

Monotropa Hypopitys L. — an Epiparasite on Tree Roots

By

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Monotropa Hypopitys L. has long been an enigmatic plant. Both plant physiologists and, in particular, specialists in mycorrhizal research, have devoted considerable attention to it, and widely varying views have been held regarding its biology. Since it is devoid of chlorophyll, it was originally regarded as a root parasite. This theory was refuted by Graves, Curtis and Hooker (1826), among others, who showed that *Monotropa* roots were quite free from the tree roots although intermixed with them in the soil. The first to observe that *Monotropa* roots are normally enveloped by fungal hyphae was Elias Fries (1832), who also gave the fungus a separate name — *Tuburcinia Monotropa*.

In 1840, however, also Unger regarded *Monotropa* as a parasite — a view that was not directly contradicted by the large number of researchers who, in the ensuing decades, treated the problem with special reference to the association of the roots with soil fungi. In 1873, Drude spoke of adventitious roots from *Monotropa* that penetrated into the roots of both beech and spruce. It was not until 1881—82 that Kamiensky established definitely that *Monotropa* lives quite independently of the tree roots. He also elucidated and made drawings of the structure of the roots, and called attention to their striking similarity with tree roots, which probably accounted for the anatomical mistakes which had earlier been made by Drude, among others. He demonstrated, too, that both tree roots and the roots¹ of *Monotropa* are normally enveloped

by fungal hyphae which penetrate between the root cells, and spoke of a typical symbiosis. McDougal (1899) established the occurrence of haustoria that penetrated into the outer root cells, and considered that the fungus, via these threads, mediated nutrients from the humus to the host plant. In his opinion *Monotropa* was to be regarded as a typical saprophyte, which view has been generally recognized by subsequent workers, including Francke (1934), who has made the most important contribution thus far to our knowledge of its biology.

According to Peklo (1908) an exchange of nutrients occurs via the fungal mantle, the hyphae of which absorb from the soil nutrients which are then transported to the epidermal cells of the roots. On the other hand the intercellular hyphae of the fungus — still according to Peklo — absorb from the root cells, by osmotic means, dissolved carbohydrates and other substances necessary for the fungus. Neger (1913) held that *Monotropa* is able, via its root fungus, to assimilate organic substance from the humus, while the fungus receives water and carbohydrates from the root. In Rexhausen's (1920) view nutrient salts are, as in the ectotrophic mycorrhiza of conifers, directly absorbed by the fungus and diffuse to the root cells. Christoph (1921) too, considered that the fungal hyphae absorb nutrients from the soil.

Due to the presence of intercellular hyphae, the mycorrhiza of *Monotropa* must be described as ectotrophic; but in view of the concomitant haustoria from the hyphal mantle that penetrate into the outer root cells, an endotrophic mycorrhiza must also be considered to have been formed. Hence it would probably be most correct to describe the *Monotropa* mycorrhiza as eciendotrophic (*cf.* Melin 1925).

Since the achlorophyllous host plant cannot readily supply its root fungus with carbohydrates, as is normally the case in a typical mycorrhiza of green plants, the question of what the fungus stands to gain from the symbiosis has been a matter of pure conjecture. In the opinion of Kamiensky (1881—82) the fungus merely utilizes the *Monotropa* root as a substrate (a "base commodity") and receives nothing from it; and even Francke (1934), who studied the contents of root and fungus tissues in detail, observed that the fungus was unlikely to obtain from the host plant appreciable amounts of nutrient material; though *Monotropa* is not, in his view, a parasite on its mycorrhizal fungus, but rather a typical symbiosis exists, since the development of the fungus is nevertheless stimulated by the host plant.

Francke (1934) made a special study of the haustorium-like hyphae which penetrate from the mantle into the root cells (one in each cell) and there discharge their contents. Via these hyphae the root cells are accordingly supplied with nutrients, which were found to consist mainly of proteins and glycogen that are immediately dealt with by the intracellular enzymes. After

¹ According to Velanovsky (1907) the underground organs of *Monotropa* are neither stem parts nor true roots, and he therefore terms them "procambones". For the sake of simplicity, however, they will here be called roots.

discharging its contents into the cell the haustorial hypha is completely cut off, and no further penetration of hyphae takes place. Such an organization has been termed ptyrophyagy and occurs in certain forms of endotrophic mycorrhizae. Probably these haustorial hyphae originally have a more or less parasitic function but, due to the host's reaction, reverse their role and supply their nutrient contents to the root cells, as in many orchids.

That the absorption of water and nutrients in *Monotropia* proceeds via the fungal hyphae is unquestionable, for the roots are invariably enshrouded completely by a fairly thick hyphal mantle (Figure 1); but none of the hypotheses as to the nature of the symbiosis have been able to account satisfactorily for the kernel of this enigmatic behavior of *Monotropia*: How can a plant possessing no nutritional store of its own and no possibility of producing one, grow so rapidly during late summer (northern Europe) that, from insignificant underground germs, it reaches its full development in a week or so? Much greater attention should be given to the energy problem inherent in the biology of *Monotropia*. The substantial amount of energy that has to be mobilized swiftly for development of the plant can scarcely be acquired from the meager and rapidly decreasing supply of soluble carbohydrates available in the soil.

