosphorous, potassium, calcium, and nitrogen).

With this relationship as a background Stahl built his theory around a number of assumptions which many students have questioned. (a) He believed that in infertile soils competition becomes severe between plant roots and soil fungi for the nutrients essential to the metabolism of both. In this struggle fungi were assumed to have a great advantage. The truth of this assumption has been confirmed by many subsequent investigators (see Waksman, 1932). (b) Stahl assumed that mycorrhizae, because of this superiority of fungi, are more efficient than non-mycorrhizal roots in absorbing mineral salts from infertile substrates. Proof of the correctness of this assumption for coniferous seedlings has been provided in this paper (also Hatch, 1936) and by Young (1936) and recently by Mitchell (1937). (c) To account for differences observed in the mycorrhizal developments of different species of plants growing in the same soil Stahl assumed that plants differ in respect to the extensiveness of their root systems and in the rapidity of their transpiration currents (it being further assumed that nutrient intake is proportional to water intake). He actually found that plants with extensive roots and (or) rapid transpiration streams tended to be autotrophic, while the possession of restricted root systems and slow transpiration streams favored mycorrhizal development. I have observed that seedlings of *Pinus insularis,* which possess relatively extensive root systems, have considerably fewer mycorrhizae than *Pinus Strobus* seedlings, which have much more restricted root systems, when the two pines are grown together in one soil. A few months ago Hitchcock and Zimmerman (1936) demonstrated that the absorption of water and of molecules dissolved in it is directly proportional to the rapidity of the transpiration current. *There remains not a single serious criticism of the mineral salt theory which has not been logically refuted.*

In the writer's opinion, therefore, the Stahlian theory is by far the most accurate picture of mycotrophy in pine that has yet appeared. My own interpretation differs from Stahl's only in that it goes farther and attempts to explain in greater detail the mechanics of the superiority of mycorrhizal roots in nutrient absorption.

V. INTERPRETATION[1]

Nutrient acquisition in pine is accomplished in two fundamentally different ways, depending on nutrient availability.

In the most productive agricultural soils, in which fertility is artificially maintained, absorption occurs through the root hairs of long-roots in the classical manner portrayed in botanical texts. The problem of nutrient acquisition is extremely simple because roots have merely to absorb from the soil solution the nutrient elements which are actively bombarding the plasmic membranes of absorbing cells. Short-roots are here of little significance because of their extremely limited size.

This picture changes in infertile soils because as soils become progressively less fertile there are correspondingly fewer readily available nutrients in the soil solution. Instead, an increasing percentage of these nutrients are adsorbed to colloids and other base-exchange materials or are still contained in undissolved rocks. Under these conditions long-roots, although more extensive, are less efficient. Eventually, in soils which we are accustomed to regard as of average fertility, a point is reached where long-roots are wholly inadequate, and here in the absence of mycorrhizal fungi trees perish.

In progressing from highly fertile to infertile soils, but in the presence of mycorrhizal fungi, there first appear a few short-roots which have become mycorrhizal. These are small and unbranched. By the time soils of average

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[1] Written with a tone of finality, this section represents merely a personal opinion and like those of my predecessors subject to gross uncertainties. It is provided in the belief that it will prove a more useful working hypothesis for further experimentation than other contemporary theories. (*Author, June, 1937*).

fertility are reached all short-roots have become mycorrhizal and most of them are profusely branched. In many their surface areas are further increased by mycelium which radiates from these mycorrhizae into the surrounding soil and in some mycorrhizae rhizomorphs connect them to fungal mycelia which permeate a large volume of soil.

Finally in infertile soils the entire nutrition of the tree is accomplished by the short-roots; the long-roots serve merely to extend the root systems into new soil and to bear short-roots. These short-roots often expose several hundred times as much absorbing surface as the non-mycorrhizal short-roots grown in fertile soils. Their cortical cells possess no contact with soil. All nutrients and water which reach their conducting elements must be absorbed by fungus mycelium, pass through a compact parenchyma-like fungal mantle, and though or around cortical cells which are wholly isolated from each other by fungus mycelium.

