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ORIGINAL PAPER

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# On the nutritional dependence of certain trees on root symbiosis with belowground fungi (an English translation of A.B. Frank's classic paper of 1885)

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To promote the possibility of truffle cultivation in the Kingdom of Prussia, His Excellency, the Minister of Agriculture, Domains and Forestry, commissioned me to approach the matter systematically. I was to begin with scientific studies on the conditions of occurrence and development of these fungi. Certain facts had already been established through observations and experience. For example, true truffles occur only with living trees, and in the Prussian truffle districts these investigations had established a strong relationship between truffle occurrence and particular tree species: beech, hornbeam and oak. Above all else was the union of *Elaphomyces* mycelium with pine roots, as recognized by Rees (Sitzungsber. D. physik.-med. Soc. zu Erlangen, 10 May 1880).

From the outset these facts pointed the research towards determining whether the true truffles also establish a connection of the mycelium with living tree roots. As this communication will show, the question must begin much farther back, because it presupposes knowledge about the nature and nutrition of plants not heretofore even slightly suspected by science. This shall be nearly the only topic of my present paper. It concerns the fact that certain tree species, above all the Cupuliferae, do not nourish themselves independently in the soil but regularly establish a symbiosis with fungal mycelium over their entire root system. This mycelium performs a "wet nurse" function and performs the entire nourishment of the tree from the soil. Surprising though this may sound, it is solidly based on the scope of my research.

When one examines feeder rootlets in the soil—the root system's end branches representing the actual organs of nutrient uptake—of any of our native oaks, beech, hornbeam, hazel or chestnut, it is evident they are generally composed of two disparate components: a core, representing the actual tree root, and an organically united mantle of fungal hyphae. This fungal mantle completely encloses the rootlet, forming a continuous cover even over the growing tip. It grows along with the root tip and behaves in every respect as an organically united, peripheral tissue belonging to the root. The entire structure is neither tree root nor fungus alone but resembles the lichen thallus, a union of two different organisms into a single, morphological organ. It can be appropriately designated as a "fungus-root" or "mycorrhiza."

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Translation from German of "Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durch unterirdische Pilze" from Berichte der Deutschen Botanischen Gesellschaft (1885) 3:128–145, revised from an earlier translation in Molina R (ed) Proceedings of the 6th North American Conference on Mycorrhizae (1985)
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Translator's note: This is not a literal translation, because the style of nineteenth century German scientific writing often does not translate comfortably into twenty-first century English. For example, the original German title of Frank's paper word-for-word in English would read, "About the on-root-symbiosis-depending nutrition of certain trees through underground fungi." In adjusting syntax in the translation, I have paid particular attention to preserving Frank's original intent and meaning. Frank used the family name "Cupuliferae," which was interpreted in the nineteenth century to include the present Betulaceae and Fagaceae, or sometimes only the Fagaceae, but has long since been discarded. Frank used it in this paper generally in reference to *Carpinus*, *Corylus*, *Castanea*, *Fagus* and *Quercus* spp.). In deriving the term "mycorrhiza" from the Greek μυκοριζα, Frank transliterated it with a single "r". Transliterating the Greek ρ (rho) into Latin letters ideally requires that the "r" be doubled and followed by an "h" in certain compound words to make the meaning and derivation clear, as discussed by W. T. Stearn [Botanical Latin, 4th Addition (1992) Timber Press, Portland, Ore., p 261). However, many authors, including Linnaeus and Frank, omitted the additional "r." For purposes of the science of mycorrhizae, it is trivial: whether with one "r" or two, our communication is equally effective. Similarly, whether one uses the Greek plural "mycorrhiza," the Latin plural "mycorrhizae," the English plural "mycorrhizas," or the plural endings of other languages is of no consequence. "Rhiza" is feminine in Greek; Frank retained that gender in German, using German feminine case endings. To reflect his usage, I have used the English rather than Latinized possessive and plural endings in this translation.

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## Structure of the mycorrhiza

In surface view the mycorrhiza resembles many fungal sclerotia in fine structure. It shows a pseudoparenchyma constructed of irregularly and very tightly interwoven hyphae, the cells ranging by diameter or length about 2.4–100 μm (Fig. 3). The cell walls are relatively thin, sometimes nearly colorless, and other times bright or dark brown, so that the mycorrhiza appears bright to brownish or nearly black. The pseudoparenchyma is usually multi-layered, forming a rather thick mantle as seen in cross- or longitudinal section. This mantle does not simply lie intimately on the root epidermis; hyphae from it penetrate between the epidermal cells of the root itself (Fig. 6). The cortex, including the epidermis, generally consists of about four cell layers. The epidermis and the subepidermal layer, or only the former, are composed of relatively broad, radially expanded cells; as a rule only the epidermis is intergrown with hyphae. The inner cortical cells always remain free. I could never detect hyphae in the endodermis or fibrovascular bundles. These endophytic hyphae always grow only at the cell wall surface. They never enter the cell lumen but completely weave around all sides of most cells. The hyphae are only 1.2–2.4 μm in diameter, considerably thinner than those of the fungal mantle but clearly recognizable as a continuation of the mantle. The thin walls of the peripheral root cells restrict their growth to a small diameter. Accordingly, one sees them most clearly in the surface view of the cell walls. Because they are normally arranged in a tight, almost pseudoparenchymatous layer, they seem at first to be a delicate reticulation of the root cell wall. Every cross-section through the cell wall, however, demonstrates that this structure originates from the intercellular hyphae appressed to the cell wall.