Since the investigations of Francke (1934) may safely be said to have confirmed that the entire intake of nutrients proceeds via the mycorrhizal fungus, the identity of that fungus will be of very great interest, for fungi differ considerably, especially with regard to their carbohydrate source. While some fungi are able to assimilate nutrients from complex carbohydrates as for example cellulose, others can utilize only soluble carbohydrates, notably glucose. If we are here concerned with a fungus of the latter type, it seems quite inconceivable that the host could obtain from the soil a carbohydrate supply as great as that which it demonstrably requires; this not only because the fungus itself needs abundant energy for its own growth, but also because the supply of soluble carbohydrates in the soil is greatly restricted through competition with various types of micro-organisms, as for instance fast-growing mould fungi.

Francke in his paper of 1934 demonstrated that the mycorrhizal fungus of *Monotropia Hypopitys* — his investigations suggested that only a single fungus was implicated — is dependent on soluble carbohydrates and is unable to break down starch or cellulose. The fungus that he cultivated and which demonstrably formed mycorrhiza on *Monotropia*, was probably, in his opinion, a *Boletus* species. From the investigations of Melin and others we know that fungi of this genus are usually able to utilize only soluble carbohydrates, chiefly glucose. True, it has been shown that this genus does include transitional types with respect to carbohydrates — for *Boletus subtomentosus* cer-

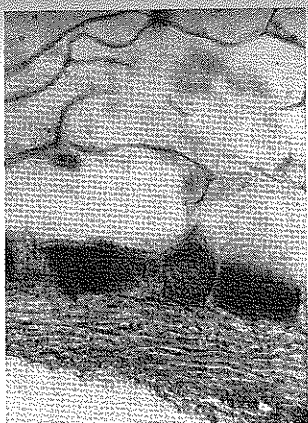


Figure 1.



Figure 2.

Figure 1. Longitudinal section through a root of *Monotropia Hypopitys L.* with the hyphal mantle to the extreme right. The fungus has penetrated only into the outermost root cells. 450X.

Figure 2. Roots of *Monotropia Hypopitys L.* with ectotrophic mycorrhiza and roots of *Picea Abies L.* with typical ectotrophic mycorrhiza. The *Monotropia*-roots are invariably enveloped by masses of fungal mycelia in which there are always tree roots bearing mycorrhizae. 1X.

tainly is able in some degree even to utilize cellulose (*cf.* Lindberg 1948) — but in the main the *Boletus* genus assuredly constitute very typical "sugar fungi".

A most interesting consideration is that species of this genus are extremely common mycorrhizal fungi of forest trees. The characteristic "nodule" mycorrhiza ("type C" of Melin 1927) of *Pinus* is composed of *Boletus* species (*Boletus luteus*, *B. bovinus*, *B. variegatus*, etc.; *cf.* Melin 1925, 1936, Hatch 1937, Modess 1941).

According to the theory propounded by Björkman (1942, 1944, 1949) respecting the conditions for the formation of mycorrhizae on forest trees (notably pine and spruce), mycorrhizae are formed when the host has ample light as well as under ecological conditions associated with some deficiency of, above all nitrogen or phosphorus in the soil; the result being that the tree or plant is unable by itself to utilize all of the formed sugar, but rather, a surplus of soluble carbohydrates collects in the roots and can be utilized by the mycorrhizal fungi. These fungi thus obtain, according to the theory, soluble carbohydrates from the host plant, which under certain conditions produces an excess of them in the assimilation of carbon dioxide. According to this view, which in principle was already held by Frank (1885) and was verified by Melin and Nilsson (1957) using the isotope technique, the fact that mycorrhizal fungi can satisfy their energy requirements in the tree roots is the direct requisite for the formation of mycorrhizae. The fungal mycelia are

attracted to the surface of the roots by various root exudates, for example carbohydrates, amino acids and vitamins, which have approximately the same action whether they have been discharged from, say, pine roots or tomato roots (Melin 1954). To all appearances the vitamin requirements of the trees' mycorrhizal fungi can scarcely be primary and specific just for those fungi, since a similar effect on the growth may also be observed in non-mycorrhiza-forming soil fungi that are able to attack cellulose and lignin — the litter decomposing fungi. The characteristic which has proved to be common to those soil fungi that form typical ectotrophic mycorrhizae on trees is, however, precisely their dependence upon soluble carbohydrates, although no sharp distinction can be made between mycorrhizal and litter decomposing fungi (*cf.* Norkrans 1950).

The mycorrhiza of *Monotropa*, which in its typical ectotrophic part exhibits very great anatomical similarities to the ectotrophic mycorrhiza of trees, is, in the light of these considerations, difficult to explain physiologically in the same way. For the achlorophyllous *Monotropa* cannot offer the mycorrhizal fungi any carbohydrates; on the contrary, the situation here must be such that the host plant itself receives large amounts of energy materials via the mycorrhizal fungus.