The concentration of salts within the cells of the pine roots is higher, as a result of absorption by mycorrhizal short-roots, than in the soil solution. Some nutrients are actually lost to the soil by the long-roots. On the other hand, the concentration of salts in the fungal hyphae is greater than in the root cells and much greater than in the soil solution. In mycorrhizae osmotic diffusion is from the mycelium toward the conducting elements of the root. Fungi are highly efficient in acquiring these nutrients because of the vast numbers of their hyphae, their tremendous surface/volume ratios, their intimate contacts with mineral bearing rock, soil colloids, and other base-exchange materials, and because of their ability to decompose and utilize the inorganic materials from humus. These attributes enable them to thrive in infertile soils where trees, lacking mycorrhizae, perish by starvation.

The utility of the relationship is based on direct osmotic acquisition by roots of nutrients from fungus mycelium and (or) on digestion of the fungus hyphae within cortical cells. The proposal by Burges (1936) that the fungus liberates the nutrients into the soil and the tree roots acquire these despite rather than because of the fungus is wholly contrary to the facts. This is the rôle of the vast multitude of non-symbiotic soil fungi whose activities enable tree growth in the absence of mycorrhizae only in fertile soils. In infertile soils soil fungi utilize all the nutrients which they liberate from complex soil materials and as a result trees starve. By *symbiosis* with these efficient mycorrhizal fungi, which are on a nearly equal footing with other soil organisms in competing for nutrients, trees are capable of existence in infertile substrates.

Mycotrophy in pine represents a symbiotic mode of nutrition in which the advantages of two distinct modes of life are pooled with mutual benefit. The vascular plant parasitizes mycorrhizal fungi for water (Cromer, 1935), soil nutrients (Mitchell, 1937), and even for carbohydrates (Falck, 1923; Francke, 1934). The fungus parasitizes tree roots chiefly (probably) for complex materials in the nature of growth-promoting substances (Melin, 1925) and possibly, under some conditions, for more simple carbohydrates. In both partners damages undoubtedly result but usually the advantages of reciprocal parasitism far outweigh these damages. In the absence of either partner the other is incapable of existence in all but the most favorable and artificially maintained environments where the problem of life is extremely simple.

VI. SIGNIFICANCE IN FORESTRY

The writer believes that several conclusions fundamental to forestation are made obvious by perusal of this paper.

Firstly, every afforestation project involving the establishment of members of the Abietineae, Fagaceae, Betulaceae, Salicaceae, the genus Hickora, and a few other trees by seeds in new nurseries is predestined to failure unless provision is made for the introduction of suitable mycorrhizal fungi along with the trees. The almost complete failure of the early pioneers to establish trees from seeds in the American prairies may be attributed primarily to the lack of these fungi in prairie regions. The Timber Culture Act of Congress in 1873 was made futile more by man's ignorance of the mechanics of tree nutrition than by the inability of trees to thrive in prairie regions. It need hardly be pointed out that the commendable measure recently adopted for excluding pathogens from new nurseries established in the American prairies, namely, that of raising all stock from seeds, at the same time dooms the nurseries in which these fungi are not already present.

Secondly, in the reforestation of long denuded forest lands the same provision—introduction of mycorrhizal fungi—-is apparently necessary to success. The mycorrhizal fungi of trees disappear from soils with the removal of their suscepts.

Thirdly, the direct seeding methods of re- and afforestation are similarly predestined to failure except where provision is made for the introduction of mycorrhizal fungi (Samual, 1926) or except on areas so recently logged that these fungi still exist in the soil (Steven, 1934).

Fourthly, a large percentage of planting failures, the world over, are probably due to the possession by nursery-raised seedlings of mycorrhizae which are not capable of surviving in the sites in which the seedlings are planted. Mycorrhizal fungi are often more exacting in their site requirements than are the trees with which they are associated (Romell, 1930). The fungal symbionts of the seedlings raised in favorable nursery environments may be wholly incapable of survival in the soil of a ridge top. Death of the fungus assures death of the seedling as well, unless other mycorrhizal fungi capable of existing in the soil are present. In the latter event death or retarded growth of the seedlings may occur during the period of conversion from one type of mycorrhizal fungus to the other.

