FURTHER STUDIES ON THE ECTENDOTROPHIC MYCORRHIZA

OLAVI LAIHO

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Preface

The laboratory and field work on which the following paper is based was completed during my year (1963—64) in the United States as an A.S.L.A.-Fulbright grantee at Oregon State University in Corvallis. All necessary facilities were kindly provided by the Department of Botany and Plant Pathology. Numerous field trips, arranged by the Forestry and Botany Schools, served as an excellent way of obtaining field samples from the Pacific Northwest. Additional samples from different parts of the United States, as well as from Canada, Puerto Rico, England and Germany, were collected on the return trip.

The topic of this study was suggested by Prof. PEITSA MIKOLA. It relates closely to the mycorrhizal studies which he has been carrying on in Finland, and in which I have assisted him. The competent advice given by Prof. MIKOLA has been of great help in planning the work and preparing the manuscript. Dr. B. ZAK of the Forestry Sciences Laboratory in Corvallis, with his expert knowledge of local mycorrhizal conditions, followed my work closely. In various ways I was also helped by Dr. E. WRIGHT, Oregon Forest Research Center, Corvallis, Dr. J. TRAPPE, Pacific Northwest Forest and Range Experiment Station, Portland, and Drs. L. ROTH, Prof. of Plant Pathology, W. FERRELL, Prof. of Forest Ecology, and C. YOUNGBERG, Prof. of Soils, all from Oregon State University. The language was checked by Mrs. J. M. PERTTUNEN, B. Sc. and Mr. T. BEEBE, B. S.

I wish to express my sincere gratitude to the persons mentioned above, as well as to nurserymen and many others not mentioned by name. I would also like to thank the Department of Silviculture, University of Helsinki, where this work was completed, and the Society of Forestry in Finland for publishing this study in its series of periodicals.

Helsinki, Department of Silviculture, March 1965.

Olavi Laiho.

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Introduction

The term »ectendotrophic mycorrhiza» was introduced by MELIN. He described three essentially different forms of this type of mycorrhiza. One of them is characterized by a thin mantle, coarse Hartig net and strong intracellular infection, particularly deeper in the cortex (MELIN 1923 b, p. 95). The so-called »C» mycorrhiza of pine, which has a very thick mantle, is, according to him, another ectendotrophic form (p. 105). The third form, found in birch, has, besides a normal mantle and Hartig net, two kinds of intracellular hyphae, viz. thin ones and thick »protein» hyphae (1923 a, p. 483). A feature common to all these forms was the digestion of intracellular hyphae at a certain stage of development of the association, and it is actually this digestion that renders the relationship symbiotic (1923 b, pp. 100, 269).

Similar associations, but under different names, had been described before, for instance by VON TUBEUF and MCDOUGALL (see MELIN 1923 b, p. 108). Recently, LEVISOHN (1954, 1963) has used the term pseudomycorrhiza to refer to them. There has not been complete agreement as to what is meant by ectendotrophic mycorrhizae. On the whole, there is a wide variety of opinion among authors on mycorrhizal terminology (MIKOLA 1965). In this work the definition used by MIKOLA is followed. Ectendotrophic mycorrhizae are short roots with a Hartig net and intracellular hyphae in the cortex. A mantle and digestion of intracellular hyphae may be found but are not necessarily present. Even this definition, simple as it is, leaves room for different interpretations. The line separating ectendo- and ectotrophic mycorrhizae is not sharp: the latter may also have intracellular hyphae (MELIN 1923 b, p. 108). Secondary infections may sometimes also be difficult to distinguish.

Ectendotrophic mycorrhizae seem to be very rare (MÖLLER 1947); many authors do not mention them at all. Important trees in the northern coniferous region in general have ectotrophic mycorrhiza (TRAPPE 1962 b). Therefore it was surprising that in connection with a nursery study (LAIHO & MIKOLA 1964) abundant ectendotrophic mycorrhizae were found. A new study was started (MIKOLA 1965) and ectendotrophism was found to be very common in Finnish nurseries on Scotch pine (*Pinus silvestris* L.). On Norway spruce (*Picea abies* (L.) KARST.) it was never encountered; in the forest it was rare even in pine. Morphologically, these mycorrhizae were always the same: mantle thin or lacking, Hartig net present, and intracellular infection strong, particularly deeper in the

cortex. All these formations were caused by the same coarse mycelium which was later isolated. All 150 isolates were similar sterile mycelia, so far unidentified, but probably belonging to the same species. In this paper they are called E-strains.

These results aroused strong interest in the phenomenon. Was it a local one or would it be found in other countries as well? What tree species were involved? The answers were not to be found in the literature; field work was necessary. A grant given to the author at a very convenient time made it possible to start the work.

As mentioned, little information is available in the literature and what exists is scattered and consists mainly of short passages. In the list given on p. 6 all the references found to »ectendotrophic» trees are mentioned, as well as similar associations reported under other names. These references give the impression that

List of references to ectendotrophic mycorrhizae, by tree species

Pinaceae

Abies balsamea (L.) MILL. — KELLEY 1950, p. 168: mycorrhiza with intracellular hyphae

A. firma SIEB. et ZUCC. — MASUI 1926a: ectendotrophic mycorrhiza (from KELLEY 1950, p. 123)

Larix decidua MILL. — BERGEMANN 1955: mycorrhizae with intracellular hyphae in pot seedlings

— LAIING 1923: endotrophic and semiectotrophic mycorrhizae in plantation

— MELIN 1922, p. 163: ectendotrophic mycorrhiza in plantation

L. laricina (DU ROI) K. KOCH — LEVIS 1924: endotrophic infection in nature

Picea abies (L.) KARST. — BERGEMANN 1955: mycorrhizae with intracellular hyphae in pot seedlings

— BJÖRKMAN 1940, 1942: ectendotrophic mycorrhizae in varied material

— LINDQUIST 1937, p. 307: »Dn» mycorrhizae with intracellular hyphae in old growth

— MELIN 1923b, p. 106: ectendotrophic mycorrhiza in forest

— PEKLO 1913: endotrophic mycorrhiza (from MELIN 1923b, p. 88)

P. engelmannii PARRY — MCDOUGALL & JACOBS 1927: ectoendotrophic mycorrhiza in forest

— THOMAS 1943: ectendotrophic mycorrhiza in forest

P. glauca (MOENCH) VOSS — LEVIS 1924: endotrophic infection in nature

P. sitchensis (BONG.) CARR. — BERGEMANN 1955, 1956: endotrophic mycorrhiza in pot seedlings

Pinus banksiana LAMB. — MCCOMB 1943: ectendotrophic mycorrhiza in nursery seedlings

P. caribaea MORELET — YOUNG 1938: ectendotrophic mycorrhiza in nursery seedlings

P. cembra L. — VON TUBEUF 1903, p. 81 Fig. 2: ectendotrophic mycorrhiza (see MELIN 1923b, p. 108)

P. contorta DOUGL. — LEVISOHN 1954: haustorial pseudomycorrhiza in pot and nursery seedlings

— MCDOUGALL & JACOBS 1927: ectoendotrophic mycorrhiza in forest

P. monophylla TORR. et FREM. — MCDOUGALL & JACOBS 1927: endotrophic mycorrhiza in forest

P. mugo TURRA — MÜLLER 1903: endotrophic mycorrhiza (see MELIN 1923 b, p. 88)

P. nigra ARNOLD — ALDRICH-BLAKE 1930: ectendotrophic mycorrhiza (from KELLEY 1950, p. 123)

— LEVISOHN 1954: haustorial pseudomycorrhiza in pot and nursery seedlings

P. ponderosa LAWS. — GOSS 1960: ectendotrophic mycorrhiza in varied material

P. radiata D. DON — LEVISOHN 1954: haustorial pseudomycorrhiza in pot and nursery seedlings

P. silvestris L. — BERGEMANN 1955, 1956: pseudomycorrhizae in pot seedlings

— BJÖRKMAN 1940, 1942: ectendotrophic mycorrhiza in varied material

— ENDRIGKEIT 1937, p. 65: pathogenic pseudomycorrhiza in plantation (see BJÖRKMAN 1942, p. 58)

— LAIHO & MIKOLA 1964: ectendotrophic mycorrhiza in nursery seedlings

— LAIING 1923: endotrophic mycorrhiza in nature

— LEVISOHN 1954, 1963: haustorial pseudomycorrhiza in varied material

— MELIN 1923b, pp. 95, 105: ectendotrophic mycorrhizae in nature

— MELIN 1927, p. 444: »E» mycorrhiza in pot seedling

— MELIN 1927, p. 452: »D» mycorrhiza with intracellular hyphae in pot seedling

— MÖLLER 1902: endotrophic mycorrhiza in varied material

— MIKOLA 1965: ectendotrophic mycorrhiza in varied material

— MIKOLA, LAIHO, ERIKÄINEN & KUVAJA 1964: ectendotrophic mycorrhiza in seedlings

— PEKLO 1913: endotrophic mycorrhiza (from MELIN 1923b, p. 88)

— RAYNER 1934, p. 101: ectendotrophic mycorrhizae in varied material

— RAYNER & NEILSON-JONES 1944: ectendotrophic mycorrhizae in varied material

P. strobus L. — HACSKAYLO & PALMER 1957: ectendotrophic mycorrhiza in plantation

— MCCOMB 1943: ectendotrophic mycorrhiza in nursery seedlings

Pseudotsuga menziesii (MIRB.) FRANCO — LINNEMANN 1955, p. 402: ectendotrophic mycorrhiza in seedlings

— MCDOUGALL & JACOBS 1927: endotrophic mycorrhiza in forest

Tsuga heterophylla (RAF.) SARG. — LAIING 1923: semiectotrophic mycorrhiza in nature

Betulaceae *Alnus* sp. — MASUI 1926b: ectendotrophic mycorrhiza (from KELLEY 1950, p. 123)

Betula pendula ROTH. — MELIN 1923a, p. 483: ectendotrophic mycorrhiza in forest

Cornaceae *Cornus florida* L. — KELLEY 1950, p. 166: ectendotrophic mycorrhiza

Fagaceae *Fagus silvatica* L. — HARLEY 1959, p. 33: coralloid infection with high level of intracellular hyphae

Leguminosae *Gleditsia triacanthos* L. — THOMAS 1943: ectendotrophic mycorrhiza in forest