Does this, then, constitute two incompatible antitheses? Some workers have asserted that such is indeed the case, and that the mycorrhiza formation of *Monotropa* and that of trees cannot have a single explanation. Björkman (1949, 1956), however, has doubted the validity of this contention on the grounds that the vitamin production of *Monotropa* may be the principal explanation of the mycorrhiza formation, provided that the plant's energy problem is resolved in some other way. Reference has been made here to the known behavior of other achlorophyllous plants, as for example certain orchids that are able to produce substances essential for, and having a marked stimulating effect on, the growth of other organisms which cannot themselves synthesize them but which nevertheless have access to sources of energy. In some achlorophyllous orchids, therefore, even cellulose- or lignin-decomposing fungi of the genera *Marasmius*, *Fomes*, and *Armillaria* can form mycorrhizae (Kusano 1911, Hamada 1940), whereby the supply of energy to the host plant may be assumed to proceed via these fungi, which, in the decomposition of wood, liberate simple carbohydrates. It may just as safely be assumed that the same fungi seek contact with orchid roots (and also with the roots of other plants) because they are stimulated by certain substances given off by those roots (*cf.* Melin 1954, Melin and Das 1954). In green orchids too, the carbohydrates are supplied via the mycorrhizal fungus before chlorophyll has had time to form — which process, as for instance in *Goodyera repens*, may take several years (Montfort and Küsters 1940) — but when the

host plant has acquired chlorophyll and hence the ability to assimilate carbon dioxide, the carbohydrate flow changes direction so that the fungus instead obtains nutrition from the host plant (Burgeff 1936).

The essential factor for an understanding of the mycorrhiza formation of *Monotropa* seems to be, in any circumstances, the energy problem. As early a worker as Kamiensky (1881—82), and subsequently Romell (1939) as well as Björkman (1949, 1956, 1959) have considered it not unlikely that the fungus which constitutes the mycorrhiza of *Monotropa* may be identical with that of the tree (pine, spruce, beech, and others) with which it is associated in some way as yet unexplained (Figure 2). If so, then the obscure energy problem of *Monotropa* would be resolved.

In order to test this hypothesis certain preliminary experimental ecological and physiological investigations were conducted between 1953 and 1958, the results of which are reported here.

Experiments are also going on with the American species *Monotropa uniflora* and mycelia isolated from the roots of this plant.

A. Isolation of *Monotropa* from Tree Roots at Natural Habitats

As long ago as 1823 an experiment was conducted at Glasgow Botanical Gardens with the view to investigate the dependence of other plants upon *Monotropa*, for which purpose *M. uniflora* specimens were transferred to the gardens, where they grew "in a box of earth which was sent containing other rarities from the neighborhood of Montreal" (Hooker 1825). The identity of the other plants was not mentioned, nor did Hooker state how long the specimens lived. Graves (Curtis and Hooker 1826) transferred large groups of *Monotropa* plants from a forest habitat to another site, and observed that plants regrew for six or seven years. This result was ambiguous, however, since fresh communications with trees may have developed rapidly at the new site.

With the aim of studying the question on a more satisfactory basis, a number of root isolation experiments with groups of *Monotropa* were conducted in a spruce forest at Bjurfors, central Sweden, and in pine forests at Södertörn and Österåker, near Stockholm. For this purpose circles of sheet metal 80 cm. in depth and 35 cm. in radius were carefully forced 70 cm. into the soil, so as to enclose groups of vigorous *Monotropa* specimens. Every precaution was taken to ensure that the *Monotropa* roots were not disturbed and that all tree roots were totally isolated from them.

On investigation the following year, some regrowth of weakly plants was observed in a few of the root-isolated areas, whereas untreated groups had

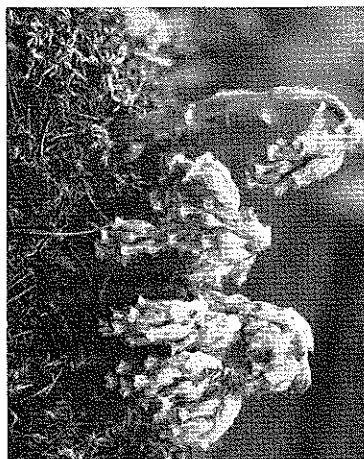


Figure 3.



Figure 4.

Figure 3. Typical group of *Monotropa* plants that regularly return at the same site each year after developing rapidly in about one week.

Figure 4. Growing specimens of *Monotropa* in a group whose original appearance was as in Figure 3 but which, the previous year, had been isolated from all tree roots by sheet metal sunk to a depth of 70 cm. around the group.

shot up normal vigorous specimens from the same "stems" as the preceding year (Figure 3). A few such weakly plants are illustrated in Figure 4. The results accordingly suggest that some growth of new individuals may occur in the absence of communications with tree roots, but that development of normal *Monotropa* plants requires some form of contact with trees.

B. Injection of Radioactive Isotopes into Tree Stems Adjacent to *Monotropa* Plants

To test the hypothesis that *Monotropa* receives its energy from the trees via a mycorrhizal fungus common to both, experiments were conducted with radioactive carbon in the form of C^{14} , which was injected into the phloem of spruce and pine trees which had groups of *Monotropa* at varying distances from the stems. The experiments were carried out in August 1957 and August–September 1958.