The mycorrhiza surface is often perfectly smooth, so that the fungal mantle is sharply differentiated from its surroundings (Fig. 3). The tightly appressed fungal mantle prevents formation of root hairs: I have never observed them. However, root hairs are often replaced by similar structures of the fungal mantle: its superficial cells extend as filaments that elongate by tip growth to spread among the surrounding soil particles. The manner in which this occurs varies greatly. Sometimes the mycorrhiza is irregularly clad with a thick, loose felt of bright or pale brown filaments similar in diameter to those of the mantle but varying between specimens from 1.2 to 3.6 μm in diameter. These meander back and forth, generally spreading among the soil particles (Fig. 4). Often they are enlarged where attached to small soil particles in the manner of lichen rhizoids or plant root hairs. Sometimes a multitude of long, straight, robust hyphae, brown or concolorous with the fungal mantle or blacker, extend into the soil, so that the mycorrhizas seem to have a broom or tuft of hairs at their tips when suspended in water. Sometimes the hyphae coalesce here and there into strands ranging from a few hyphae to forming rhizomorphs almost as thick as the mycorrhiza itself (Fig. 7). Without careful examination, these can be easily confused with the mycorrhizas themselves. The hyphae of these rhizomorphs, commonly corresponding in color and size to those of the mycorrhizal mantle, often emanate from the rhizomorph surface into the surrounding soil as numerous, individual filaments. In the truffle districts, they customarily abound in the soil, especially near a fruiting truffle. They form a system of innumerable branches and anastomoses spreading through the soil. Their connection with the mycorrhizas of any Cupuliferae present in the soil is easily confirmed.

## Development of the mycorrhiza

Longitudinal sections show that the fungal mantle continues to the tip of the mycorrhiza and likewise completely encloses it. The core of the tip elongates through a clearly developed growing point, thus showing all characteristics of a true root (Fig. 5). Therefore it must be assumed that the fungal mantle can expand in pace with the elongation of the root that it encloses. Indeed, it has its growing region where the growing point and zone of elongation of the root lie. The cells that comprise the fungal mantle over the root tip are always much smaller than those farther back, where the root is no longer elongating, i.e. 0.8–2.4 μm broad and up to about 5 μm long. They gradually grade to the larger cells farther back from the tip (Fig. 5). The fungal mantle thus enlarges by the continuing insertion of new hyphae between the existing ones at the tip of the mycorrhiza, and the cells of the pseudoparenchyma thus constructed broaden to their final size. Both parts of the mycorrhiza keep up with each other in this growth pattern, so that the fungal mantle always compactly overlies the growing root tip.

Growth of hyphae into the epidermis first occurs where length growth of the rootlet has stopped, not in its growing region. Progressing from younger to older regions in longitudinal sections, one can clearly follow the gradual penetration of the endophytic hyphae from the surface of the epidermis.

In terms of growth phenomena, we differentiate the tissues common to all root tips: apical meristem, procambium, protoderm, and root cap. And, we see how these meristems change to the usual permanent tissues of the root: the central fibrovascular cylinder, the root cortex, and the epidermis. The classification of these meristems follows the types that apply to most dicotyledons, as discussed in de Bary's *Comparative Anatomy*, where the root apex with a sharply differentiated apical meristem and procambium is covered by a common layer of initials for the protoderm and root cap (Fig. 5).

The weak development of the root cap is remarkable: often at a given time only a single cell layer is present, while as soon as the next one begins to split off from the protoderm, the layer becomes disorganized. Remnants of the older cap layers can often be recognized as thin, brown masses compressed by the surrounding fungal mantle and soon becoming unrecognizable. Reduced root cap formation may be understandable in this case, because the fungal mantle occupies the space needed for root cap development and necessarily replaces the root cap. Still, it is interesting to see that the inherent histological differentiation of the root remains intact despite the symbiosis.

How the mycorrhiza develops in the soil with the young plant is another question. Naturally, the fungi do not colonize the radicle of the embryo in the seed. The tap root of the first stage of germination is also free of fungus. The tap root soon develops lateral roots, which remain rather slender and form numerous short, repeatedly branched, feeder rootlets that appear almost coralloid. The fungal colonization progresses on these lateral second and lower order roots. At individual sites the hyphae at first appress to the root epidermis for a short distance and, as they branch and spread further over the root, they anastomose with themselves and other hyphae. The fungal mantle is gradually constructed from such starting points.