Robinia pseudoacacia L. — THOMAS 1943: ectendotrophic mycorrhiza in forest

Oleaceae *Fraxinus pennsylvanica* MARSH. — THOMAS 1943: ectendotrophic mycorrhiza in forest

Rosaceae *Cercocarpus montanus* RAF. — THOMAS 1943: ectendotrophic mycorrhiza in forest

Prunus virginiana L. — THOMAS 1943: ectendotrophic mycorrhiza in forest

Salicaceae *Populus tremula L.* — MELIN 1923a, p. 480: ectendotrophic mycorrhiza in forest

P. tremuloides MICHX. — MCDOUGALL & JACOBS 1927: endotrophic mycorrhiza in forest

Tiliaceae *Tilia americana* L. — MCDOUGALL 1914: heterotrophic mycorrhiza in nature (see MELIN 1923b, p. 108)

the type of ectendotrophic mycorrhiza studied by MIKOLA had been observed earlier by many other investigators on pine. On the other hand, ectendotrophic spruce mycorrhizae remain a mystery, as do the answers to such basic questions as how common they are, how many types of them there are and what fungi are involved. As far as fungi are concerned there is not even complete agreement as to whether there are one or more fungi in one and the same ectendotrophic mycorrhiza (BJÖRKMAN 1956, p. 269). These fungi have been isolated only twice, viz. by

References to fungus species forming ectendotrophic mycorrhizae

Amanita muscaria (L. ex FR.) PERS. ex HOOKER — MELIN 1923a, p. 502: ectendotrophic mycorrhiza in aseptic synthesis with *Betula pendula*

Cenococcum graniforme (SOW.) FRED. & WINGE — LINDQUIST 1937, p. 307: »Dn» mycorrhizae with intracellular hyphae on *Picea abies* in forest

— MELIN 1927, p. 452: »D» mycorrhiza with intracellular hyphae in a pot seedling of *Pinus silvestris*

— MIKOLA 1948, pp. 83—4: »Dn» mycorrhizae with intracellular hyphae in aseptic synthesis with *Betula verrucosa*

— TRAPPE 1962a, 1964: ectendotrophic »Dn» mycorrhizae with many tree species in aseptic synthesis and in nature

E-strains — MIKOLA 1965: ectendotrophic mycorrhiza in aseptic and semiaseptic synthesis with *Pinus silvestris*

Leccinum aurantiacum (BULL.) S. F. GRAY (*Boletus rufus*) — MELIN 1923a, pp. 498—9: ectendotrophic mycorrhiza in aseptic synthesis with *Betula pendula* and *Populus tremula*

L. scabrum (BULL. ex FR.) S. F. GRAY (*Boletus scaber*) — MELIN 1923a, pp 497—8: ectendotrophic mycorrhiza in aseptic synthesis with *Betula pendula* and *Populus tremula*

Lyophyllum immundum (BERK.) KÜHN. (*Tricholoma fumosum*) — NORKRANS 1950, p. 75: ectendotrophic mycorrhiza in aseptic synthesis with *Pinus silvestris*

Mycelium radicis silvestris α (*Boletus* sp.) — MELIN 1923b, p. 173: ectendotrophic mycorrhiza in aseptic synthesis with *Picea abies* and *Pinus silvestris*

Mycelium radicis silvestris β (clamps) — ectendotrophic mycorrhiza in aseptic synthesis with *Larix decidua* (MELIN 1922, p. 185), *Picea abies* and *Pinus silvestris* (MELIN 1923b, p. 173)

Mycelium radicis silvestris γ (clamps) — ectendotrophic mycorrhiza in aseptic synthesis with *Betula pendula* (MELIN 1923a, p. 507), *Picea abies* and *Pinus silvestris* (MELIN 1923b, p. 173)

Rhizoctonia silvestris — MELIN 1923b, p. 193: ectotrophic mycorrhiza with intracellular hyphae in aseptic synthesis with *Pinus silvestris*

Rhizoctonia silvestris type — DOAK 1934: strong infection in aseptic synthesis with *Pinus radiata, P. resinosa, P. strobus* and *P. taeda* (see Goss 1960, p. 42)

Rhizoctonia sp. — LEVISOHN 1954, 1963: haustorial pseudomycorrhiza in greenhouse synthesis with *Pinus contorta, P. nigra, P. radiata* and *P. silvestris*

Tricholoma flavobrunneum (FR.) KUMM. — MELIN 1923a, p. 500: ectendotrophic mycorrhiza in aseptic synthesis with *Betula pendula*

Suillus granulatus (L. ex FR.) O. KUNTZE. (*Boletus granulatus*) — YOUNG 1938: ectendotrophic mycorrhiza with *Pinus caribaea* in a nursery experiment

LEVISOHN and by MIKOLA (see list on p. 8). Many other fungi reported to have formed ectendotrophic mycorrhizae in aseptic synthesis are better known as ordinary ectotrophic symbionts. It is not certain whether all the cases mentioned in the above list should be classified as ectendotrophic or even whether the fungi listed form similar associations in nature.

There are two methods to be followed in solving the problems relative to ectendotrophic mycorrhizae. One is to sample mycorrhizae to see how common ectendotrophism is, and what forms of it exist in nature. The other is to isolate the fungal symbionts involved and let them produce mycorrhizae with different tree species to see what forms belong together. In this study both approaches were used.

The question of the true nature of ectendotrophic fungus-root associations has been the object of much speculation. Two different opinions have been presented. Because of the digestive process observed by him, MELIN considered it to be a true symbiosis (1923 b, pp. 100, 269). BJÖRKMAN, like many others, did not notice any digestion, and regarded the relationship as only partially symbiotic (1949, pp. 237—8). The fungus involved was not available to the authors mentioned, and physiological experiments, the importance of which is emphasized by MIKOLA (1965) and many others, could not be made. LEVISOHN (1954) did make some, however, and she regarded the relationship as one-sided parasitism. On the other hand, MIKOLA (1965) could find no harmful effects of the fungus but neither did he observe any clear benefits from its presence. More experiments under different conditions were needed.

In short, the following questions were studied in this work:

1. What kind of ectendotrophic mycorrhizae exist in forests and forest nurseries outside Finland, particularly in North America?

2. What kind of mycorrhizae, if any, do E-strains isolated from Scotch pine and Norway spruce form with other tree species?

3. Are these associations symbiotic or one-sidedly parasitic?

Methods and material

The mycorrhizal material used in this study is of two kinds, i.e. synthetic and natural. Synthesis experiments were conducted under both aseptic and semiaseptic conditions. Natural root material was collected from different forest stands as well as from forest nurseries.

Aseptic synthesis is the only sure way to prove that a certain mycelium forms mycorrhizae. Therefore, it was included in this study. A slight modification was made in MELIN's (1936) technique: instead of quartz sand, vermiculite was used as a substrate, mixed with 20 per cent peat moss (by volume) to keep the pH at about 5.0. The nutrient solution was that of NORKRANS (1949). Seeding was done

on Nov. 5, 1963, inoculation on Dec. 17, and harvesting on April 9, 1964. The inoculum was grown on Hagem agar from three stock cultures of Finnish E-strains, described as follows:

E—15. Isolated fall 1962, from an ectendotrophic mycorrhiza on a Scotch pine nursery seedling.
E—35. Isolated fall 1962, from a similar Scotch pine seedling.
E—57. Isolated fall 1962, from an actotrophic mycorrhiza on a Norway spruce nursery seedling.

All these strains, comparable in their morphology and physiology, had previously formed similar ectendotrophic mycorrhizae with Scotch pine in aseptic synthesis (MIKOLA 1965). In this study they were tested with 14 tree species.

Aseptic synthesis has two major shortcomings, however: it is laborious and unnatural. Morover, the reactions of the host cannot be studied (BJÖRKMAN 1942, pp. 129—30, footnote). Therefore, a semiaseptic method was employed, using autoclaved soil and seedlings grown in open pots and inoculated from pure cultures. The soil used in this experiment was taken from a stand of Douglas-fir (*Pseudotsuga menziesii* (MIRB.) FRANCO). For ponderosa pine, (*Pinus ponderosa* LAWS.) soil from a ponderosa stand was also used. No fertilizers were added. According to an analysis made when the experiment was over, the nutrient level of both these soils was relatively high, but unbalanced (Table 1). The amounts of available nutrients were about the same as recommended for forest nurseries by WILDE (1958, p. 360) and somewhat lower than the average in Finnish nurseries (MIKOLA 1957), but a severe phosphorus deficiency is evident. The possible effect of autoclaving on available nutrients was not visible any longer (5.5 months later).

Table 1. Some properties of the soils used in the semiaseptic experiment.

Origin	Treatment	Loss on ignition		N	Exchangeable		
					K_2O	CaO	P_2O_5
		%	pH	%	mg/100 g		
Surface soil (loam) from a 200-yr.-old Douglas-fir stand. Site index II. Alsea Basin area, Oregon.	Autoclaved	12.3	5.7	0.15	22.4	101	0.1
	Not autocl.	13.2	5.5	0.15	24.8	111	0.4
Surface soil (pumice sand) from a 200-yr.-old ponderosa pine stand. Site index III. Pringle Falls, Oregon.	Autoclaved	8.2	6.2	0.12	25.6	141	0.5
	Not autocl.	7.1	6.1	0.11	28.8	131	0.5

To tie up this experiment to those made in Finland, Scotch pine and Norway spruce were included. The other 22 species, most of them common in the Pacific Northwest, were chosen so as to include both conifers and broad-leaved trees reported to have ecto-, endo- and ectendotrophic mycorrhiza in nature. The seed

used was from test samples sent to the Oregon State Seed Laboratory, Corvallis. Sterilization was done by immersion in 30 % hydrogen peroxide (10—60 min. depending on seed coat), thereby also quaranteeing higher and faster germination (TRAPPE 1961). Seeding was done Nov. 3, 1963, inoculation between Dec. 17 and Jan. 20, and harvesting about April 20, 1964. E-strains 15, 35 and 57 were also used in this experiment. The inoculum was grown on sand moistened with MOSER's (1958) solution. In some cases Hagem agar colonies were used. Both types worked well when a square centimeter of the colony was buried near roots close to the soil surface. Three different treatments were included:

1. Soil autoclaved (three hours at 109° C), seedlings inoculated with an E-strain. Structure of mycorrhizae and reaction of seedlings were studied.
2. Soil autoclaved but not inoculated. In this control, seedling development was studied.
3. Soil not autoclaved and not inoculated. In this control, structure of mycorrhizae and reaction of seedlings were studied.