1. Experiments of 1957

These experiments were confined to spruce trees with underground *Monotropa*. The trees selected for the purpose were in a closed spruce stand with a moss cover principally composed of *Hylacomium splendens* and *Pleurozium Schreberi*. It was located at Banmarboda, in the parish of Österåker, north of Stockholm. Three

groups of *Monotropa* were chosen for the experiment. Group 1 was composed of fifteen younger, still growing specimens at a distance of 1.1 m. from an approximately 45-year-old spruce and 3.8 m. from the next nearest tree. Group 2 comprised eleven almost fully developed specimens at a distance of 0.9 m. from an approximately 60-year-old spruce and 3.5 m. from the next nearest tree. Group 3, lastly, consisted of thirteen old, almost fully developed specimens situated at a distance of 1.6 m. from a 50-year-old spruce and 3.2 m. from the next nearest tree. A control group of *Monotropa* was selected at a distance of 35 m. from the nearest experimental group.

The C^{14} was supplied in the form of labelled glucose (D-glucose- C^{14}) by the Radiochemical Centre, Amersham, England. It was dissolved in water to give a concentration of 0.1 mC per 10 ml. This solution was portioned between three trees.

In the bark of each tree a shallow hole was bored as far as the phloem, after which an angulated glass tube was inserted and the joint well sealed with vaseline. Into the tube was then dropped with a pipet about 3.5 ml. of the water solution, which had been absorbed after an hour or so. The tube was left in its position. All *Monotropa* specimens selected for the experiment as well as the control group were collected after five days. The material was broken into small pieces and divided into parallel samples, each with a dry weight averaging 3–4 g.

For conversion of C^{14} in organic form to CO_2 and $BaCO_3$, the following method was used, *ad modum* van Slyke and Folch (1940), Claycomb, Hutchens and van Buggen (1950), Evan and Huston (1952) and Comar (1955). The material was first dried at $40^\circ C$ for 12 hours to remove all water. The samples were thereafter placed in a flask and a mixture of H_2SO_4 , H_3PO_4 and CrO_3 added *in vacuo*, whereby all carbon was oxidized to CO_2 . The formed gas was conducted into another flask containing 0.5 M NaOH, whereafter formed carbonate was precipitated with $BaCl_2$ and $0.4 N NH_4Cl$ as $BaCO_3$. This precipitate was then tested for radioactivity in a Geiger-Müller counter (LKB).

Results. — The results of the radioactivity determinations are condensed in Table 1.

In Table 2 are condensed the results of an analysis of variance (Bonnier and Tedin 1957) for the total material with respect to the difference in radioactivity between samples from *Monotropa* plants adjacent to treated trees and samples from plants situated some distance therefrom.

Table 1. Radioactivity per gram dry weight in samples of *Monotropa Hypophytys* collected at varying distances from mature spruces injected with C^{14} . The determinations are the means of six duplicate tests of each kind. The samples were collected five days after injection of C^{14} . Experiment conducted 5th–10th August 1957.

Monotropa group	Distance from treated tree in m.	Counts per minute (CPM)	Actual CPM (CPM—background)
1. Younger plants.....	1.1	33.4	3.7
2. Mature plants.....	0.9	32.7	3.0
3. " ".....	1.6	35.6	5.9
4. Control ".....	35.0	31.3	1.6
Background.....		29.7	

Table 2. Analysis of variance between the radioactivity in *Monotropa* plants adjacent to spruce trees injected with C^{14} and in plants at some distance from treated trees. Cf. Table 1.

Variation source	Degree of freedom	Sum of squares	Mean square
Between treatments	1	5.685	5.685
Within " "	22	5.571	253
Total	23	11.256	

Variance ratio 22.47 0.001 > P***

With a view to checking these results, a further experiment of the same type was conducted at the end of August 1957 in the experimental area of the School of Forestry at Stensången, Bogesund. The experimental site here was an approximately 50-year-old spruce stand with ground vegetation consisting of *Hylocomium splendens* and *Pleurozium Schreberi* as well as occasional *Vaccinium myrtillus* in the field layer. The experimental trees were treated as before with a similar radioactive solution injected into the phloem. *Monotropa* group 5, which comprised fourteen fully developed specimens, was situated at a distance of 1.1 m. from a treated tree; group 6, composed of eleven specimens, at a distance of 0.9 m. from another treated tree. This time, samples of *Monotropa* were collected four days after the injection. The results are presented in Table 3.

As will be seen in Table 3, the actual CPM amounted to 10.1 and 5.5 respectively per gram dry weight for the *Monotropa* plants from beneath the treated trees, but zero for the controls. An analysis of variation between the radioactivity readings for all *Monotropa* samples from groups beneath C^{14} -treated trees, and the readings for samples from the control plants, showed the following values (Table 4).

The experiments accordingly demonstrated that carbohydrate deriving from the trees had been absorbed by the *Monotropa* plants beneath them. If that had not taken place via a common mycorrhiza fungus, the *Monotropa* Table 3. Radioactivity per gram dry weight in samples of *Monotropa Hypopitys* collected at varying distances from mature spruces injected with C^{14} . The determinations are the means of six duplicate tests of each kind. The samples were collected four days after injection of C^{14} . Experiments conducted 25th—27th August 1957.

<i>Monotropa</i> group	Distance from treated tree in m.	Counts per minute (CPM)	Actual CPM (CPM—background)
5. Younger plants	1.1	44.1	10.1
6. Mature plants	0.9	39.5	5.5
7. Control	28.0	33.8	0
Background		34.0	

Table 4. Analysis of variance between the radioactivity in *Monotropa* plants adjacent to spruce trees injected with C^{14} and in plants at some distance from treated trees. Cf. Table 3.