The colonization seems to progress most rapidly on *Carpinus*; as a rule, the whole absorbing root system of 1-year-old plants is converted to mycorrhizas. It occurs more slowly on *Quercus*, so one can easily follow the process: 1- and 2-year-old plants or individual parts of the root system of older plants are often only partially colonized. These fungus-free feeder rootlets are clad with root hairs, as are the feeder rootlets of plant species that never form mycorrhizas. Still, such fungus-free roots of the Cupuliferae are relatively infrequent. Moreover, at least the tips of such rootlets are often colonized, while the mycelium spreads mainly towards the younger part of the root system. It soon is able to colonize tips of the young rootlets, because they grow slowly and always remain short. Only the stronger and vigorously growing long roots, which penetrate root-free parts of the soil and bear the actual feeder rootlets, usually remain free of fungus. As with young plants, the fungi colonize feeder rootlets on older parts of the root system directly from the soil.

## Fungus-induced changes in the root

The mycorrhiza differs in form from the noncolonized rootlet. When we cultivate broad-leaved trees under fungus-free conditions, as will be discussed below, the feeder roots are relatively thin and long, their lateral branches emerge monopodially at fairly distant intervals, and they resemble the parent root in shape and branching. In contrast, the mycorrhiza shows a very slow length growth but attains greater thickness, because the cell layers of the apical meristem and procambium proliferate somewhat more, and the epidermal cells reach a greater width to form a short, relatively thick structure. Moreover, a stronger tendency to branching develops, so that the lateral rootlet branchlets occur at short intervals close behind the tip. These branchlets behave similarly to the parent rootlet in growth, form and branching. The mycorrhizas thereby form more or less coralloid growths (Fig. 1) that often develop into large clusters (Fig. 1).

The branching of the mycorrhiza occurs by the endogenous mode common for roots, and the new growing tip that emerges from the parent rootlet thus is clad with the parent rootlet's fungal mantle from the beginning. From then on the fungal mantle continues growth with the new rootlet branch as described for the parent rootlet. The branching is strongly monopodial: in spite of the coralloid growth form, no dichotomy is to be seen.

The first branchlet always forms behind the tip where length growth has ceased. Branching proceeds acropetally, so the branchlet nearest the tip is always the youngest and shortest. These branchlets form rather clearly in longitudinal rows as is usual for roots: sometimes in two, sometimes in three, sometimes in four rows, occasionally in only one row, patterns that may partly depend on prevailing conditions of space. In addition, gradations occur in the changes of form produced by the fungus. Sometimes the feeder rootlets approach the form of noncolonized roots (Fig. 1a). In that case the fungal mantle still envelops them but is not as thick as on roots with the most pronounced coralloid form.

## Subsequent fate of the mycorrhiza

The distinctive combined organ of fungus and root as described above generally has a limited life span. Still, it shares this property on the whole with feeder rootlets of woody plants. As the trees expand their root systems with age and invade new areas of soil, the feeder rootlets of the aging parts are lost, while new ones develop on growing parts of the system in other areas of the soil. The mycorrhizas, which have determinate growth, cease growing after a while or regrow only on individual branchlets until they stop altogether. Eventually they die off to shrivel and turn dark-brown to black and brittle. Despite that, as already mentioned, they can form anew at other places, often close to a deceased mycorrhiza cluster.

How long mycorrhizas remain alive depends on a number of circumstances and may vary considerably. They may persist for many years: one often finds huge nests of mycorrhizas which, considering their slow growth rate, must have taken a long time to form. As with nonmycorrhizal tree roots in advanced age, the cortical cells turn brown in the oldest parts of a mycorrhiza as the dying process commences, while the fibrovascular strand continues to function within the protective endodermis. Death of the fungal mantle goes hand in hand with that. The stronger growing tips of some mycorrhizas may convert to perennial, lignified branches of the root system through further lengthening and thickening. They establish a cork cambium beneath the endodermis and a vascular cambium in the fibrovascular cylinder, in the process losing their fungal mantle. The mantle is only for the younger rootlets, particularly those involved with nutrient uptake.

Berichte d. Deutschen Bot. Gesellsch. Bd. III.