The experiment totaled 300 ordinary clay pots (diam. 4″), which were kept in a greenhouse and watered daily. In the fall, the seedlings suffered from low temperature (about 15° C) and reduced light, although an additional light of 200 foot-candles (18 hour photoperiod) was used. In the spring, growth conditions were more favorable.

The seedlings numbered ten per pot or, in some species, where germination was low, less. When harvested, they were individually examined under a binocular microscope (magnification x 5—20). Lest any possible contamination should escape notice, special emphasis was placed on the homogeneity of the mycorrhizae. For all treatments, the following quantitative determinations were made:

Relative number of mycorrhizal short root tips
Relative number of dichotomous short root tips
Length of epicotyl
Dry weight (dried at room temperature), shoot and roots seperately

Additional notes were made of shoot color, root hairs, etc. Representative seedlings were fixed for later microscopic examination. The macroscopic short root classification was checked by microscopic examination. Above all the anatomy of the mycorrhizae was studied.

Short root samples from forests and nurseries were mainly collected during field trips arranged by the Forestry and Botany Schools. Thus, experts were available to help in finding representative stands. The most critical point is to know with certainty the identity of the roots being sampled. Therefore samples from seedlings were taken by lifting the whole seedling; from older trees only pure stands were sampled. All told, 108 samples were taken from forest stands and 44 from forest nurseries, a total of 41 tree species being included. Most of these samples (details given in Tables 5 and 6) are from a relatively small area,

mainly in Oregon. Nevertheless, there was a wide range of types included, varying from coastal »rain forests» to dry ponderosa stands in East Oregon, from the lowlands to the Cascade and Siskiyou Mountains, from fertile clay to dune sand and from pumice to serpentine soils. Samples from other areas are relatively few and mainly from nurseries and their environs. Of these, Saratoga (New York), Albany (Georgia), Puerto Rico, Kennington (England) and Halstenbek (Germany) may be mentioned. Most samples were taken from within the natural range of the species. Both plantations and old growths were included. Sampling was done the year round.

From these samples, the number of symbionts and the general structure of the short roots were determined under a binocular microscope. Representative samples were fixed and the anatomy of mycorrhizae studied microscopically. A total of 628 short roots were sectioned with a paraffin microtome. The thickness of the sections was 5—10 microns. Double staining with safranin and fast green was used, according to MIKOLA and PERSIDSKY (1951). The same technique was also used in connection with the aseptic and semiaseptic experiments, the number of sections being 61 and 390, respectively. Thus the total number of short roots sectioned exceeds a thousand.

Finally, some attempts were made to isolate fungal symbionts of ectendotrophic mycorrhizae. MELIN's (1936) technique was used with slight modifications. The best of these, which is practically the same as the one successfully employed on a previous occasion by MIKOLA (1965), is given below. With this medium

Treatment of mycorrhizae	*Medium*		
Washed in running water	KH2PO4	1	g
1 min. in 70 % ethanol to remove air bubbles	NH4C1	0.5	g
10 sec. in 0.1 % $HgC1_2$	MgS04	0.5	g
Three rinses in sterile water	Fe-citrate	10	mg
Cutting into pieces and plating	Glucose	5	g
	Agar	5	g
	Gelatin	10	g
	Yeast extract	100	mg
	Coconut milk	20	ml
	Water (dist.)	1 000	ml
	Streptomycin (after autocl.)	50	ppm

ectendotrophic symbionts were isolated, particularly if the mycorrhizae used were young, large, smooth and fresh. On one occasion, 600 mycorrhizae were plated; 80 of them yielded *Mycelium radicis atrovirens*, and various molds and bacteria. In twenty plates uncontaminated E-strains grew from the mycorrhizae; none of the others yielded any outgrowth. Thus, on the average every thirtieth mycorrhiza plated gave a symbiont. In Sitka spruce (*Picea sitchensis* (BONG.) CARR.) the yield was about 50 % (13 out of 27), a very high rate even as compared with isolation experiments on ectotrophic symbionts (ZAK & MARX 1964). The time

required for these isolates to grow out of mycorrhiza was surprisingly short, only 6—12 days. For many ectotrophic symbionts it takes weeks or even months to do the same (MELIN 1923 b, p. 126).

Results

Aseptic mycorrhiza synthesis

Primarily, 60 flasks and 14 tree species were included in this experiment. At harvesting, five months later, mycorrhizae were found in nine flasks representing seven tree species. Furthermore, even in these only a few mycorrhizae were found, which microscopic examination (Table 2) showed to be very diverse in nature. In pine, the typical ectendotrophic mycorrhiza was present (Fig. 1), as well as ectotrophic ones and even short roots surrounded by a loose mantle only. Mycorrhizae formed by E-strains with spruces and Douglas-fir were all ectotrophic, but the mantle was only occasionally present.

Table 2. Microscopic structure of mycorrhizae formed by E-strains in aseptic synthesis. Seeded Nov. 5, 1963, inoculated Dec. 17, harvested April 9, 1964. Corvallis, Oregon.

Tree species	Of mycorrhizae sectioned				
	Ectendotrophic		Ectotrophic		
	Without mantle	With mantle	Without mantle	With mantle	Mantle only
Picea abies (L) KARST.				3	
P. engelmannii PARRY			6	1	
P. pungens ENGELM.			2	1	
Pinus ponderosa LAWS.			3		
P. silvestris L.	2	3			
P. strobus L.					6
Pseudotsuga menziesii (MIRB.) FRANCO			2		

Among those not producing mycorrhizae were species of pine, spruce, fir, larch and sequoia. This does not definitely prove that they were incapable of forming mycorrhizae with E-strains. Contamination may have killed the seedling or the inoculum. The low aeration, as evidenced by numerous lenticels, may also have prevented mycorrhiza formation, as well as affecting the structure of those formed. Finally, the time was too short.

Despite their shortcomings, these results show that E-strains are cabable of forming mycorrhizae with other species besides pines. The mycorrhizae formed are not necessarily ectendotrophic but may also be ordinary ectotrophic ones. This is in line with the fact that some E-strains are isolated from ectotrophic spruce mycorrhizae. Similarly, in earlier synthesis experiments (MIKOLA 1965)

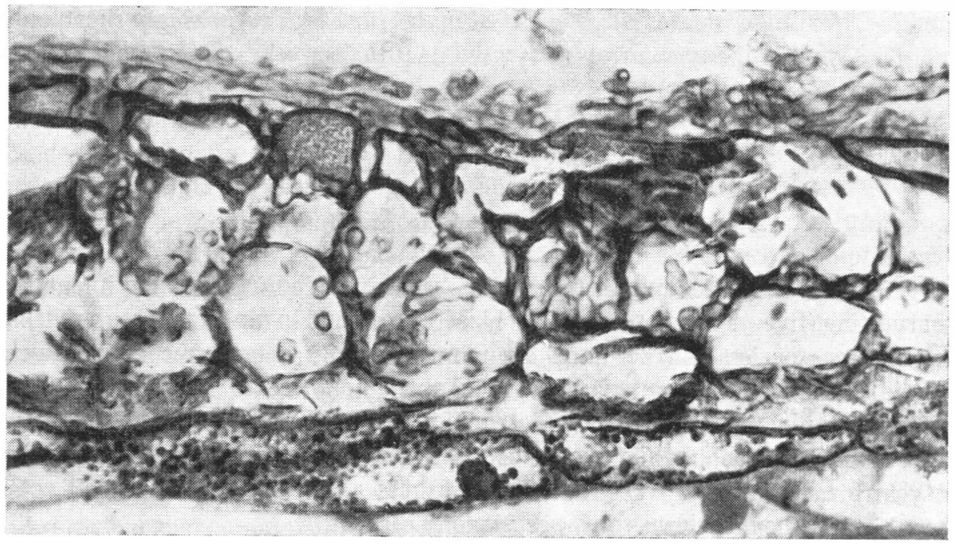

Fig. 1. Longitudinal section of an aseptically synthesized ectendotrophic mycorrhiza. The loose mantle may be noted. *Pinus silvestris* and E-15. Magnification x 550.

E-strains have without exception formed ectotrophic mycorrhizae with Norway spruce. On the other hand, it seems very likely that E-strains do not infect species with endotrophic mycorrhiza. In many uncontaminated flasks E-strain was growing around the roots of the giant sequoia (*Sequoia gigantea* (LINDL.) DECNE) but did not even penetrate the surface cells.

Semiaseptic mycorrhiza synthesis

Contamination

There are two essential qualifications which a semiaseptic mycorrhiza synthesis must fulfill: the inoculum must be successfully introduced, and outside contamination by mycorrhizal fungi must be prevented or eliminated. When this experiment was harvested, it turned out that one-quarter of the autoclaved control pots was contaminated (see list below).

Soil autoclaved, uninoculated: mycorrhizae in 12 pots out of 46
Soil autoclaved, inoculated with E-strains: mycorrhizae in 79 pots out of 82
Soil not autoclaved, uninoculated: mycorrhizae in all 65 pots

Inoculated pots were almost all mycorrhizal. Thus the inoculum took well, but on the other hand there was contamination. In autoclaved uninoculated pots the contamination was usually restricted to a small part of the pot and was of two kinds; the mycorrhizae were either ectotrophic with a thick white mantle or, more often, similar to the mycorrhizae in the inoculated pots. Thus in most

cases contamination had originated from inoculated pots (the pots were kept side by side). Of course, there were white contamination mycorrhizae in the inoculated pots also, but even there it was possible to recognize them. It was of great help that the mycelium of E-strains, which is easy to recognize, was visibly growing in many inoculated pots. Finally, E-strains were reisolated from ectendotrophic ponderosa pine mycorrhizae and ectotrophic mycorrhizae of Sitka spruce and western hemlock (*Tsuga heterophylla* (RAF.) SARG.).

All pots in which contamination was discovered were excluded from further consideration, so that the information given below refers only to mycorrhizae formed by E-strains.

Structure of mycorrhizae

The results obtained by microscopic examination of the mycorrhizae formed by E-strains in this semiaseptic synthesis show many distinct features (Table 3).

Table 3. Microscopic structure of mycorrhizae formed by E-strains in a semiaseptic synthesis. Seeding (in most cases) Nov. 3, 1963, inoculation between Dec. 7 — Jan. 20, harvested about April 20, 1964. Seedlings raised in open pots in a greenhouse. Corvallis, Oregon.