Variation source	Degree of freedom	Sum of squares	Mean square
Between treatments	1	31.930	31.930
Within " "	10	8.549	855
Total	11	40.479	

Variance ratio 37.35 0.001 > P***

plants apparently had not been able to absorb these small actual amounts of soluble carbohydrates in competition with all sugar-demanding fast-growing soil organisms.

2. Experiments of 1958

a. C^{14} experiments. — Corresponding experiments with injection of C^{14} were conducted in 1958, though on pine instead of spruce trees. Some were carried out at Bogesund and others at Bammarboda in the parish of Österåker. At Bogesund the experimental area consisted of a mixed stand of pine and spruce with *Hylocomium splendens* and *Pleurozium Schreberi* in the bottom layer and chiefly a sparse growth of *Vaccinium myrtillus* and *Calluna vulgaris* in the field layer. At the Bammarboda site there was no *Vaccinium* but patches of *Cladonia rangiferina* and *C. sibirica*.

Three groups of *Monotropa* were selected at each site for the experiments, as shown in Table 5. Group 8' comprised seven plants at a distance of 1.2 m. from the treated pine, which was about 90 years old; group 9', six plants at a distance of 1.4 m. from the same tree. Control group '10', consisting of thirteen specimens, was at a distance of 22 m. from the experimental tree. Group 11', numbering eighteen specimens, and group 12', composed of nine plants, were growing at a distance of only 0.5 m. from the treated tree, an approximately 50-year-old pine. Control group '13' (fourteen plants) was 17.5 m. away from the experimental tree. Samples from groups 8 and 11 consisted of younger plants; those from groups 9 and 12, older plants. The latter showed signs of wilting — that is, darkening of stems and flowers.

The injections were as in the preceding experiments, and the plants were collected after four days. The samples were also prepared as before. The time required for 10,000 impulses was determined in the Geiger-Müller counter; hence variations in the readings could be ignored and only one sample of each type needed to be tested. The results are condensed in Table 5.

Following injection on August 18, there had been an appreciable uptake of C^{14} by younger *Monotropa* plants situated 1.2 m. from a treated tree, but a much lesser uptake by older plants of the same group. Following injection 16 days later, when the individual *Monotropa* had reached its full development and the older plants had already begun to wilt, only very slight traces of C^{14} or none at all were detectable in the latter plants; in the younger ones,

Table 5. Radioactivity per gram dry weight in samples of *Monotropa Hypopitys* collected at varying distances from mature pines injected with C^{14} . The samples were collected four days after the injections, which had been made on August 18 (samples 8 and 9) and September 3 (samples 11 and 12) 1958.

Monotropa group	Distance from treated tree in m.	Counts per minute (CPM)	Actual CPM (CPM - background)
8. Younger plants	1.2	40.0	9.2
9. Mature plants	1.4	36.0	5.2
10. Control	22.0	31.2	0.4
11. Younger plants	0.5	34.6	3.8
12. Mature plants	0.5	32.5	1.7
13. Control	17.5	31.5	0.7
Background		30.8	

on the other hand, the C^{14} uptake was distinct though, in view of the short distance from the injected tree, fairly slight.

The experiments thus corroborated the previous year's results, besides throwing some light on the significance of the distance from the tree and on the development stage of the plants as well as their nutritional requirements.

b. *Experiments with P^{32}* . — Since it has now been demonstrated (Colwell 1942, Biddulph and Markle 1944, and others) that not only organic material is transported in the phloem, but also salts that have entered the stem, another run of experiments was conducted with injection of radioactive phosphate (P^{32}), which was introduced into a pine stem exactly as had been done with C^{14} .

The amount of P^{32} was calculated to be 2.0 mC in a solution of 20 ml. orthophosphate in dilute hydrochloric acid two days before the solution was used. In the solution used for injection the concentration of P^{32} was 0.05 mC.

In the experiment, which was conducted at Bogesund, P^{32} -solution was injected into a pine tree beneath which a vigorous *Monotropa* group was growing at a distance of 2 m. from the stem. Samples of *Monotropa* were collected 3 days after the treatment. Following incineration of the samples and preparation of the ashes (cf. Tamm 1955, Oksbjerg 1958) the radioactivity was

Table 6. Actual CPM per gram dry weight in samples of various plants collected at a distance of 2 m. from an approximately 70-year-old pine, into the phloem of which P^{32} had been injected. Experiments conducted 17th—20th August 1958.

Plant	Actual CPM
<i>Monotropa Hypopitys</i>	5.5
<i>Vaccinium Vitis-idaea</i>	0.8
<i>Vaccinium Myrtillus</i>	0.3
<i>Calluna vulgaris</i>	0

determined in a Geiger-Müller counter. Samples of leaves of *Vaccinium myrtillus*, *V. vitis-idaea* and *Calluna vulgaris*, growing at the same distance from the tree, were collected and treated in the same way. In a parallel series, similar samples were collected from corresponding plants 60 m. away from the injected tree. The results of the experiment are summarized in Table 6.

Under these experimental conditions *Monotropa* was thus able even to absorb phosphate from the tree via its fungal hyphae, whereas the other experimental plants having no direct communication with the tree roots had done so only to a very slight extent or not at all.