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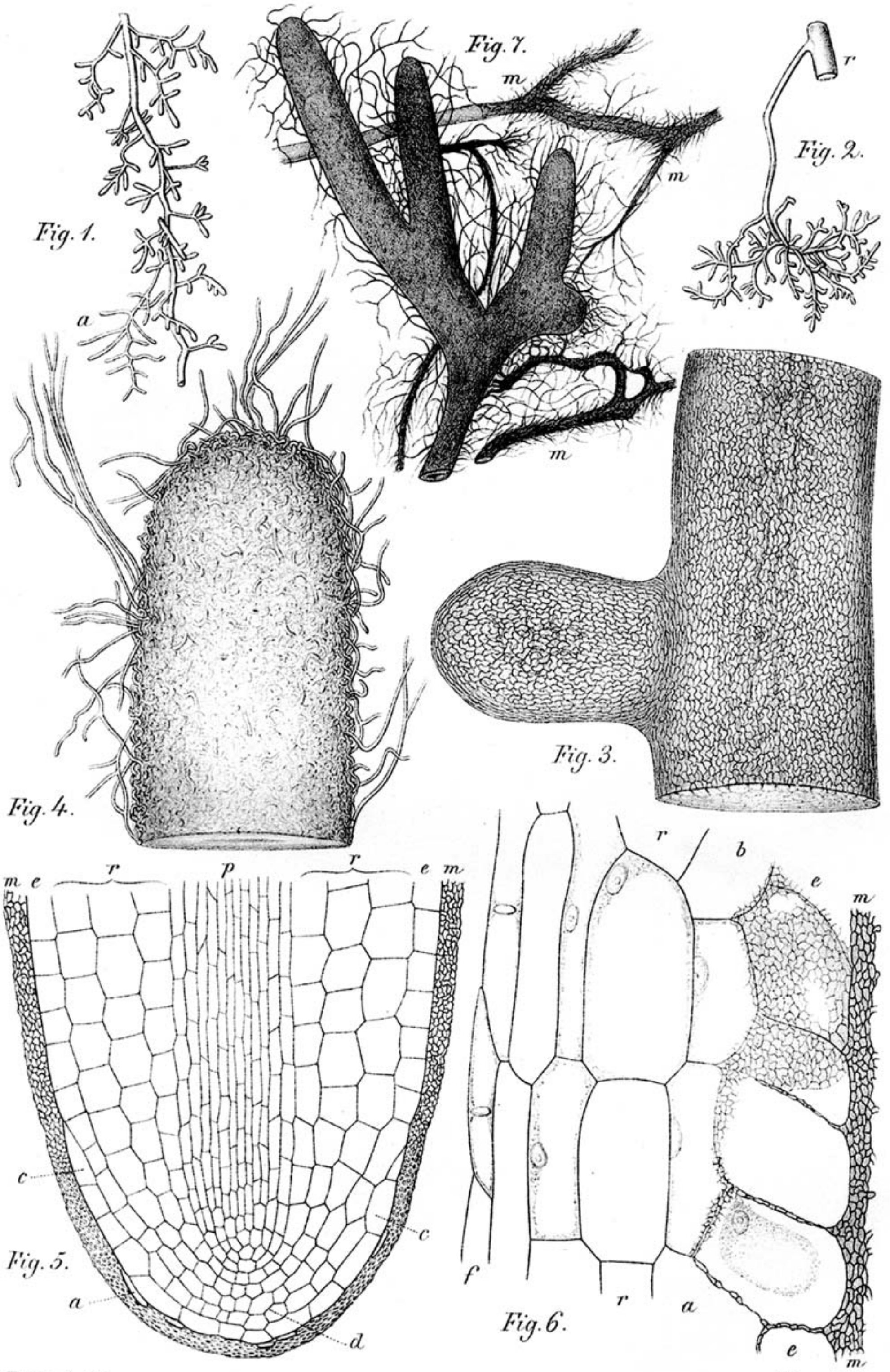

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◀ **Fig. 1** Root of *Carpinus betulus* laterally bearing mycorrhizas that are mostly coralloid thickened. *a* A branch system that is thinner but also colonized by fungi. ×1

**Fig. 2** Cluster of mycorrhizas on a lateral root emerging from a taproot (*r*) of a 1-year-old *Carpinus betulus.* ×1

**Fig. 3** Part of a mycorrhiza of *Carpinus betulus* with a short lateral branch; the surface view illustrates the structure of the outer, smooth fungal mantle composed of a small-celled, pseudoparenchymatous tissue, through which in places the contours of the underlying root epidermis can be seen. ×145

**Fig. 4** Tip of a mycorrhiza of *Fagus sylvatica*, showing the form of the fungal mantle with loosely arranged, superficial hyphae that grow out irregularly into the soil. ×145

**Fig. 5** Longitudinal section through a growing mycorrhizal tip of a 1-year-old *Carpinus betulus*. *p* Procambium; *r* root cortex; *e* epidermis; *c* ephemeral root cap layer tha covers the root apex; *d* general layer of initials of the protoderm, root cap and epidermis and from which forms the root cap layer through periodically repeated divisions to the outside; *m* fungal mantle, comprised of pseudoparenchymatous tissue that surrounds the entire growing tip of the root and the cells of which are narrower at the root tip, the youngest part of the root, than further back on the root. *a* Detached remnants of the once contiguous cells of the now detached root cap layer surrounded by hyphae of the fungal mantle

**Fig. 6** Part of a longitudinal section through an older portion of a mycorrhiza of *Carpinus betulus*, leading through the epidermis (*e*), the cortex (*r*), and the cells that border the fibrovascular strand (*f*). *a* The direction towards the root tip, *b* towards the root base, *m* the fungal mantle, which forms the surface of the mycorrhiza and from which a pseudoparenchymatic cell layer continues inward to weave around the walls of the epidermal cells; in the lower part of the figure one sees the same in cross section of the epidermal cell wall, in the upper part a surface view, not unlike a fine, reticulate thickening of the wall. ×480