Family	Of mycorrhizae sectioned			
	Endotrophic	Ectendotrophic	Ectotrophic	
		All without mantle	Without mantle	With mantle
Pinaceae. *Abies lasiocarpa* (HOOK.) NUTT.			4	
A. procera REHD.			8	
Larix occidentalis NUTT.		9	4	
Picea abies (L.) KARST.			2	
P. engelmannii PARRY			4	
P. glauca (MOENCH) VOSS			7	
P. pungens ENGELM.			3	1
P. sitchensis (BONG.) CARR.			12	1
Picea sp.			1	
Pinus edulis ENGELM.		1	4	1
P. monticola DOUGL.		4		
P. ponderosa LAWS.		26	1	
P. radiata D. DON		3		
P. silvestris L.		4		
P. strobus L.		1	3	
Pseudotsuga menziesii (MIRB.) FRANCO			22	
Tsuga heterophylla (RAF.) SARG.			5	1
Cupressaceae. *Chamaecyparis lawsoniana* (A. MURR.) PARL.	0	0	0	0
Libocedrus decurrens TORR.	0	0	0	0
Taxodiaceae. *Sequoia gigantea* (LINDL.) DECNE	0	0	0	0
Aceraceae. *Acer macrophyllum* PURSH	0	0	0	0
Betulaceae. *Betula verrucosa* EHRH.			3	3
Salicaceae. *Populus trichocarpa* TORR. et GRAY			1	

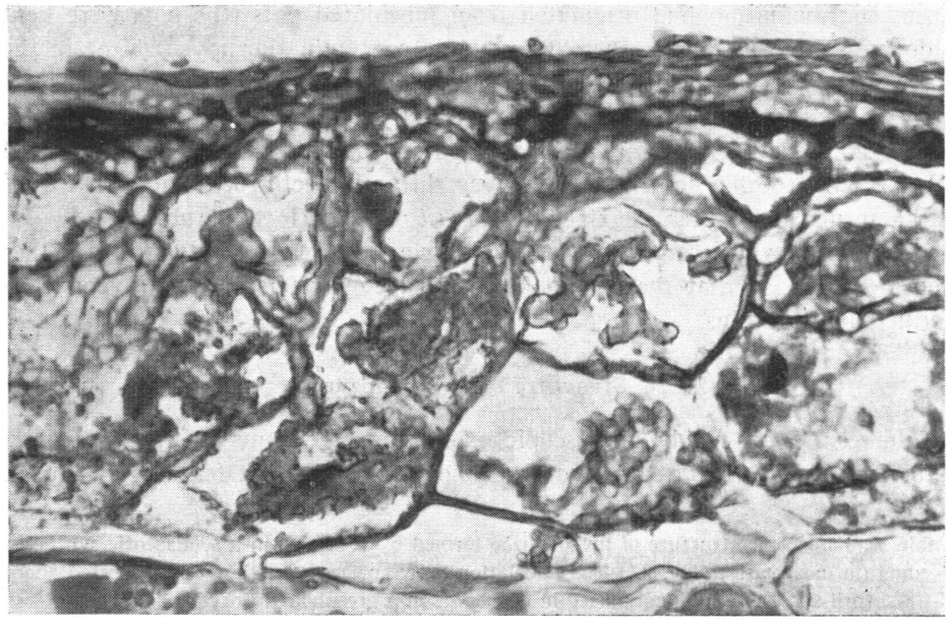

Fig. 2. Longitudinal section of an ectendotrophic mycorrhiza. No mantle, all intracellular hyphae not clearly visible. *Pinus ponderosa* and E-35 in semiaseptic synthesis. Magnification x 550.

Mycorrhizae were formed with 19 out of the 20 tree species included which had been reported to have ectotrophic mycorrhiza in nature. However, they were very different from each other. On the other hand, no mycorrhizae were formed with the four species reported to have endotrophic mycorrhiza. Thus the results essentially confirm those obtained in the aseptic synthesis.

In all six pine species ectendotrophic mycorrhizae were formed; nearly all of them were well developed (Fig. 2). One exception was a short root in which the cortical cells were filled with fungal pseudoparenchyma (Fig. 3). In pinyon (*Pinus edulis* ENGELM.) and eastern white pine (*Pinus strobus* L.) ectotrophic mycorrhizae, similar to that illustrated in Fig. 5, were present. Ectendotrophic mycorrhizae formed with the same species contained only a few intracellular hyphae. Thus, not all pine species seem to be equally inclined towards ectendotrophism. According to MIKOLA (1965), E-strain mycorrhizae on Scotch pine are always ectendotrophic. In this experiment, this species was the first to be mycorrhizally infected (seeding Nov. 3, first mycorrhizae about Jan. 15) and even those very first mycorrhizae were clearly ectendotrophic. Thus, although the amount of intracellular hyphae increases somewhat as the mycorrhizae grow older (MIKOLA 1965), it does not affect classification in this and many other species. In species

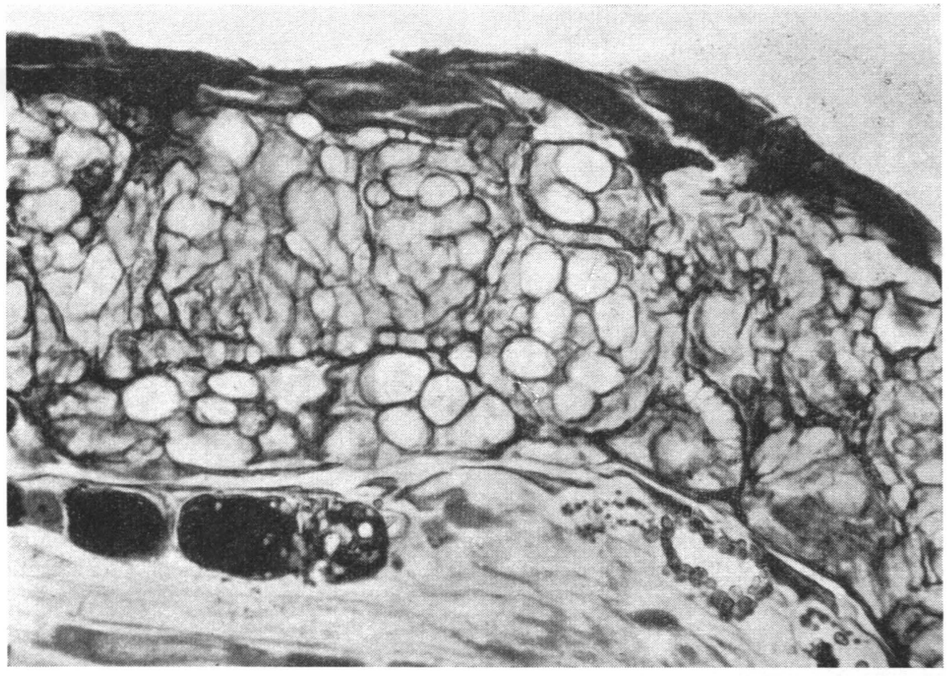

Fig. 3. Longitudinal section from the base of an ectendotrophic mycorrhiza. No mantle, Hartig net throughout the cortex, many cortical cells filled with fungal pseudoparenchyma. *Pinus silvestris* and E-57 in semiaseptic synthesis. Magnification x 450.

like pinyon and eastern white pine it probably does, however. Their E-strain mycorrhizae are recognized as ectendotrophic only after the intracellular infection increases with age.

In larch (*Larix occidentalis* NUTT.), ectendotrophic mycorrhizae similar to those in pine were observed (Fig. 4), but some young mycorrhizae were of the ectotrophic type, too. The mycorrhizae of spruce, fir, hemlock and Douglas-fir were universally ectotrophic. In all of these latter genera, E-strain mycorrhizae were consistent in character: a coarse Hartig net throughout the cortex, mantle and intracellular hyphae lacking (Fig. 5).

In the case of birch (*Betula verrucosa* EHRH.), the situation was somewhat different. It was characteristic of the species that a Hartig net surrounded the hypertrophied cells of a single cortex layer (Fig. 6). Sometimes there was a mantle, but intracellular hyphae were not found. The mycorrhizae examined must be regarded as ectotrophic but from this it can not be concluded that no other types exist, because the mycorrhizae formed on this species were all very young still (birch, cottonwood (*Populus trichocarpa* TORR. et GRAY) and alder (*Alnus sinuata* (REG.) RYDB.) were exceptionally seeded on Jan. 19 and harvested on

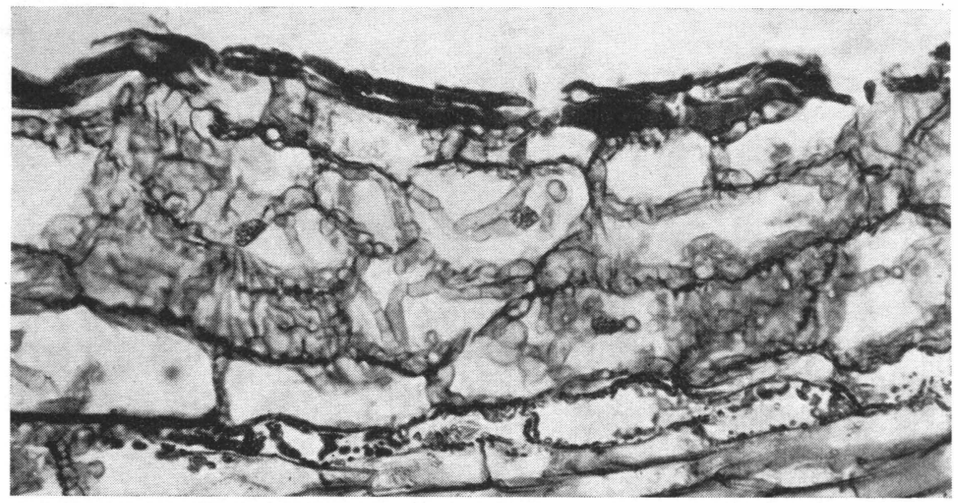

Fig. 4. Longitudinal section of an ectendotrophic larch mycorrhiza. Mantle lacking, intracellular hyphae as well as nucleai clearly visible. *Larix occidentalis* and E-35 in semiaseptic synthesis. Magnification x 410.