The science of forestation is apparently still in its infancy and it will probably remain so until our knowledge of tree nutrition, of mycotrophy, and of the influence of environment upon the survival of root symbionts has been widely explored. *The obvious need today is for precise information on the influence of different species of mycorrhizal fungi upon the growth of our most important trees planted in a wide variety of habitats.* The problem, therefore, is not one which may be solved by one or even several individuals. It requires local and international study by many organizations engaged in forestation. At the present time the Governments of Great Britain, Australia, and the Netherlands' East Indies are alone engaged in such programs. It may require many years for them to gain the information necessary to achieve the greatest benefits, but this knowledge once gained may prove more useful in forestry than has a similar knowledge of the root nodule habit of legumes in agriculture.

VII. SUMMARY

1. For a decade serious nursery failures in widely separated continents have resulted from the lack in specific soils of a biological factor believed to be mycorrhizal fungi. Since the failures occurred in characteristically mineral rather than in humus soils, questions have arisen as to the correctness of the organic nitrogen theory. Furthermore, the probability is attractive that a more intimate knowledge of mycorrhizae might lead to applications in forestry which parallel those found for nodule bacteria in agriculture.

2. A careful review of the literature, pursued with the object of summarizing observational and experimental data rather than theories, revealed three significant facts which are not generally appreciated.

A. Without realizing their dual nature early foresters regularly encountered and widely regarded as the normal absorbing organs of trees structures which we now know to be mycorrhizal roots. Indeed the original (1840-1851) bases for the modern system of classifying tree roots into *short*- and *long*-roots, were differences which today are recognized as those which separate respectively mycorrhizal and non-mycorrhizal roots.

B. The belief, although erroneous, is general today that controversy has long existed on the prevalence of the mycorrhizal habit in trees. This belief has gained wide acceptance largely because (1) we have not segregated statements in the literature which represent actual field observations from those which are merely opinions, and because (2) our concepts of root structure and morphology have been inaccurate. The truth is that one observer only (McDougall, 1914-1928) has repeatedly maintained, on the basis of field observations, that mycorrhizae are infrequent structures in trees. The methods employed in collecting data in this case were largely indirect and they involved assumptions which are wholly erroneous. All other persistent observers, that is, all who have published more than one account of field observations, report essentially the same finding, namely, that mycotrophy in forest-grown trees is widespread and habitual.

C. The experiments of Melin reported in 1927 and of several earlier workers yielded results which are contrary to those predicted by the organic nitrogen theory. A satisfactory explanation of the known peculiarities of mycorrhizal distribution has not appeared subsequently.

3. Four methods were selected for investigating the mycorrhizal relationship:

A. The soil culture method, with forest soils, for examining the influence of the nutrient environment on the development of mycorrhizae and on seedling growth.

B. The soil culture method, with prairie soil, for determining the effect of introduced pure cultures of mycorrhizal fungi on the growth of seedlings in a soil environment.

C. The pure culture method for discovering which fungi produce mycorrhizae and for quantitative physiological studies on the utilization of organic nitrogen and mineral salts by mycorrhizal and non-mycorrhizal plants.

D. Comparative studies of the morphology and anatomy of mycorrhizal and non-mycorrhizal root systems.

4. Soil culture experiments revealed that the abundance of mycorrhizae on the roots of pine seedlings is determined in normal forest soils by the availability of mineral salts. They were produced in abundance under conditions of low availability of any one or more than one of four elements—nitrogen, phosphorous, potassium, and calcium—or of lack of balance in the availability of these elements.

5. These results supply the first quantitative experimental demonstration of the correctness, in plants possessing ectotrophic mycorrhizae, of the observations on which Stahl in 1900 built the mineral salt theory to account for mycotrophy throughout the plant kingdom. They suggest a completely satisfactory explanation of the observed facts of distribution in nature, namely: except as saturation, excessive dryness, or unfavorable acidities of soils affect the ability of mycorrhizal fungi to survive, the distribution of ectotrophic mycorrhizae in nature is determined by the availabilities of nutrient elements.