C. Laboratory Experiments with a Mycorrhizal Fungus of *Monotropa* and with Some Other Fungi

A mycorrhizal fungus occurring in *Monotropa* roots was cultured by the technique employed by Melin (1936), the same fungus obtained from six different samples. The fungus produced a sterile white mycelium which, as mentioned above, Francke (1934) ascribed to a *Boletus* species. In synthesis experiments using Melin's method, this mycelium was found to be capable of forming typical ectotrophic mycorrhizae in pine plants, and probably in spruce plants too, though in these the Hartig network was not clearly distinguishable. Cf. Figure 6 demonstrating incipient mycorrhiza formation on pine plants with the same fungus using Lundebergs (1960) inoculation method.

With this mycelium — nothing, however, contradict the occurrence of different mycorrhiza fungi on different forms or species of *Monotropa* or at different sites — a run of preliminary laboratory experiments was conducted with the principal aim of studying its vitamin requirements in comparison with those of some other fungi. The fungi thus investigated were *Boletus subtomentosus* Fr., *B. granulatus* L. and *B. variegatus* Schwartz, which have been shown, or assumed, to be mycorrhiza producers in conifers (Melin 1936), as well as the typical litter decomposing fungus *Psalliota arvensis* Fr., and the well-known decay fungus *Fomes annosus* Fr.

The flasks used in the experiments had volumes of 100 ml. and contained 25 ml. of the following nutrient solution *ad modum* Melin and Lindeberg (1939):

Glucose	10 g.	$MgSO_4 \cdot 7 H_2O$	0.5 g.
NH_4 -tartrate	1 "	$FeCl_3$ (1% solution)	10 drops
KH_2PO_4	0.5 g.	Distilled water	1,000 ml.
pH after autoclaving 5.5			

In each flask were placed small pieces of agar of uniform size which had been cut out of thin agar cultures of the respective fungi. The flasks — eight of each combination — were stored at +25°C.

Table 7. Dry weight in milligrams of mycelia of various fungi cultured in nutrient solution with addition of extract of *Monotropa Hypopitys* and of vitamin B₁.

Fungus	Duration of experiment in days	Without extract	Without vitamin B ₁						With vitamin B ₁ 1 µg per flask
			With <i>Monotropa</i> -extract of						
			Flowers		Flowers and stems		Roots		
			0.4 %	4.0 %	0.4 %	4.0 %	0.4 %	4.0 %	
Mycorrhiza fungus of <i>Monotropa</i> ..	28	110.5 ± 6.1	145.6 ± 7.7	156.2 ± 9.1	164.4 ± 9.2	212.2 ± 8.8	151.6 ± 11.2	221.3 ± 9.3	154.4 ± 7.6
	34	135.2 ± 7.0	143.3 ± 6.8	168.1 ± 8.7	151.7 ± 6.3	223.5 ± 11.4	162.5 ± 7.9	220.2 ± 8.2	161.3 ± 8.2
<i>Boletus subtomentosus</i>	27	3.8 ± 0.4	15.6 ± 0.9	20.5 ± 1.7	—	—	22.1 ± 2.1	24.6 ± 1.9	30.8 ± 2.2
<i>Boletus granulatus</i>	28	3.2 ± 0.2	6.0 ± 0.4	11.8 ± 0.6	8.8 ± 0.5	14.0 ± 1.1	26.1 ± 1.7	29.7 ± 1.3	34.6 ± 2.6
	35	3.8 ± 0.3	—	—	16.4 ± 1.2	28.7 ± 2.1	—	—	41.1 ± 1.7
	27	4.0 ± 0.2	9.1 ± 0.5	19.2 ± 1.1	12.3 ± 0.7	22.3 ± 1.3	27.2 ± 1.6	39.2 ± 2.4	42.2 ± 2.7
<i>Boletus variegatus</i>	35	6.8 ± 0.4	13.5 ± 0.6	20.5 ± 1.8	—	—	23.1 ± 0.9	35.4 ± 1.6	61.8 ± 4.1
	26	4.8 ± 0.4	—	7.3 ± 0.6	—	9.1 ± 0.7	—	10.3 ± 0.6	46.4 ± 3.1
<i>Psalliota arvensis</i>	23	4.1 ± 0.5	4.7 ± 0.5	9.0 ± 0.7	5.7 ± 0.4	9.3 ± 0.8	6.1 ± 0.5	8.7 ± 0.6	28.1 ± 1.8

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In order to test the influence of *Monotropa*, extracts were prepared from flowers, from entire plants minus the roots, and lastly from the roots alone, which were first carefully washed in distilled water. In preparing the extracts a Turmix apparatus was used first; then followed leaching in distilled water for three days at +2°C. The extract was thereafter run through a Seitz filter, and added to the nutrient solution after autoclaving. In the preliminary experiments reported here only two concentrations of the extracts were used — 0.4 per cent and 4.0 per cent — of which at least the first cannot have had any coincident nutritional effect.

The relevant mycelia were tested both in these substrates and in the same nutrient solution with the addition of 1 µg. of vitamin B₁ per flask; for it had earlier been shown that not least *Boletus* species are markedly stimulated by vitamin B₁ (Melin and Lindeberg 1939, Melin and Nyman 1940, 1941, and others), even though various strains of *Boletus granulatus*, for example, to all appearances may differ in their ability to synthesize that substance (Melin and Nyman 1941). The results of the experiments are condensed in Table 7.

