

ECTOMYCORRHIZA – NATURE AND SIGNIFICANCE FOR FUNCTIONING OF FOREST ECOSYSTEMS

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Abstract

The ectomycorrhiza has a great influence on vegetation and for the forest ecosystems it is essential. The present review aims to provide brief information about main participants – myco- and phytobionts in such a well-organized mutualistic association, their structural elements, function and role in the natural processes. It also emphasizes that ectomycorrhizas has no equal value through the fungal species. Furthermore the last complement each other in their ecological niches and as a result we can observe optimal exploration of site which is very outlined in habitats, where there is a deficit on one or more mineral elements. Describing ectomycorrhizal mycocoenosis is very challenging task and provide valuable information about all the community as a whole. For this, the molecular markers are excellent tools but must be combined with morphological and anatomical studies, because only thorough studies can bring better understanding of the driving processes at ecosystem levels.

Key words: common mycelial network, extraradical mycelium, Hartig net, mantle, mycorrhizosphere.

Introduction

Mycorrhiza (pl. mycorrhizas, mycorrhizae) (fungus root) is a symbiotic relationship that is not pathogenic or is slightly pathogenic, resulting in a fungus root association between plant and fungus (Kirk et al. 2008). The term mycorrhiza was derived from the Greek 'μυκόσ' – fungus 'ρίζα' – root and was used for first time by Frank in 1885 in his work 'On the nutritional dependence of certain trees on root symbiosis with belowground fungi' to describe two different organisms forming a single morphological structure – fungus root (Frank 2005). There is a high accuracy in this

first attempt on morphological description of mycorrhiza.

Mycorrhizas differ significantly through various groups of plants, in the way of interaction between fungus and plant, respectively within the fungal species involved in this association and the type of obtaining nutrients. Therefore, they are classified in different types (Harley 1991, Agerer 1995, Smith and Read 2008). One of these types of mycorrhizas, most often studied and of great importance for the boreal regions, is the ectomycorrhiza.

According to Molina et al. (1992) there are six features characterizing the ecological phenomenon of mycorrhiza. These

are: 1) dependence–independence – determines whether the plant is involved in mycorrhizal symbiosis or not, under natural conditions; 2) facultative and obligatory symbiotrophic relationship – determines the ability and inability of symbiotrophs to complete the whole cycle of development in the absence or not of mycorrhiza or mycorrhizas; 3) mycorrhizal type – determined by the fact that most of symbiotrophs form a certain type of mycorrhiza, but some of them (the mycobionts and photobionts – together or separately) can participate in the formation of two or more types of mycorrhizas and this depends on abiotic and biotic conditions, and the ontogenetic stages of development; 4) photobiotic range – it is determined by the

scope that go from narrow (usually family) to medium, and finally wide, which include plants that spread beyond the family, including a few close or more distant ones, as well as a separate order or above order; 5) mycobiontic compatibility – determines the number of mycorrhizal fungi that are associated with certain photobiont and according to Molina et al. (1992) there are two groups: plants, associated with low number and plants associated with high number of fungi; 6) ecological specificity – it is determined by the influence of abiotic and biotic factors on the ability of plants to form functional mycorrhiza with fungi under certain conditions of the natural environment.

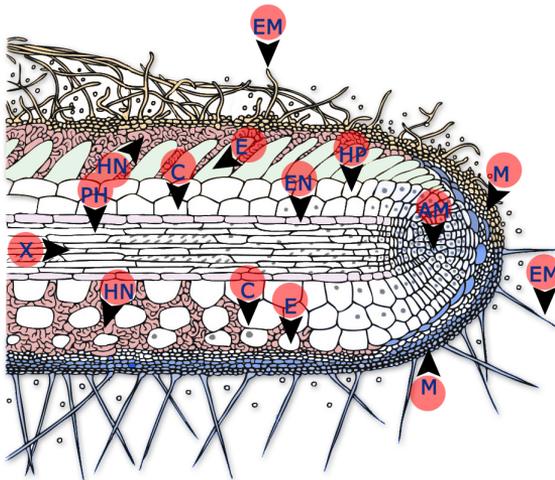


Fig. 1. The main features of ectomycorrhiza – view in longitudinal section in gymnosperms (lower half) and angiosperms (upper half).

Note: M – mantle (blue and yellow), HN – Hartig net, EM – extraradical mycelium, E – epidermal cells, HP – hypodermis, C – cortex cells, EN – endodermis, PH – phloem in the main cylinder, X – xylem in the main cylinder, AM – apical meristem.

Structural Features of Ectomycorrhiza

The ectomycorrhizal associations are called ectotrophic associations or ectomycorrhizas. They are a mutualistic relationship between fungi and vascular plants. From a morpho-anatomical point of view, ectomycorrhiza forms a functionally separate unit, acting alone, represented by fungus hyphae and root cells located outside the endodermis (Fig. 1). The main differentiating features from other types of mycorrhiza is the presence of three structural elements: 1) a veil or mantle that covers the root surface; 2) Hartig net – a labyrinth-like structure, built by branching hyphae and situ-

ated between dermatogen and periblem, and 3) extraradical mycelium and its formations, representing a system of hyphal components, located outside the mantle and making the connection to the sporophores of ectomycorrhizal fungi.