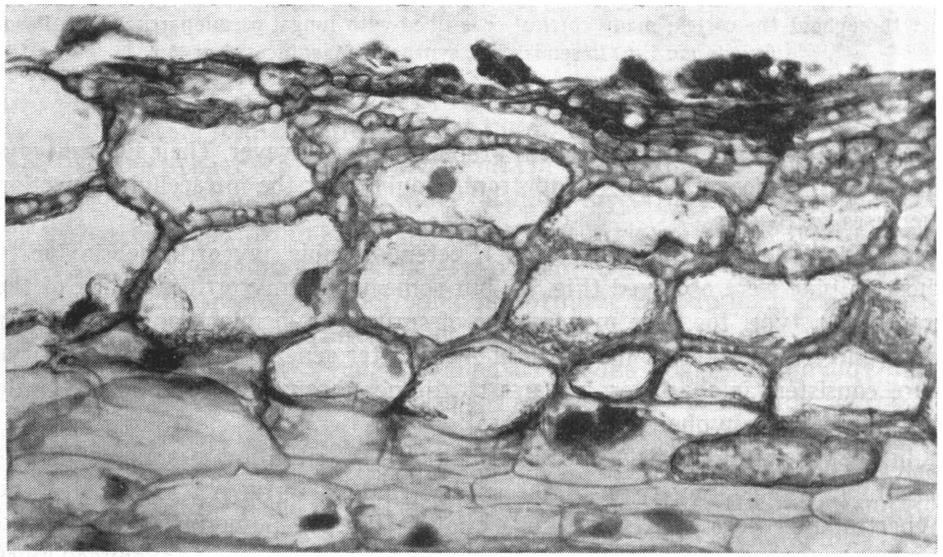

Fig. 5. Longitudinal section of a typical ectotrophic E-strain mycorrhiza. No mantle, coarse Hartig net throughout the cortex. *Picea sitchensis* and E-35 in semiaseptic synthesis. Magnification x 410.

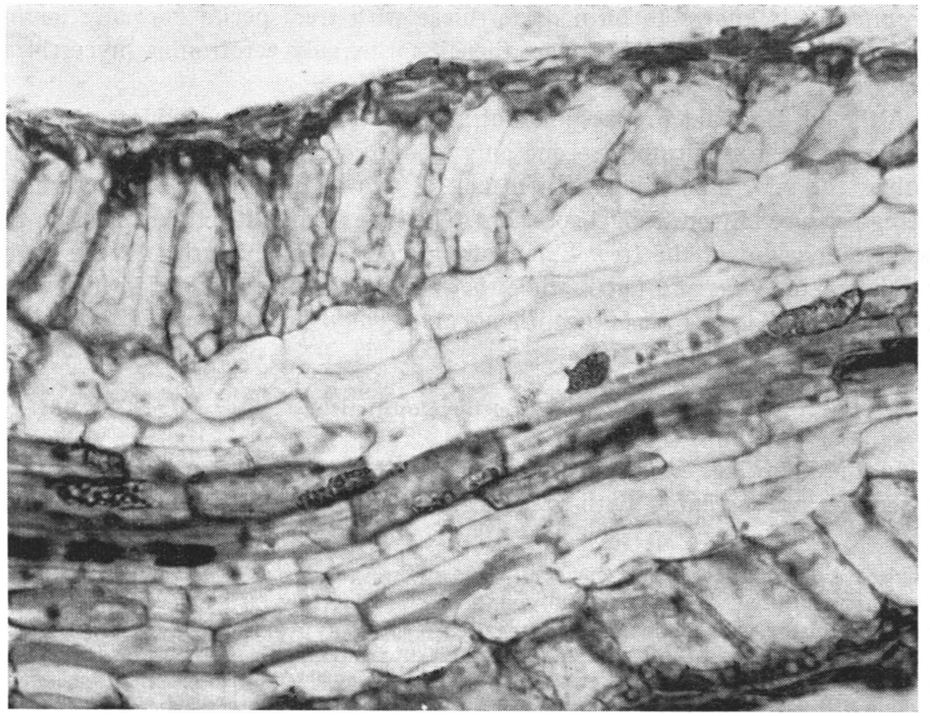

Fig. 6. Longitudinal section of a birch mycorrhiza. Mantle lacking, Hartig net one cell layer deep, no intracellular infection. *Betula verrucosa* and E-35 in semiaseptic synthesis. Magnification x 480.

June 5). In the case of cottonwood, the conclusion is even more uncertain, although the sample examined was ectotrophic. For alder the time was too short for mycorrhizae to develop, even in the unautoclaved control.

The E-strains showed a strong tendency to form mycorrhizae. As a matter of fact, it is possible that they do so with all tree species that have ectotrophic mycorrhiza. On the other hand, this experiment gave fairly convincing evidence that endotrophic species are not infected. Representatives of local maple, white-cedar, incense-cedar and sequoia species had abundant mycorrhizae in the unautoclaved controls, but the inoculated pots showed no signs of infection. In some cases these seedlings were even grown in the same pots with pine seedlings that had E-strain mycorrhizae to guarantee that there was a healthy inoculum near the roots. This did not change the negative result, which was, in fact, to be expected. Endotrophic symbionts, as far as is known (Mosse 1963), are essentially unrelated to the ectotrophic type (Trappe 1962 b). According to Bergemann (1956), ectotrophic symbionts sometimes infect roots endotrophically; this, however, remains a mere hypothesis. Only one ectotrophic symbiont, *Cenococcum*

graniforme, is known to form mycorrhizae with tree species normally having endotrophic mycorrhiza, but even then it forms only ectotrophic mycorrhizae (TRAPPE 1964).

Although E-strains probably do not infect species that have endotrophic mycorrhizae, their spectrum is second only to *Cenococcum* in width. Further, it involves the very interesting phenomenon of two distinct kinds of mycorrhizae being produced by one fungal symbiont. The type formed strictly depends on the species or genus of the tree. Ectendotrophic E-strain mycorrhiza seems to be restricted to *Pinus* and *Larix*, while the ectotrophic form is found in the genera *Picea*, *Abies*, *Tsuga*, *Pseudotsuga*, *Betula* and *Populus*.

Seedling development

In the following, two groups of seedlings from the semiaseptic synthesis experiments are compared, both of them grown in autoclaved soil from a Douglas-fir stand. In half of them E-strains had formed mycorrhizae (77 % of the number of short root tips), while the other half, which were uninoculated, remained non-mycorrhizal. On the average, there were seven seedlings per pot. The mean weight of the shoots in each pot was used as an index of growth. The mycorrhizal pots and their controls were laid out in pairs. All pairs not destroyed by contamination are included in the results (Table 4); Fig. 7 shows some typical seedlings.

Table 4. Average shoot weight (some with standard error) of mycorrhizal and non-mycorrhizal seedlings. Soil (from Douglas-fir stand) autoclaved. Seeding Nov. 3, 1963 (*P. edulis* Jan. 10, 1964), inoculation with E-strains. Seedlings raised in open pots in greenhouse. Corvallis, Oregon.

Tree species	Inoculated	First. mycorrh.	Harvested	Number of pots	Number of seedlings	Shoot weight, mg	Shoot color
Abies procera	Jan. 20	Febr. 30	Apr. 21	1	4	160	green
—»— Control	—	—	Apr. 21	1	3	83	green
Picea abies	Jan. 20	Febr. 20	Apr. 21	1	5	50	green
—»— Control	—	—	Apr. 21	1	7	20	pale
P. pungens	Dec. 17	Febr. 30	Apr. 21	1	10	50	green
—»— Control	—	—	Apr. 21	1	5	25	pale
P. sitchensis	Jan. 6	Febr. 10	Apr. 18	8	78	68 ± 4.0	green
—»— Control	—	—	Apr. 18	8	75	24 ± 1.7	green
Picea sp.	Jan. 20	Febr. 30	Apr. 21	1	10	25	green
—»— Control	—	—	Apr. 21	1	10	18	green
Pinus edulis	Jan. 30	March 20	Apr. 21	1	2	210	blue-green
—»— Control	—	—	Apr. 21	1	5	152	blue-green
P. ponderosa	Dec. 17	Febr. 30	Apr. 19	15	103	180 ± 11	green
—»— Control	—	—	Apr. 19	15	111	148 ± 8.6	green
P. radiata	Dec. 17	Febr. 10	Apr. 20	1	4	193	green
—»— Control	—	—	Apr. 20	1	7	192	green
P. silvestris	Jan. 20	Febr. 20	Apr. 21	1	8	86	green
—»— Control	—	—	Apr. 12	1	5	28	violet
P. strobus	Jan. 20	Febr. 30	Apr. 21	1	10	118	green
—»— Control	—	—	Apr. 21	1	10	69	pale
Pseudotsuga menziesii	Dec. 17	Febr. 20	Apr. 19	11	99	150 ± 10	green
—»— Control	—	—	Apr. 19	11	107	95 ± 6.2	green
Tsuga heterophylla	Jan. 20	Febr. 30	Apr. 17	5	8	88 ± 11	green
—»— Control	—	—	Apr. 17	5	7	19 ± 2.2	pale

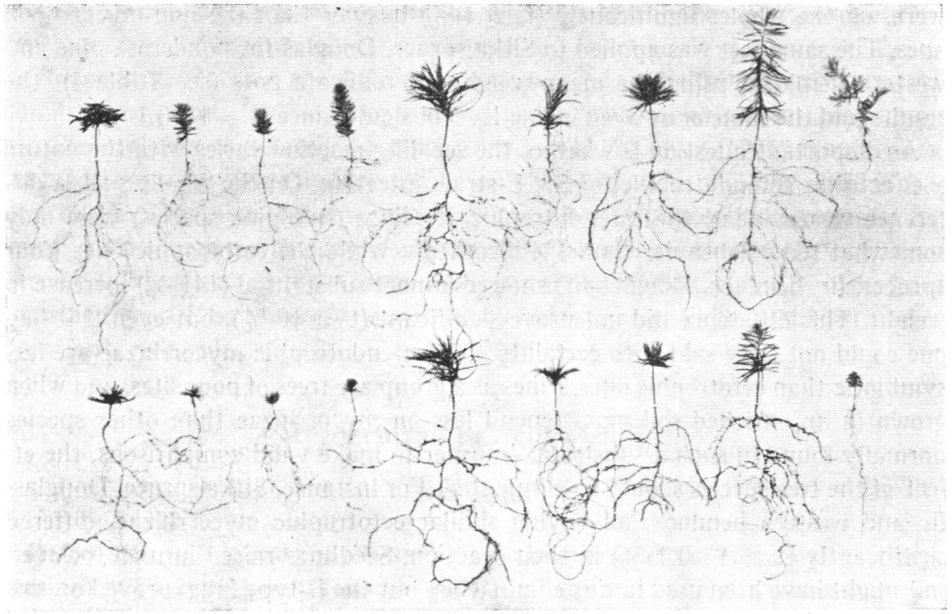

Fig. 7. Representative seedlings (above mycorrhizal, below non-mycorrhizal) from the semiaseptic synthesis. From the left: *Abies procera*, *Picea abies*, *P. pungens*, *P. sitchensis*, *Pinus ponderosa*, *P. silvestris*, *P. strobus*, *Pseudotsuga menziesii* and *Tsuga heterophylla*. Soil autoclaved, mycorrhizal seedlings inoculated with E-strains. All seedlings grown in a greenhouse and harvested at the age of 5.5 months. 1/7 natural size. Corvallis, Oregon.