**Fig. 7** A mycorrhiza of *Fagus sylvatica* from Alefeld in the vicinity of a truffle (*Tuber aestivum)*, with a very strongly developed fungal envelope, which extends through the soil partly as free mycelial filaments and partly as almost rhizomorph-like mycelial strands that in turn disperse as free mycelial filaments. ×40

## Regular presence of the fungus in all ages and on all roots of the tree

To study the roots of Cupuliferae at various ages, I acquired 1-, 2- and 3-year-old seedlings of oak, beech, hornbeam and hazel from various localities, as well as root samples with feeder rootlets of older trees: a 120-year-old beech, a 100-year-old hornbeam, and a 40-year-old hazel. The feeder rootlets of all these trees at all ages were developed as mycorrhizas, accompanied by the fungus through the entire life of the root. It was interesting on the older trees how the fungus behaves at the various soil depths in which the roots occur. I pursued that especially with the beech and hornbeam. In the uppermost soil layer, about 5 cm thick and relatively humus-rich, the rootlets customarily form the largest number of feeder rootlets, and these, as already mentioned, are always developed as mycorrhizas. These mycorrhizas are surprisingly abundant in this soil layer, especially at truffle sites, so that the ripe truffles rest on, and in, a thick weft of mycorrhizas. In deep soil, one can follow how the frequency of feeder rootlets decreases with depth, at first gradually but then increasingly so. The stronger roots freely penetrate deep layers, but there they form feeder rootlets only sparingly or do so mostly on branches that have penetrated upward into more shallow soil layers. In forest soil with the parent material at a depth of 50 cm, roots could be traced in the parent material but formed feeder rootlets only sparingly. Even there, however, the feeder rootlets develop as mycorrhizas. One could explain this by a distribution of the root-colonizing fungus in all soil layers. More simply, however, it can be clarified by penetration of the parasite into the soil along with the root, which is always colonized as it elongates into the deeper soil layers.

## Occurrence of the root fungus on plant species

It is extremely interesting that this soil-inhabiting fungus selects the rootlets that it colonizes strictly by species and thus abides by a strong systematic constraint. For example, in the soil of a beech stand, only the beech roots developed as mycorrhizas. All the herbaceous vegetation that occurred there, such as *Oxalis acetosella*, *Mercurialis perennis*, *Anemone nemorosa*, *Asperula odorata*, *Viola canina*, *Convallaria multiflora*, etc., as well as other woody plants, e.g., *Hedera helix* and *Acer pseudoplatanus*, have roots completely fungus-free and with root hairs, as is usual for plant roots. This is even the case when those roots are near or growing through a mycorrhizal cluster.

To accurately determine the spectrum of host plants used by the root fungi, I examined most of our woody plant genera and can specify those where the root fungi fail: *Betula alba*, *Alnus incana*, *Ulmus campestris*, *Morus alba*, *Platanus occidentalis*, *Juglans regia*, *Pyrus malus*, *Sorbus aucuparia*, *Crataegus oxyacantha*, *Prunus padus*, *Robinia pseudoacacia*, *Tilia europeae*, *Acer platanoides* and *pseudoplatanus*, *Rhamnus cathartica*, *Cornus mas*, *Fraxinus excelsior*, *Syringa vulgaris*, *Sambucus nigra.* Thus the great majority of plant families to which the native trees belong are free of the root fungus, as judged by the representatives here examined.

Because the limitation to a small spectrum of plants always points to the Cupuliferae, I studied its most important representatives in this regard and verified the presence of root fungi without exception. The roots of these trees show an essentially constant fungal colonization of the kind described above: *Carpinus betulus*, *Corylus avellana*, *Fagus sylvatica*, *Quercus pedunculata* and *sessiliflora*, *Castanea vesca*, from samples from the Rheinland as well as the Berlin Botanical Garden, and the American *Quercus rubra* from the local botanical garden. From these results one may assert that the root fungi are a special feature of all the Cupuliferae. Indeed, this symbiosis is so constant for this family that one could be tempted to regard it as a systematic criterion. At any rate it is worth mentioning that the inclusion of the Betulaceae in the Cupuliferae, as accepted by the newer taxonomists, does not seem to be supported as judged by the occurrence of the root fungi.

On the other hand, it also interesting to see a hint through the occurrence of these fungi beyond the Cupuliferae, of a certain kinship of some other families with the Cupuliferae: the Salicaceae and the Coniferae. I have found mycorrhizas also with them, but not so generally as with the Cupuliferae. *Salix viminalis*, *S. caprea*, and *S. aurita*, as well as *Populus tremula*, originating from many sites, were colonized by the fungi in varying degrees, although no colonization was evident at other sites. I found roots of *Taxus baccata*, *Juniperus communis*, and *Larix europaea* in the vicinity of Berlin to be free of the fungi, but those of pine, spruce, and white fir near Berlin to be mostly but not everywhere colonized in the typical manner. Rees (loc. cit.) has similarly described this colonization on pine roots in sites where *Elaphomyces* occurs, but it is evident that the root fungus is much more broadly distributed on conifers than Rees believed, including sites where no sporocarps of *Elaphomyces* have been found.