It is evident from Table 7 that the isolated mycorrhiza fungus of *Monotropa* was greatly stimulated by extracts of *Monotropa* but not to any major degree by addition of vitamin B₁. On the other hand, all *Boletus* species that were studied exhibited very pronounced responses to vitamin B₁, but also to *Monotropa* extracts. The disparities in mycelial growth suggest that the *Monotropa* mycelium is not assignable to any of the *Boletus* species tested; yet at the same time its similar reaction to the *Monotropa* extract points to a certain relationship with those species. Each of the other two fungi, *Psalliota arvensis* and *Fomes annosus*, was markedly stimulated by vitamin B₁, though not so much as the mycorrhiza-forming *Boletus* species were by the *Monotropa* extract.

Of great interest, of course, is the chemical character of the stimulatory factor present in *Monotropa*. In analyses of *Monotropa* extracts performed by Dr. Niels Nielsen, Kabi, Stockholm, the vitamin B₁ content was determined both as free thiamine and following acid hydrolysis. In extracts from flowers the total content was found to be 40 µg. per 1 ml., and the free amount to 20 µg. per 1 ml.; in stems the corresponding figures were 25 and 15 µg. per 1 ml. respectively. These amounts must be considered minute and could scarcely exert any stimulating effect on micro-organisms. It is possible, on the other hand, that the substance occurring in *Monotropa* may be identical with or related to Melin's "M factor" (Melin 1954, Melin and Das 1954), which shows little signs of being species specific.

In the opinion of Melin the M factor may be one important cause not only of the vigorous development of the hyphae of the mycorrhizal fungi around conifer roots, but also of their penetration into the roots — although this has not been demonstrated conclusively. In full accord with such a hypothesis is that the factor — here called "*tuburcin*" after the old name *Tuburcinita* — that attracts the fungus which forms mycorrhizae in *Monotropa*, also is

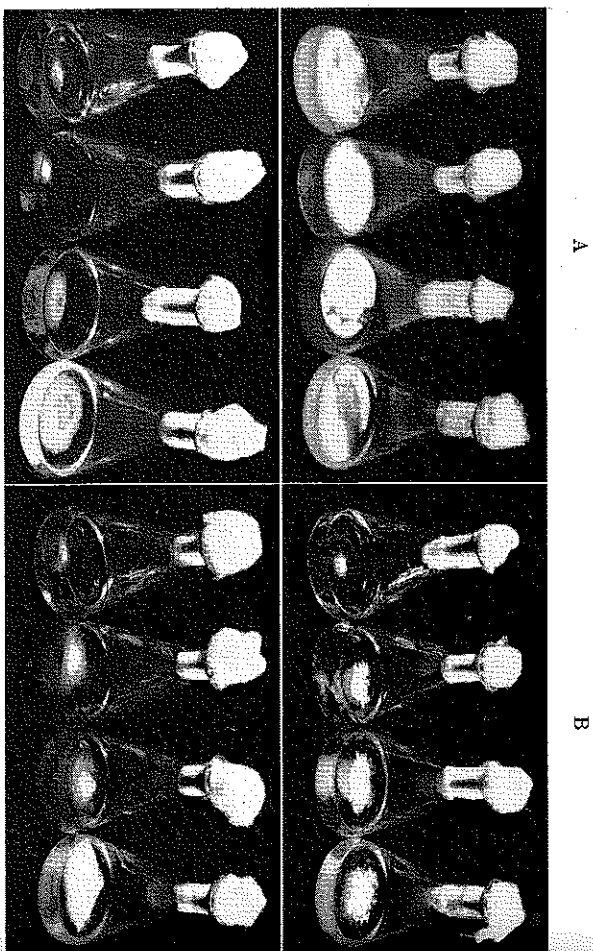
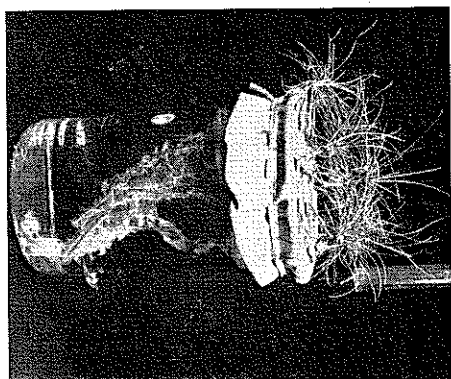


Figure 5. Mycelia of different fungi cultured in flasks containing, from left to right, pure nutrient solution; the same plus extract of *Monotropa* flowers; the same plus extract of *Monotropa* roots; and the same plus vitamin B₁ (cf. Table 7). a. A mycorrhizal fungus of *Monotropa*. b. *Boletus submentosus*. c. *Boletus granulatus*. d. *Boletus partegatus*.

responsible for its penetration into the roots. This penetration is not, however, fully comparable with that of the hyphae in the typical ectotrophic mycorrhiza of, for example, pine and spruce, since the fungus of *Monotropa* penetrates only to the outermost cell layer (Figure 1). In a complete and typical ectotrophic mycorrhiza the fungal hyphae often penetrate all the way to the endodermis and envelop all cells of the primary cortex, where exchange of nutrients occurs by diffusion. But if such a complete ectotrophic mycorrhiza is to be formed, an excess of soluble carbohydrates will have to be available in the cells of the host plant, as demonstrated by Björkman (1942). And since no such surplus can be found in *Monotropa* roots, the fact that the mycorrhizal fungus of *Monotropa* affects only the outermost cell layer, where the "tubercin" is presumably given off from the roots to the rhizosphere, is fully consistent with the hypothesis. Mycelia, incidentally, occur in abundance round *Monotropa* roots, whether the plants are growing in humus-rich soil or in sand; and normal concomitants are fine tree roots surrounded by, to all appearances, identical hyphae, in great part transformed into typical mycorrhizae.