The development of the seedlings was followed closely, observations being made daily. About two months after inoculation it became evident that the seedlings in the inoculated pots were doing better than the controls. In certain species this difference was very clear and increased as time went on, while in others no difference could be observed. At the end of the experiment the mycorrhizal seedlings were on the average twice as heavy as the non-mycorrhizal ones. In hemlock the difference was nearly fivefold. In no species were the mycorrhizal seedlings smaller than their controls.

The mean seedling weights were paired (pot inoculated with E-strain and control) and the weight difference expressed as a percentage of the mean weight in the control pot. Application of the t-test showed that the mycorrhizal seedlings

were, on the whole, significantly ($t > 1$ %) heavier than the non-mycorrhizal ones. The same test was applied to Sitka spruce, Douglas-fir, ponderosa pine and western hemlock, using the mean weights of replicate pots (see Table 4): the results had the same or an even higher level of significance ($t > 1$—0.1 %).

An important question is whether the seedling reaction varies with the nature (i.e. ecto- or ectendotrophic) of the E-strain infection. On the whole, such a difference was evident: the ectendotrophic seedlings (five pine species) were only somewhat (68 %) heavier than their controls, while the ectotrophic ones (four spruces, fir, hemlock, Douglas-fir) showed a more substantial (141 %) increase in weight. This difference did not prove significant ($t < 10$ %), but even if it had one could not have said with certainty that ectendotrophic mycorrhizae are less symbiotic than ectotrophic ones. Pines as a group are trees of poor sites, and when grown in an enriched soil may depend less on mycorrhizae than other species normally found in such a substrate. In order to make valid comparisons, the effect of the tree species should be eliminated. For instance, Sitka spruce, Douglas-fir and western hemlock, all having similar ectotrophic mycorrhizae, differed significantly ($t > 1$—0.1 %) in their reaction. Seedlings raised in unautoclaved soil might have been used in this elimination, but the E-type fungi proved on this occasion to be too ubiquitous. In both the Douglas-fir and ponderosa stands from which the soil was obtained, the mycorrhizae were examined and found to be ectotrophic. The E-type fungi must have been present in these soils in some form, because the great majority of mycorrhizae in the unautoclaved treatment were of either the ectendotrophic or the corresponding ectotrophic type, and E-strains were isolated from them (on p. 14 the chance of contamination is eliminated from consideration). Thus the chance was lost to compare seedlings having E-strain mycorrhizae (both types) with the ectotrophic ones formed by other symbionts, and the seedling size in the inoculated and unautoclaved soils was about the same.

No other seedling characteristics will be given here, since they would not add anything essential to the facts already presented. It is enough to mention that if one were to use the theoretically best characteristic, the growth differences arising in the 1.5—2.5 months during which mycorrhizae were present, the reactions would be of still greater magnitude. As an example, non-mycorrhizal hemlock seedlings did not visibly grow at all during that period.

Among the possible reasons for these results, it must be mentioned that temperature, light and moisture were optimal during the mycorrhizal phase, and thus enabled differences to develop. The soil, on the other hand, was unbalanced in its nutrient status and particularly low in phosphorus. It is under these conditions that the benefits of mycorrhizae reach their peak (HATCH 1937, BJÖRKMAN 1942); the ectotrophic mycorrhizae have been shown to be quite effective in absorbing phosphorus, for instance. Probably the same holds true for the ectendotrophic E-strain mycorrhiza, as suggested by the typical phosphorus deficiency symptoms

showing in non-mycorrhizal Scotch pine seedlings but absent in mycorrhizal individuals.

The positive reaction of seedlings recorded here is clearly different from that noted by LEVISOHN (1954), who claimed that this association was pseudomycorrhizal or even completely harmful. In many of MIKOLA's (1965) experiments, the reaction was either indifferent or only slightly positive. The short duration and unfavorable growing conditions during the mycorrhizal phase of his investigation are given as reasons why no major growth reactions could have been expected. Thus the E-type fungi have been reported to play all roles, from one-sided parasitism to very beneficial symbiosis. More experiments of longer duration are needed.

Mycorrhizae in field samples

Nurseries

Before this material was collected, it was known that the ectendotrophic mycorrhiza in Finland is only found in pine seedlings and mainly in forest nurseries (MIKOLA 1965). Therefore, as many nursery samples as possible, were collected, giving first preference to pines. A total of 44 samples from 12 nurseries was obtained.

As in the synthesis experiment, microscopic examination revealed ectendotrophic mycorrhizae only in pine and larch (Table 5). Such mycorrhizae were very common in almost all the samples of these species, always with an essentially similar coarse mycelium and no mantle. More important, they were similar to those produced by the E-strains in the synthesis experiments and to those found in Finnish nurseries. This morphological similarity was confirmed by isolations of the fungus. Eight strains were isolated from ponderosa and western white pine (*Pinus monticola* DOUGL.) seedlings from the Wind River nursery. They were all similar to each other and to E-strains isolated in Finland. In an aseptic synthesis they have formed ectendotrophic mycorrhizae with Scotch pine. Thus ectendotrophic mycorrhizae from Oregon and Finland are indistinguishable. It seems probable that the same symbiont might have been isolated from many other nurseries, such as Saratoga, Kennington, and Halstenbek. However, for lack of time and facilities, such tests could not be made.

Although samples of other tree species are few, there is strong evidence that, as might be expected from the synthesis experiments, this type of ectendotrophic mycorrhiza does not exist in Sitka spruce and Douglas-fir in nurseries. In the Greeley nursery, pine and spruce were growing mixed in the same row with their roots entangled with each other. The pine mycorrhizae were ectendotrophic, while those of the spruce were ectotrophic. Both samples of Douglas-fir also came from nurseries where ectendotrophic mycorrhiza dominated in pine. However,

Table 5. Microscopic structure of mycorrhizae in certain nursery seedlings.

Nursery	Tree species	Age in years	No of samples	Of mycorrhizae sectioned			
				Ectendotrophic		Ectotrophic	
				Without mantle	With mantle	Without mantle	With mantle
Industrial Forestry Association: Colonel W. B. Greeley Nursery, Nisqually, Wash.	*Picea sitchensis*	2	1			4	
	Pinus ponderosa	2	1	4			
U.S. Forest Service: Bend Nursery, Bend, Ore.	*Pinus ponderosa*	1	7	22		4	5
U.S. Forest Service: Wind River Nursery, Carson, Wash.	*Pinus monticola*	1	2	7			
	P. ponderosa	1	2	10		1	
The Oregon Forest Nursery, McDonald Forest, Corvallis, Ore.	*Pinus attenuata*	2	1	3			
	P. contorta	2	1	3		1	
	P. lambertiana	2	1	3			
	P. nigra	2	1	1	1	1	1
	P. ponderosa	2	1	5			
	P. silvestris	2	1	4			
	Pseudotsuga menziesii	2	1			3	1
Saratoga Tree Nursery, Saratoga, N.Y.	*Larix leptolepis*	3	1	2		3	
	Pinus resinosa	3	2	6			
	P. silvestris	2	1	5			
	P. strobus	2	2	2		10	
Georgia Forestry Commision: Herty Nursery, Albany, Ga.	*Pinus elliottii*	1	3			5	12
	P. palustris	2	2			8	2
Nursery of the Institute of Tropical Forestry, Rio Piedras, Puerto Rico. Seedlings raised in plastic bags (see Fig. 8).	*Pinus caribaea*	1/3	2			13	1
Kennington Nursery, Oxford, England.	*Pinus contorta*	3	1	2		5	
	P. nigra	2	1	4		1	
	P. silvestris	1	1	5			
	Pseudotsuga menziesii	2	1			1	
Alice Holt Nursery (Headley Section), Farnham, Surrey, England.	*Pinus silvestris*	2	1			5	
Eduard Heins Nursery, Halstenbek, Germany.	*Pinus silvestris*	1	1	5			
Gustaf Lüdemann Nursery, Halstenbek, Germany.	*Pinus silvestris*	1—2	2	1		3	2
	Quercus robur	2	1				4
Pein & Pein Nursery, Halstenbek, Germany.	*Pinus silvestris*	1—2	2	11			2

presumably these species were also associated with E-type fungi, since their mycorrhizae were quite similar to those synthesized with E-strains. Similar ectotrophic mycorrhizae without a mantle have also previously been found in nurseries where the ectendotrophic type dominated in pine, viz. from Sitka spruce (LEVISOHN 1954) and Norway spruce (MIKOLA 1965). In the latter case, E-strains were also isolated from them, for instance the number E-57 used in this work.

As was mentioned above, the ectendotrophic mycorrhiza was common; actually it was only in the Alice Holt nursery that no sign of ectendotrophic mycorrhizae or E-type fungi was found. In the Herty nursery there were mantleless mycorrhizae formed by a coarse mycelium, although these were not a dominating type. These mycorrhizae closely resemble the ectotrophic pine type formed by E-strains in the aseptic synthesis. In Puerto Rico, similar mycorrhizae were found to be dominant. Although microtome sections did not reveal distinct intracellular hyphae (Fig. 8), samples taken from the same seedling lot by Dr. MIKOLA contained an occasional mycorrhiza that was distinctly ectendotrophic although not rich in intracellular infection. It seems possible, if not probable, that these are also examples of the same or very similar fungi. Of course, this can only be conclusively established by further isolations.