## Geographic distribution of the root fungi of the Cupuliferae

Once the fungi under consideration were found on the Cupuliferae in sites that produce no truffles, so that the occurrence of those fungi appeared to be more and more a general phenomenon, it was appropriate to systematically clarify their distribution. By arrangement with His Excellency, the Minister, I have received for study roots of all species of Cupuliferae occurring in a large number of forest districts, representing as much as possible the variety of soil conditions and geographical situations in the Kingdom of Prussia. The plants were mostly 1–3 years old, but samples of older trees were also included. The primary result can be revealed at the outset: the mycorrhizas occurred in all regions, and no Cupuliferae were free of root fungi.

Beech, hornbeam and oak collected from various compartments of the forest district at our most southwestern border near Saarbrücken were colonized by the fungi without exception. So were beech from Rügen Island, hornbeam from the Brödlauken Forest District in the Gumbinnen Administrative District at our eastern border, and indeed from all the regions in between from which Cupuliferae were examined. The results show that differing elevations and soil types do not limit the fungus: it occurs in the flood plains, e.g., in the flood zone of the Elster of the Schkeuditz Forest District and in the Elbe lowlands near Gräfenhainchen in the Merseburg Administrative District. It occurs outside of floodplains as well as in completely flat areas or gently rolling hills, e.g., it was constant on plants from the forest complex of the Dübener Heath between the Elbe and Mulde, south of Wittenberg from the Zöckeritz Forest District near Bitterfeld, from near Berlin as well as from the Jülich flatlands.

In hill and mountain regions the fungus was found at all elevations and exposures, on the plateaus as well as in the valleys and hollows and on slopes, equally on north, south, west and east aspects, and without difference between gentle and steep slopes. The fungus also ascends with the beech into the higher mountain regions.

Considering soil conditions, both shallow and deep soil are suitable for the fungus; its behavior here has been discussed above. Also, none of the geologic conditions of the soil exclude the parasite. It is constant in diluvial soil from various regions, indeed in strongly humic river loam no less than in light, more or less humus-poor sand (e.g., from various places near Berlin), as well as in the intermediate formations of sandy loam and loamy sand with varying humus contents. Further, it is in the loamy sand soil that is the weathering product both of carbonaceous sandstone (e.g., from Münster) and of the new red sandstone (e.g., from Saarbrücken); in greywacke soil, e.g., from the Eifel, Westerwald, etc.; then in the red loam soil weathered from the red sedimentaries at Sangerhausen. Finally, it occurs on all types of limestones, namely shell limestone (e.g., Freiburg on the coast, Heldrungen, Wanfried on the Werra, Friedland on the Leine), platey limestone (from southern Hannover, e.g., Alefeld, etc.), and no less on Eifel limestone (e.g. Schleiden on the Eifel) as well as chalk soils of Rügen Island.

It merits mention that the fungus always seems to develop most luxuriantly on limestone substrates. Finally, it must be emphasized that no vegetation type hinders the appearance of the fungus on the Cupuliferae present; it occurs equally in high forests of trees grown from seed, mixed high-coppice forests and coppice forests, in fields, and no less outside the forest where Cupuliferae are raised, such as parks, gardens, tree nurseries, etc. Indeed, I found the fungus growing along with roots of plants I had potted with soil in flower pots about 2 years before and then had allowed to grow.

It might seem odd that botanists have missed the root fungus of the Cupuliferae before now, as it has such a wide distribution. Plant roots, especially the root tips, have been studied botanically many times, but in general only seedling radicles have been used. When that is done with the Cupuliferae, the roots are at a developmental stage that precedes appearance of the fungus. Researchers dealing with root diseases of the Cupuliferae could hardly miss it, however. Much contemporary plant pathology work is conducted uncritically; this innocent fungus was found accidently in pathology work, and, although no studies were conducted on its significance, it was assumed and declared to cause some anomalous growth phenomena. However, it is an inalienable part of every beech and oak tree, and, as we will see, serves as an important "wet nurse" for their nutrition.

Misinterpretation happened in fact with the ink disease of chestnut that occurs particularly in upper and central Italy. It is called that because it commences as an initial blackening and general dying and rotting of the roots. In truth, the cause of this disease is not investigated at all. Gibelli [*Nuovi studi sulla malattia del castagno detta dell'inchiostro* (1883) Bologna], who has been much occupied with this disease, believes it to lie in fungi that appear on the rotting chestnut roots; he would characterize them as

*Torula exitiosa* de Seyn., *Diplodia castaneae* Sacc., and *Melanoma gibellianum* Sacc. The evidence on the causal relationship of these fungi to the disease is lacking, so much the more so because these and similar fungus formations occur as decomposers on plant parts rotting on and in the soil after death by any cause.