6. The susceptibility of short-roots to infection by mycorrhizal fungi in soil and in pure culture experiments is apparently controlled by the concentration of nutrient elements within the two symbionts. The combination of low or unbalanced concentrations in the vascular plants and high nutrient concentrations in the fungus lead to susceptibility. Such a combination of internal conditions in the two symbionts appears typically in soils characterized by soil solutions with low concentrations of nutrient ions. Such soils may be infertile, or high in unsaturated base-exchange materials, or they may contain difficulty soluble nutrients.

7. When comparisons were made between size and mycorrhizal developments of seedlings raised in *different nutrient environments,* it was found that in general (that is, except where soil conditions inhibited the growth of mycorrhizal fungi) the smallest and least thrifty seedlings were most abundantly mycorrhizal. The differences in size of seedlings, however, could be attributed to the known differences in the nutrient environments rather than to injury by the root fungi.

8. Correlation analyses with seedlings raised in *one nutrient environment* (but differing in mycorrhizal equipments) failed to reveal that mycorrhizae either increased or decreased growth. From these data it was

concluded that an answer to the question whether or not mycorrhizae increase plant growth can be obtained only in an environment where access to infection is the sole variable. Such an environment was obtained in a prairie soil.

9. Soil culture experiments with prairie soil, which lacked mycorrhizal fungi but in which pure cultures of mycorrhizal fungi were introduced in parallel series, provided a positive answer to this question. Seedlings lacking mycorrhizae were small and yellow and contained only minute quantities of phosphorous, potassium, and nitrogen. The percentage contents of two of these elements were lower than has hitherto been found in pine grown in any soil, however infertile. Mycorrhizal seedlings were large, dark green, and healthy, and contained normal quantities of mineral salts, ranging from 86 to 234 per cent more than was found in the non-mycorrhizal plants.

10. The prairie soil experiment, therefore, provides acceptable and quantitative evidence (a) that mycorrhizae are concerned with the absorption of mineral salts and (b) that pines are incapable of absorbing sufficient nutrients to maintain growth in some soils when mycorrhizal fungi are lacking.

11. For pure culture studies special apparatus and technique were devised whereby seedlings could be grown in quantity under optimum (sand culture) physical environments. The special features of this apparatus include facilities for constant aeration, change of nutrients, substrate aeration, and exposure to direct solar radiation while maintaining constant temperatures by partial or complete submergence in water.

12. Quantitative evidence was obtained in pure culture that pine seedlings are apparently capable of utilizing peptone with facility under the conditions of these experiments. A similar but qualitative result was recorded with nucleic acid. In both cases ammonium did not ap-

pear in the substrates and hence nucleic acid and peptone molecules were apparently absorbed by the intact pine roots.

13. In pure culture mycorrhizae were produced in quantity in a sand substrate which possessed appreciable base-exchange properties and which was rich in undissolved minerals (fluvio-glacial sand). Mycorrhizae were not produced in an inert sand substrate (silica sand) which possessed a low base-exchange capacity, which lacked insoluble minerals, or to which dissolved minerals were added.

14. Comparative study of the structure and morphology of mycorrhizal and non-mycorrhizal root systems of pine seedlings raised in pure and open sand cultures and in soil cultures, and of roots in nature, led to the discarding of the idea that infection decreases the absorption surface areas.

15. The ''absorbing'' surface area of short-roots is increased through infection by mycorrhizal fungi in five different ways as follows: (a) by continued elongation, (b) by increase in diameter, (c) by multiple tip development, (d) by increasing the life of the cortex through the action of the fungus in delaying suberization of the endodermis and cortex, and (3) by acquiring the surface areas of the mycorrhizal fungi either (1) as a parenchyma-like mantle, (2) as individual hyphae extending from the mantle into the soil, or (3) as mycelium connected to the mycorrhizae by rhizomorphs.

16. Integration of these numerous data and of the literature has led to the following tentative and in part theoretical conclusions (interpretations):

A. The mycotrophic relationship in pine, and presumably in other plants possessing ectotrophic mycorrhizae, is a symbiotic mechanism which increases, chiefly by physical and therefore by relatively non-selective means, the absorption of soil nutrients.