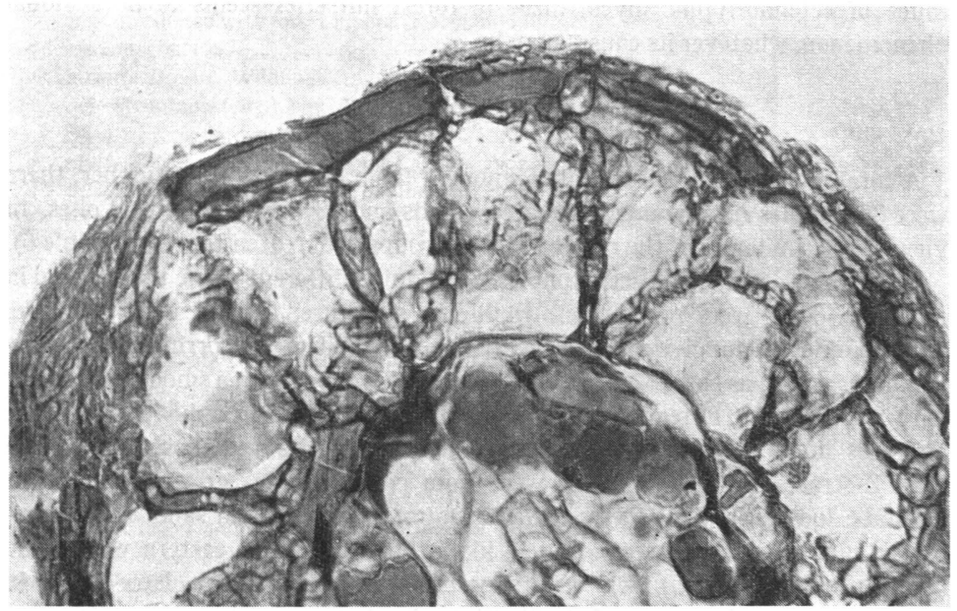

Fig. 8. Transverse section of a typical mycorrhiza from the nursery of the Institute of Tropical Forestry in Puerto Rico. No mantle, mycelium coarse, Hartig net bulbous, intracellular hyphae mainly lacking but occasionally present. *Pinus caribaea* (Honduras seed source.) Seedling raised in vermiculate in a plastic bag, inoculated with forest humus (Maryland mixture, see BRISCOE 1959). Magnification x 390.

As stated by ZAK and MARX (1964), the number of symbionts in forest nurseries is usually low, with one species dominating. This observation was confirmed by the present investigation. In most samples the mycorrhizae were all of the same type, if one excludes an occasionally encountered »C» or »Dn» type. Once microscopic examination has shown most of these pine mycorrhizae to be ectendotrophic, the only possible conclusion to be drawn is that E-types usually dominate in nurseries. In pine nursery stock, the E-types usually seem to form 50—70 % of the total number of mycorrhizae; occasionally the percentage may approach 100. Other species besides pine and larch are also included.

As in the synthesis experiments, the tree species clearly determined the types of mycorrhizae. It is of particular interest that eastern white pine mycorrhizae here were again low in intracellular hyphae (see p. 16). Besides tree species, some minor factors may also have an effect on the appearance of these mycorrhizae. In the Bend nursery a large amount of organic matter (litter, bark, sawdust, 10—100 tons/acre) had been added to the soil; many ectotrophic pine mycorrhizae seemed to be formed by an E-type fungus. In the Herty nursery, too, forest litter is regularly added to the soil and perhaps this helped the E-type to form ectotrophic mycorrhizae.

These findings strongly support those of MIKOLA (1965). The common appearance of ectendotrophic mycorrhizae in forest nurseries seems to be a global phenomenon, whatever its causes may be.

Forest samples

A total of 108 samples were taken, most of them (49) wildlings. Further, there were 36 samples from young cultivated stands and 23 from old natural ones. In view of the similarity of the results, all the groups are treated together (Table 6).

Mycorrhizae in these forest samples were quite different from those found in nurseries. They were mainly ectotrophic in structure, usually with a mantle. Many species of fungi were clearly involved. The »Dn» mycorrhiza was fairly common, as well as the »C» and many other whitish types with a smooth mantle or with rhizomorphs. Ectendotrophic mycorrhiza was found in only four samples, and was in all cases similar to those found in nurseries and those synthesized with E-strains. All four samples were from pine wildlings growing in diverse habitats: lodgepole pine (*Pinus contorta* DOUGL.) from coastal sand dunes, ponderosa pine from the Cascade and Siskiyou Mountains and eastern white pine (again a weak infection) from a nursery arboretum at Saratoga, New York. In two samples (ponderosa pine from the Cascades, lodgepole pine), ectendotrophic mycorrhiza seemed to dominate. However, it was not found in other seedlings in the same area, or even in similar localities, although about ten samples of both were taken. Thus, this survey clearly supports the idea that ectendotrophic mycorrhizae in forests are rare (MIKOLA 1965).

Table 6. Microscopic structure of mycorrhizae in some forest samples

Family	Tree species	Description of samples	No. of samples	Of mycorrhizae sectioned				
				Endotrophic	Ectendotrophic	Ectotrophic		
						Without mantle	With mantle	Mantle only
Pinaceae.	*Abies amabilis* (DOUGL.) FORBES.	Wildling (Cascade Mountains, Ore.)	1				3	
	Larix decidua MILL.	Plantation (Peavy arboretum, Corvallis, Ore.)	1			3	2	
	L. laricina (DU ROI) K. KOCH.	Plantation (Nursery arboretum at Saratoga, N.Y.)	1				5	
	L. leptolepis (SIEB. et ZUCC.) GORD.	Plantations (Nursery arboretum at Saratoga, N.Y. and Kennington forest, Oxford, England)	2			5	1	
	Picea sitchensis (BONG.) CARR.	Wildlings from sand dunes and one old growth from »rain forest» (Ore.)	5			9	7	
	Pinus banksiana LAMB.	Plantation (Nursery arboretum at Saratoga, N.Y.)	1			1	2	
	P. caribaea MORELET	(Honduras seed source). Plantations (Puerto Rico), 3—5 years old, height 5—9 m. Seedlings inoculated with forest humus (Maryland mixture, see BRISCOE 1959)	3			1	10	
	P. contorta DOUGL.	Wildlings from sand dunes and Cascade Mountains (Ore.), one old growth (Lac La Hache, B.C.)	14		2	13	36	
	P. edulis ENGELM.	Wildling (Grand Canyon, Ariz.)	1				3	
	P. elliottii ENGELM.	Shelterbelt plantation (Herty nursery, Albany, Ga)	1			1	2	
	P. jeffreyi GREV. et BALF.	Wildling (Siskiyou Mountains, Ore.)	1				1	
	P. lambertiana DOUGL.	Wildling (Siskiyou Mountains, Ore.)	1			1	1	
	P. monticola DOUGL.	Wildlings and plantations (Ore. and Wash.)	4			6	6	
	P. palustris MILL.	Wildlings and plantations (Suwannee, Fla, and Herty nursery environs, Albany, Ga)	4			4	8	
	P. ponderosa LAWS.	Plantations (Mc Donald forest, Corvallis, Ore), wildlings and old growths (mainly from East Oregon and Cascade Mountains but also from Siskiyou Mountains, Ore., and Flagstaff and Grand Canyon, Ariz.)	21		4	16	31	
	P. radiata D. DON.	Wildling (Monterey peninsula, Calif.) and plantation (sand dune, Ore).	2			4	3	
	P. resinosa AIT.	Plantations (Nursery arboretum at Saratoga, N.Y., and South Windham, Vt)	2			4	3	
	P. rigida MILL.	Old growth (Oneco, Conn.)	1			1	1	
	P. silvestris L.	Wildling and plantation from Big Bond,						

Continued

Surrey, England, and plantations from McDonald forest, Corvallis, Ore., Nursery arboretum at Saratoga, N.Y., and South Windham, Vt	5			10	11
P. strobus L. Wildling and plantation (Nursery arboretum at Saratoga, N.Y.), wildlings and old growths from South Windham, Vt, Oneco, Conn., and St. Ignice, Mich.	9		1	12	14
P. taeda L. Wildling, plantation and old growth (Troy, Ala)	3			6	10
Pseudotsuga menziesii (MIRB.) FRANCO. Old growths (McDonald forest, Corvallis, and Alsea Basin area, Ore.)	4			3	9
Tsuga canadensis (L.) CARR. Wildling (South Windham, Vt)	1				3
Ts. heterophylla (RAF.) SARG. Wildling (Alsea Basin area, Ore.)	1			2	
Ts. mertensiana (BONG.) CARR. Wildling (Cascade Mountains, Ore.)	1				1
Cupressaceae. *Chamaecyparis lawsoniana* (A. MURR.) PARL. Wildlings (sand dunes, Ore.) and one plantation (McDonald forest, Corvallis, Ore.)	3	8			
Juniperus occidentalis HOOK. Old growth (Bend, Ore.)	1	2			
Libocedrus decurrens TORR. Wildlings (Siskiyou Mountains, Ore.)	2	2			
Thuja plicata DONN. Plantation (Peavy arboretum, Corvallis, Ore.)	1	2			
Taxodiaceae. *Sequoia gigantea* (LINDL.) DECNE. Plantation (Peavy arboretum, Corvallis, Ore.)	1	1			
S. sempervirens (D. DON) ENDL. Old growths (Calif.) and a plantation (Peavy arboretum, Corvallis, Ore.)	3	2			
Aceraceae. *Acer macrophyllum* PURSH. Wildling (Corvallis, Ore.)	1	2			
Betulaceae. *Alnus rubra* BONG. Old growth (Corvallis, Ore.)	1				1
Fagaceae. *Fagus grandifolia* EHRH. Plantation (Peavy arboretum, Corvallis, Ore.)	1				4
Quercus sp. Old growth (Siskiyou Mountains, Ore.)	1			3	
Salicaeae. *Populus tremuloides* MICHX. Wildling (Nursery arboretum at Saratoga, N.Y.)	1		5		
P. trichocarpa TORR. et GRAY. Plantation (Peavy arboretum, Corvallis, Ore.)	1			5	
Verbenaceae. *Tectona grandis* L.F. Plantation (Puerto Rico)	1	2			

Particular interest attaches to samples from a few plantations. Ponderosa pine was planted 28 years ago in the vicinity of the Oregon forest nursery. These seedlings were raised in the Bend nursery, which is dominated now and probably also then by ectendotrophic mycorrhizae. There was no sign of ectendotrophism in the ten samples taken, even though it was dominant in the nursery about one kilometer away. Just as in recent experiments made by MIKOLA (1965), the fungal partner seemed to have changed.