Gibelli also noted the true mycorrhizal fungus on living feeder rootlets of chestnut. His description leaves no doubt about its identity with our fungus: the coralloid, tuberculate, swollen roots with tips capped by a pseudoparenchymatic mycelial tissue and entangled with branched rhizomorphs, as illustrated in Plates IV and V of his report. He was so biased towards the concept of a root-injuring enemy, however, that he equated this root fungus with the fungi on rotting roots listed above as a cause of the disease under consideration. Gibelli did state that he had found the characteristic root fungus to be general in Italy on roots of healthy chestnut trees as well as oaks, beech, hazels, and other Cupuliferae. But even these observations were insufficient to lead him to a different interpretation, that the actual injurious parasite had already expanded to a wide distribution in Italy and causes the disease, but the tree does not suffer from the attack as long as it is growing vigorously, rather only if it is weakened from other causes. No further confirmation that Gibelli's viewpoint is erroneous is needed beyond what has been said above and what is to be said below about the biological significance of the root fungus. For us the interesting fact emerges that Italy is included in the general geographical distribution of the mycorrhizas of the Cupuliferae.

The biological discovery of mycorrhizas of the Cupuliferae could also have been possible earlier in Germany. R. Hartig (Untersuchungen aus dem Forstbotanischen Institut zu München (1880) I. Berlin, p 1 ff.) studied a root disease of 1- to 3-year-old oaks in various sites, as reported under the title, "The oak root killer, *Rosellinia* (*Rhizoctonia*) *quercina*." He regarded that fungus as the cause of the disease, which produces a massive rotting of the taproot and the lower parts of the stem in the field and also grows on the soil surface. This fungus does not correspond with our root fungus; R. Hartig did not confuse the two, because he did not examine the oak roots well enough to discover the mycorrhizal fungus.

Because the mycorrhizal fungus occurs so generally and regularly that no Cupuliferae can be found without it, I pondered on a way to artificially free the plant from its "nurse" to force it to take up nutrients independently. I succeeded in this by water culture: 1- and 2-year old seedlings were lifted from the soil in late winter and transferred with intact root systems colonized by the fungus into a nutrient solution composed of compounds common for water culture.

After some weeks and before the buds burst, root formation began. The mycorrhizas already present grew no further, but very bright, new rootlets, easily differentiated from the previously formed, darkly colored ones, were produced laterally at various places. This is the common phenomenon: roots formed in soil do not develop further when placed in water. Rather, new roots initiate in the water. The root fungus passed over to these new rootlets in its characteristic form, partly by forming loose mantling hyphae that assumed a kind of colorless water form. But it unquestionably could no longer keep up with the rootlet formation. The base of the new rootlets still showed the extended fungal mantle, but it seemed less distinct, thinner, and often so interrupted that broad stretches of the epidermis showed only a spotty fungal mantle. The remaining surfaces were bare, thus prepared for root hair formation that would otherwise be repressed by the fungal mantle. The tips of the new rootlets were free of the fungus. A 3-year-old oak cultivated from germination in water culture and never in soil was in accord with this: its strongly developed root system was completely fungus free. From the facts related thus far, we must conclude that the fungi of the mycorrhiza thrive best on roots in soil, are generally distributed in vegetation-supporting soil, and from such soil colonize rootlets of the Cupuliferae.

## The question of the species of the root fungi

The systematic position of the fungi in question can be determined only by identification of their fruiting bodies. The occurrence of the mycelium on the roots necessarily directs attention to the hypogeous fungi, above all the Tuberaceae and many Gasteromycetes. It might appear strange that the ubiquity of the root fungus mycelium is not accompanied by a similarly general occurrence of the fungal fruiting bodies. That can be for two reasons, however. First, with extremely attentive searching, hypogeous fungi can often be found even where one does not expect them. Second and foremost, the presence of the mycelium of a fungus is not necessarily accompanied by the appearance of its fruiting body at just any time. There are examples enough that the mycelium of a fungus can grow year round without forming fruiting bodies, and that the latter appear only when certain environmental conditions are met. So, we are presented with the question of whether or not it is possible to determine the fungi by their mycelial character.

The many variations in form, thickness, color and connections of the hyphae that branch off from the mycorrhiza into the soil have been described above. More precise observations, however, quickly lead to the recognition that these characteristics are not usable for specific differentiation without elaboration, in that they may vary on the same mycorrhiza; at least in part these variations represent changes in form of a single mycelium.

If one compares the mycorrhizas of a truffle site with that of a site not bearing truffles, no sharp differences are to be seen, even in significant morphological characters. Often the differences that do exist are primarily quantitative in the biomass development of the mycorrhizas and the prevalence of the mycelium in the soil, which reaches its maximum in truffle sites. Accordingly, we can assume that the fungi, which produce truffles in many regions, are much more broadly distributed than are the truffles themselves. Perhaps they are quite common fungi and, where truffles are lacking, their fruiting body production is limited by lack of the proper environmental conditions.