Figure 6. Incipient mycorrhizal formation on pine plants by a fungus isolated from mycorrhizal roots of *Monotropa Hypophytis*. 40 days.



In the light of these facts it is probable that in nature the mycorrhizal fungus of *Monotropa* is identical with that of the tree or trees with which the plant is associated. Since *Monotropa* though achlorophyllous, nevertheless reaches its full development in a remarkably short space of time when the main growth of tree shoots and leaves is ended, manifestly it must have access to large amounts of energy in the form of soluble carbohydrates during that time. This will be possible if the accumulated energy material which is normally available in tree roots during late summer can be mobilized, as it is in the development of the trees themselves. New growth of roots as well as the most vigorous mycorrhiza formation, both of which require large amounts of energy food, normally occur in late summer in northern Europe. Mediation of soluble carbohydrates to *Monotropa* via the common mycorrhizal fungus also provides a natural explanation of the apparently obscure, swift development of that plant during late summer. *Monotropa* is accordingly to be regarded chiefly as an epiparasite on the roots of certain trees with ectotrophic mycorrhizae.

This interpretation is in full conformity with the fact that *Monotropa* will develop vigorously without abundance of humus, which it would need if it were a typical saprophyte, obtaining its principal nutriment directly from organic substances in the soil. *Monotropa* has indeed been found in pure sandy soils south of Stockholm, and on a previous driftsand site with young pines (Almqvist 1929, cf. Romell 1939), and even in pure sanddunes together with *Salix* and other shrubs (Darbishire 1924).

This interpretation of *Monotropa* as an epiparasite attaching chiefly to forest trees through its mycorrhizal fungus also explains the apparently

strange circumstances under which it thrives. The chief interest, however, lies in the principle of the mycorrhiza of *Monotropa* which, despite its special character, and out of the complicated puzzle presented by a symbiosis between such different organisms as fungi and trees or fungi and *Monotropa*, fits well into the general ecological picture.

Summary

1. The ectendotrophic mycorrhiza of *Monotropa* has long been enigmatic in regard to its sources of energy. The fungus cannot obtain sugar from the host plant, rather, the host has to obtain such material via its mycorrhizal fungus.

2. *Monotropa* roots are normally enveloped by masses of fungal mycelia, in which there are tree roots bearing mycorrhizae.

3. Some experiments were conducted to test a hypothesis that *Monotropa* and the trees with which it is associated have a common mycorrhizal fungus. In this way *Monotropa* might receive nutrients from the trees via that fungus, which in turn might be stimulated in its development by some substance present in *Monotropa* and given off by its roots.

4. By totally isolating groups of *Monotropa* from all tree roots, it was shown that the normal development of the plant actually is in some way dependent on the trees.

5. Using radioactive glucose (D-glucose-C¹⁴), which was injected into the phloem of mature pine and spruce trees beneath which grew groups of *Monotropa*, it was found that carbohydrate passed from trees to plants.

6. On similar injection of radioactive phosphorus (P³²), such phosphate was found to have been absorbed by *Monotropa* plants but not so much by *Vaccinium myrtillus*, *V. vitis-idaea*, or *Calluna* growing in their immediate vicinity.

7. A mycorrhizal fungus of *Monotropa*, cultivated from the roots, was able to produce mycorrhiza in pure culture with pine plants and, probably, with spruce plants too.

8. Extracts of *Monotropa* added to cultures of the mycorrhizal fungus of *Monotropa*, three different *Boletus* species, a litter decomposing fungus, and a wood decay fungus, had a powerful stimulating effect especially on the *Monotropa* fungus and the *Boletus* species. The growth-stimulating substance was not vitamin B₁, as otherwise might have been expected in view of its known stimulatory action on the growth of *Boletus* species. The growth factor present in *Monotropa*, called "tuburein", is not unlikely to be identical

with the "M factor" of Melin. In *Monotropa* the mycorrhiza is confined to the outermost cell layer of the roots, whereas in the typical ectotrophic mycorrhiza of conifers the fungus usually penetrates deeper.

9. The investigations suggest that *Monotropa* has its mycorrhizal fungus in common with the tree with which it is associated; that this fungus is stimulated by a substance occurring in *Monotropa*, whereby mycorrhiza formation occurs; and that nutrient material passes from the trees to *Monotropa* via their common mycorrhizal fungus. The rapid development of *Monotropa* in late summer occurs at a time when the production of mycorrhizae reaches its optimum.

10. *Monotropa Hypopitys* is therefore to be regarded chiefly as an epiparasite on the roots of certain trees with ectotrophic mycorrhizae.

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