At one corner of the Saratoga arboretum, which bordered the nursery, the situation was somewhat different. There, an ectendotrophic mycorrhiza was found in an eastern white pine wildling. In a 20-year old Scotch pine plantation coarse mantleless ectotrophic mycorrhizae were present, similar to those formed by the E-strains. It may be that for some environmental reason the symbiont formed ectotrophic mycorrhizae. In two other samples, similar mycorrhizae were also observed, namely on Monterey pine (*Pinus radiata* D. DON) growing on Monterey peninsula, California, and on eastern white pine near South Windham, Vermont, both wildlings. The true identity of these mycorrhizae can, of course, only be determined from isolations.

On the basis of the synthesis experiments one would expect to find ectendotrophic mycorrhizae in pine and larch. On the basis of the list on p. 6 one would expect to find them in many more species. Nevertheless, only four positive findings were made and the conclusion seems inevitable that E-type ectendotrophism is quite rare under forest conditions. No other types of ectendotrophism were found either in forests or in nurseries. Two reasons for this fact seem possible — either they are very rare or they were not recognized. Both reasons may be partially right. Another person examining the same slides might find more ectendotrophic mycorrhizae. In this study the rule was followed that an ectotrophic mycorrhiza can not be classified as ectendotrophic on grounds of thin hyphae appearing irregularly in the cortical cells. Furthermore, »Dn» mycorrhizae, which sometimes have ectendotrophic features, were usually not sectioned because their structure is relatively well known from previous studies (e.g. TRAPPE 1964).

Discussion

This study of ectendotrophic mycorrhizae is based on four sources of material: aseptic and semiaseptic synthesis and samples taken from nurseries and from forests. The results obtained from these different sources are completely complementary; together this body of facts makes it possible to discuss many aspects of the ectendotrophic phenomenon.

First of all, it was surprising to find only one type of ectendotrophic mycorrhiza, although over 600 short roots collected from two continents were sectioned. The type found was the same as that studied by MIKOLA (1965). If other types exist, they must be very rare, very local, or poorly defined. The type found has very sharp characteristics: the mycelium is coarse and forms a strong Hartig net; intracellular infection is heavy and clearly visible. The evidence is convincing that this structure was always formed by the same fungus species throughout. The

fungal partner has been repeatedly isolated in Finland and Oregon, 150 and 13 isolates, respectively. All of them are essentially similar; so far they are unidentified (here called E-strains) but probably do not belong to the genus *Rhizoctonia* (MIKOLA 1965).

As discussed by MIKOLA, this form of ectendotrophism must be the same as has previously been described by many authors. LEVISOHN (1946, 1954, 1963) called it the »haustorial type of intracellular root infection» or pseudomycorrhiza. Samples removed from Kennington nursery, where LEVISOHN carried out some of her experiments, show ectendotrophism typical of that described in this paper. BJÖRKMAN (1940, 1942) and Goss (1960) must also have observed this particular mycorrhiza, as well as MELIN (1923 b, p. 95) and presumably many others, including MÖLLER (1902), MÜLLER (1903), VON TUBEUF (1903), RAYNER (1934), ENDRIGKEIT (1937) and MCCOMB (1943). But, on the other hand, the list on p. 6 contains associations that can not be formed by E-type fungi. The ectendotrophic mycorrhiza is often reported in association with spruce. Mycorrhizae synthesized by E-strains with six spruce species were all ectotrophic, as were those sampled from the nature. The same was also the case with fir, hemlock and Douglas-fir, for instance. Thus, there seems to be fungi other than the E-type capable of forming ectendotrophic mycorrhizae. However, if one looks critically into the references (p. 6), it quickly becomes apparent that the ectendotrophic spruce mycorrhiza is hardly ever described in detail. BJÖRKMAN (1940, p. 49) once mentions that is was not found »fully developed» in spruce. In the same investigation, pine mycorrhizae were clearly ectendotrophic and actually of the E-type. It would be desirable for the observations on p. 6 to be confirmed and studied in detail. The fungal partners involved should be isolated, and in addition to the aseptic syntheses, as many semiaseptic ones as possible should be made. As an example of the possible variation, *Cenococcum graniforme* usually forms ectotrophic mycorrhizae but, in conditions unfavorable to the host plant, fills the cells of the outer cortex with fungal pseudoparenchyma (MIKOLA 1948, p. 84). Many other instances are known of this »reaction to environment» (e.g. MELIN 1925, pp. 89—93: LUNDEBERG 1963), and actually there is some doubt as to whether all the fungus species listed on p. 8 are truely ectendotrophic.

On the basis of this study, nothing can be said about ectendotrophic mycorrhizae in general, but the E-type, whether ecto- or ectendotrophic, proved to be a balanced symbiosis. The possibility remains that it functions as an ordinary exchange mycorrhiza although no digestion of intracellular hyphae was noted (see p. 32). The final criterion of the true nature of these associations is the reaction of the host. In this study, greenhouse seedlings bearing ectendotrophic mycorrhizae did well. Experiments made in Finland (MIKOLA 1965) have not shown any reaction, unless it be a slightly positive one. LEVISOHN (1954, 1963) has claimed similar ectendotrophic association to be clearly harmful. Other authors have based their opinions on material collected from nature. After finding these mycorrhizae mainly associated with stunted seedlings, they have concluded that the ectendotrophic mycorrhiza is harmful (e.g. BJÖRKMAN 1942, 1949). MIKOLA's large seedling material does not support their view, and neither do the results of this study. The seedlings in all the nurseries did well. Further, in physiological experiments, E-strains behave in the same way as mycorrhizal fungi in general (MIKOLA 1965).

Thus, the E-type endophytes must be regarded as true mycorrhizal fungi. Whether they are more or less beneficial than others remains an open question. Because of the thin or lacking mantle one might think that they are less beneficial than other types (BERGEMANN 1955). Whatever its true nature, the fact remains that mycorrhizae formed by E-types lack a mantle in both forest and nursery environments (Tables 5 and 6). Many other fungi develop a good mantle in both places. Actually, the belief that mycorrhizae in fields and nurseries are poor in structure as compared with forests (BJÖRKMAN 1961) rests partially on the inability of the E-types to develop a mantle. Since they dominate in nurseries, the typical mycorrhiza there is mantleless, although occasional »C», »Dn», *Thelephora* and many other mycorrhizae have a well-developed mantle.

The sharp contrast between forest and nursery found by MIKOLA (1965) was fully confirmed. In nurseries the ectendotrophic mycorrhiza dominated, in forests it was found very seldom and only in seedlings. The fact that this phenomenon has not been previously demonstrated is probably a result of too much mycorrhizal work being done without microtome sectioning. MIKOLA further demonstrated that pine seedlings, once planted out in the woods, lose their original ectendotrophic mycorrhizae within one or two years. In many cases the symbiont clearly changed. Thus, whatever the reasons, the ectendotrophic mycorrhiza does not seem to have the same competitive ability in forests as in nurseries. The detailed role of E-type fungi in the forest, however, must be considered unsolved at present. On two occasions (p. 22) an E-strain was accidentally brought in from the forest, where it had not been observed to be living symbiotically, unless perhaps it was in an unrecognizable form. Ectotrophic mycorrhizae may be one such form and although no environmental factors seem to affect the structure of the E-strain mycorrhiza in Scotch pine (always ectendotrophic, MIKOLA 1965), this does not seem to be true of all other pine species, as indicated on pp. 16, 26 and 29.

In the above, the similarity of the ectendotrophic mycorrhizae studied in this work has been emphasized (lack of mantle, coarse Hartig net, strong intracellular infection). On the other hand, differences also existed. In synthesis experiments E-strains formed either ecto- or ectendotrophic mycorrhizae, the choise depended on the tree species. There was, however, important variation in these ectendotrophic mycorrhizae, even when the differences in the amount of intracellular infection are excluded. Intracellular hyphae were namely of three kinds. In most cases, they were sharp in outline (Figs. 1 and 4, see also MIKOLA 1965) but some-

times also appearing as if they were partially dissolved in the cytoplasm (Fig. 2), much like arbuscules in certain endotrophic mycorrhizae. As a third form, a kind of fungal pseudoparenchyma was discovered (Fig. 3). It could not be decided whether these forms were developmental stages of ectendotrophic mycorrhizae, but the arbuscule-like form was exclusively found in young mycorrhizae. MIKOLA (1965) emphasizes the high amount of intracellular hyphae in older ectendotrophic mycorrhizae. Thus, even in the event that arbuscule-like structures indicate fungal digestion, the hypothesis is not supported that digestion takes place in certain later stages of the development of ectendotrophic mycorrhizae.

Presumably there will be a growing interest in ectendotrophic mycorrhizae which will keep pace with the continued spread of nurseries and forest plantations the world over. The need for an evaluation of the role which this phenomenon plays in regeneration work is urgent. Besides their practical importance, E-type fungi may have a special theoretical interest. It has been demonstrated that cellulase is produded when *Tricholoma fumosum* forms ectendotrophic mycorrhizae (NORKRANS 1950, p. 75). It may be that cellulase is the key to mycorrhizal fungi in cortical cells in general. This hypothesis could be tested with E-strains. With modern biotechniques, it might also be possible to find out how some tree species keep this fungus out of the cortical cells. This would probably be a good step towards a better understanding of mycorrhiza formation in general.

The classification of ectendotrophic mycorrhizae is a problem. Being brown in color and relatively thin because of the absence of a mantle, they are macroscopicly easily confused with pseudomycorrhizae (Goss 1960, p. 17). Microscopic examination is thus necessary to check macroscopic classification, a fact too often neglected. Even when discovered, the ectendotrophic mycorrhiza is a problem. Many workers have found this type of mycorrhiza, as revealed in personal discussions, but considered it atypical or parasitic and therefore ignored it. As far as mycorrhizae formed by E-type fungi are concerned, there is no reason for doing so: these fungi are only one further type of the ever- increasing group of known mycorrhizal symbionts. If a detailed classification is needed, the ectendotrophic mycorrhiza itself might be used as a natural unit. Morpho-anatomical systems, however accurate (e.g. DOMINIK 1959), probably do not provide the advantages of a classification based on fungal partners.

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