On the other hand, it is unwarranted to conclude without further study that the similarity of the mycelia means the fungi are everywhere the same. In keeping with the common rule for fungi that related species present no reliably differentiating mycelial characters, various species of Tuberaceae probably cannot be differentiated by their mycelium alone. However, these questions are beyond the scope of this paper and should be left for when results of pending studies are available.

## Biological and physiological significance of the mycorrhiza

The roots of the Cupuliferae and the fungal mycelium organically unite into a morphologically unique organ. The intimate, reciprocal dependence that follows the growth of both partners and the tight interrelationships of physiological functions that must exist between the two appear to be a new example of symbiosis in the plant kingdom. It goes beyond the lower organisms to the most highly developed plant form, the trees, and is incontestably both unexpected and surprising. First of all, the fungus mycelium must be regarded as an undoubted parasite on the living cupulifer root, as is evident from the entire manner of its colonization and penetration into the growing rootlet. As is the case for all parasitic fungi, the basic nutritional needs of the fungus are primarily the carbon compounds procured from the photosynthesizing tree. In contrast, the fungus is evidently independent in regard to uptake of soil minerals, in that it alone contacts the soil by its peripheral position on the mycorrhiza and the innumerable hyphae it extends into the soil to grow around soil particles like root hairs.

Now, the question of great interest must be, is the tree damaged by the fungal parasitism of its rootlets? We know from a multitude of cases that parasitic fungi damage their host plants. The morphological changes assumed by the tree rootlets under influence of the parasite can be characterized as hypertrophy or gall formation, albeit relatively weak. This suggests an irritation by the fungus on root growth. However, the root is in no way killed by the fungus, and despite its change it does not lose the capacity to function for the tree, as the prosperity of the latter adequately demonstrates. For the same reason, the idea that the fungus deprives the tree of mineral nutrients carries no weight. Were this so, healthy beeches and oaks could not exist, because each cupuliferous tree is accompanied by the fungus from its first year to advanced age. We conclude from all that, the root fungus, at least in the mycelial state, can inflict absolutely no disadvantage on the tree.

This fact imposes the stamp of symbiosis on this relationship, because both of the united organisms live together in reciprocal assistance without harm to each other. The fungus fulfills a reciprocal service for what it receives from the plant, a service of eminent significance, because it represents the most important factor in the nutrition of the tree. That soil water and nutrients needed by the tree are supplied only through the mediation of the fungus cannot be challenged: it envelops the entire surface of the feeder rootlets, and its hyphae perform the role that root hairs do for other plants in intimately contacting the soil particles. The enlargement of the volume of the root epidermal cells and their complete envelopment by the hyphae produce an arrangement probably designed for nutrient uptake by the tree. The fungus takes up soil minerals not only for its own nutrition but also for that of the tree, so we must consider that the root fungus is the sole organ for uptake of water and soil nutrients by oaks, beech, etc. It functions as a wet nurse of the tree in this respect. Thus, in contrast to autotrophic plants and trees, the Cupuliferae show a relationship that can be termed "heterotrophy," i.e., nutrition from soil with help of another organism on a truly splendid scale, known before now only with lichen gonidia and some lower algae living within higher plants.

The symbiosis of the Cupuliferae most closely parallels that of the lichens, specifically in its biological character, even allowing for the differences, i.e., the association meets both the requirements and outputs for nourishment of both partners. Indeed, the root fungus is analogous to the lichen hyphae and the tree to the lichen alga; the comparison need not be elaborated further. A complete analogy even appears to prevail in reference to what degree these symbiotic relationships are either necessary or dispensable for both partners. The lichen algae are known to exist independently of the lichen fungus and can develop as a free alga after isolation from the lichen. Similarly, as previously mentioned, the Cupuliferae can be cultivated fungus-free in water culture for years. Of course, the Cupuliferae do not develop strongly when free of fungi in water culture. Still, that is at least partly due to the unusual medium, for the same thing shows up with other land plants grown in this culture method. Whether the Cupuliferae can nourish themselves better with their fungus nurse than without is not known from these studies, because no adult Cupuliferae seem to be fungus-free. On the other hand, as the lichen hyphae do not develop strongly and, in any case, never attain typical fruiting in the absence of the algae, so also the mycorrhizal fungi seem to depend on the chlorophyllous tree for their development.

So far I have not succeeded in growing the hyphae from pieces of living mycorrhizas in water or fungal nutrient solutions such as plum decoction. Moreover, the strong dependence of the fruiting of truffles on the presence of living trees is emphatically significant here. This would not preclude the supposition that a weak, perhaps somewhat saprobic, development of the fungus is possible in the soil without the nourishing tree to explain the general distribution of the fungus in soil that supports plants. Finally, the root fungi are also reminiscent of the dependence of lichens on specific substrates. The occurrence and fruiting of many lichens are restricted to specific types of rock. So also the fruiting bodies of hypogeous fungi occur in striking relationship to soil properties aside from their dependence on the nourishing trees. For example, the edible *Tuber* species indicate with certainty an underlying limestone. The two symbiotic relationships here compared thus differ only morphologically through the differentiation and organization of the body of the tree as compared to the alga.

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