B. The greater absorption-capacity of mycorrhizal seedlings is brought about by, and is proportional to, increases in the effective absorbing surface areas of short-roots resulting from fungal invasion.

C. The magnitude of the surface area of short-roots, and therefore the relative importance of mycorrhizae in tree nutrition, is determined by the availability of soil nutrients.

D. In fertile soils characterized by luxuriant supplies of dissolved nutrients, mycorrhizae are rarely produced and long-root tips serve as the chief organs of absorption.

E. In infertile soils possessing few dissolved salts, where the nutrient elements are held in base-exchange compounds, mycorrhizal short-roots become numerous, their number and development being inversely proportional to fertility.

F. It follows that as the availability of nutrients decreases mycorrhizal short-roots take on an increasing share of seedling nutrition and eventually become the only organs of absorption.

G. The superior efficiency of fungi as compared with roots in extracting nutrients from insoluble rocks and from base-exchange materials is believed to center primarily in the fact that the ratio ''surface area/volume'' is vastly greater in fungi than in roots and only secondarily in the peculiar physiology of fungi which enables them to utilize complex organic materials.

H. The benefits which accrue to mycorrhizal fungi from association with tree roots are probably in the nature of growth-promoting substances, since in *Monotropa* carbohydrates as well as salts are obtained from the fungus.

I. Susceptibility to infection by mycorrhizal fungi is apparently controlled indirectly by the internal concentration of nutrient elements in short-roots.

J. Trees are dependent on symbiotic association with mycorrhizal fungi for their soil nutrients and therefore for their existence in all but the most fertile agricultural soils.

17. The probable significance of this knowledge in forestation is suggested.

VIII. COMMENT

THE phenomenon of mycorrhiza-formation is of outstanding scientific interest. It also has broad practical implications. Despite the fact that the mycorrhizae of forest trees have been studied for over fifty years the entire field of inquiry is characterized by confusion, controversy, and disagreement.

Great difficulty is encountered and a high degree of scholarship is required to reduce a mass of conflicting observations and hypotheses to a reasonable, clear-cut, workable philosophy or theory. Furthermore, many hazards inevitably are encountered in developing such a philosophy or theory. Sins of omission and of commission are easily made. Some observations must be discarded and rejected; other observations must be accepted. As Sir Thomas Browne remarked over two hundred and fifty years ago, ''To purchase a clear and warrantable body of truth we must forget and part with much we know.'' In this paper Dr. Hatch has discarded much and put forth a clear-cut and simple explanation of the mycorrhizal relationship in trees. When further research uncovers additional facts on the formation and function of tree mycorrhizae it may be necessary to modify somewhat his conclusions but in the present state of knowledge they appear justified and reasonable.

The Physical Basis of Mycotrophy in Pinus is a distinct forward step in a better understanding of tree mycorrhizae. The important literature on the subject has been reviewed critically and evaluated and a definite position is taken concerning the present status of the mycorrhizal problem. This in itself is a valuable contribution. To one who has followed the mycorrhizal literature more or less critically for a considerable number of years it has always appeared that most of such reviews ended largely as they began—in confusion.

The experimental work reported in this paper shows the inadequacy of the so-called organic nitrogen theory, the errors in the pathogenic theory, and strengthens and extends the so-called mineral salt theory first postulated by Stahl in 1900. Furthermore, the study has reversed completely earlier concepts on root morphology as influenced by mycorrhizal fungi. Instead of retarding development, it was found that mycorrhizal fungi stimulate root development. The greater efficiency of mycorrhizae (over root-hair-covered roots) is attributed to their much greater surface areas. This increase in absorption area is indeed concluded to be the physical basis of mycotrophy in Pinus. In brief, the experimental data show clearly that trees are dependent on symbiotic association with mycorrhizal fungi for several soil nutrients, and therefore for their existence, in all but the most fertile soils.

Dr. Hatch's results will influence greatly many silvicultural practices. More important still, they will serve as a definite point of departure for additional investigative work. *The Physical Basis of Mycotrophy in Pinus* clearly is an outstanding contribution to a better understanding of a highly important and exceedingly complex scientific problem.

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