The structural features of the mantle and extraradical mycelium are constant at least at genus level and can serve as important characters for recognition of the fungal partner (Agerer 1986–2008, Ingleby et al. 1990, Goodman et al. 1998, Agerer and Rambold 2004–2011). The ectomycorrhiza is easily distinguishable from other types of mycorrhiza on the basis of clearly noticeable absence of penetration of the hyphae into the intracellular space. If it occurs, the type of the mycorrhiza is classified as ectendomycorrhiza (Peterson et al. 2004). In some cases, one fungus is capable to form ectomycorrhiza with some plants and in other – ectendomycorrhiza. This also depends on the peculiarities of abiotic factors. For instance, *Wilcoxina mikolae* Chin S. Yang & Korf forms ectomycorrhiza with young plants of genera *Pinus* L. and *Larix* Mill. In the nurseries under natural conditions it forms an ectendomycorrhiza with species of the genera *Abies* Mill., *Picea* A. Dietr. and *Tsuga* Carr. (Mikola 1988). The species of genus *Wilcoxina* have very high ecological importance because they participate in the mycorrhizal association with species of genus *Pinus* under the extreme conditions and are probably crucial for the ecological plasticity and occurrence of trees (Fig. 2).

For example, the stalk base of the pine tree shown in Fig. 2 is under water surface near half a year and the dam is frozen for at least another three months (Fig. 2.1). A great excess of water and nutrients makes the fine roots grow mainly in the upper parts of soil. For a very limited

time, among the third and fourth quarter of the year the water level goes down, and temperatures in the ground surface rise dramatically (Fig. 2.2). Through this time there is a high risk of starting of decaying processes and ectomycorrhiza develops rapidly, protecting the finest roots. In this Scots pine tree, two morphotypes are observed: $\approx 5\%$ probably *Lactarius* sp. (Fig. 2.3) and the most abundant $\approx 95\%$ *Wilcoxina* sp. (Fig. 2.4). The last one (39AJ45) was DNA sequenced in Germany. The comparison with the known fungal databases showed only the genus *Wilcoxina* (99% match – DQ320129 – Uncultured *Wilcoxina* isolate NS30C1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence. – CLC Genomics Workbench) (see Fig. 3).

Abbreviation of codes: WM – *Wilcoxina mikolae* (Chin S. Yang & H.E. Wilcox) Chin S. Yang & Korf; WR – *Wilcoxina rehmi* Chin S. Yang & Korf; WA – *Wilcoxina alaskana* Kempton, Chin S. Yang & Korf; WS – *Wilcoxina* sp., uncultured.

The closest related known species to isolate 39AJ45, according to the phylogram is *Wilcoxina alaskana*. It forms small, 6–12 mm in diameter apothecia with ochraceous hymenium (Yang and Korf 1985).

Most ectomycorrhizal fungi in extreme conditions are able to penetrate intracellularly through the older parts of the root system or where the nutrient balance in the ectomycorrhizal association is disturbed (Smith and Read 2008). In this case, the fungus appears to be a little pathogenic. The latter can probably be observed in the individual stages of development of the plant and some combina-



Fig. 2. Ectomycorrhizal morphotypes associated with *Pinus sylvestris*, 1935 m above sea level, in extreme conditions, Belmeken dam.



Fig. 3. Phylogenetic relationships in genus *Wilcoxina*, according to the available information in the NCBI GenBank (1995–2014).

tion of influencing factors, i.e. the impact and the benefits to the partners do not always show a symmetry. Contrariwise, there are dynamic fluctuations over the

time. In most cases, a weak pathogenic effect occurs on the plant and less on the fungal partner. Many of the typical ectomycorrhizal fungi involved in symbiotic relationships with species from genera *Pinus* and *Fagus* L. are forming mycorrhiza with the ericoid species and in this case it is defined as arbutoid (Harley 1985) due to different anatomic structure of root and its influence to hyphae growth.

The three attributes for ectomycorrhiza are not constant, and there are lesser or greater variations in the manner in which the mantle is developed, Hartig net or extraradical mycelium in different plant species also vary. In some species of Asteraceae (Warcup 1980) only a part of the mantle is present and in species of the genus *Pinus*, forming the mycorrhiza with *Tricholoma matsutake* (S. Ito & S. Imai) Singer, the mantle is not observed (Yamada et al. 2006). The roots of *Pisonia grandis* R.Br. do not form a Hartig net (Ashford and Allaway 1982). All these mycorrhizal types functionally belong to ectomycorrhiza.

Plant Species Involved in Ectomycorrhiza (Photobionts)

Plant species involved in ectomycorrhiza are a relatively small number (not more than 5 % of vascular plants), but their representatives are very important because among them are nearly all woody plants that determine the vegetation appearance in large geographical areas (Meyer 1973). These include species of Pinaceae, the main components of the vast northern boreal areas and Fagaceae – dominants and subdominants in the northern (*Fagus* L., *Quercus* L.) and southern (*Nothofagus* Blume) temperate zones. In Australia the ectomycorrhizal species are all in the genus *Eucalyptus* L'Hér. (Peterson et al. 2004).

The most spread mycorrhiza is vesicular arbuscular mycorrhiza (VAM) and it occurs mainly in the herbaceous plants. Their general characters are vesicles (not always) and arbuscules – special mycelial morphological structures, who penetrates into the cellular space. Fungal partners in this type mycorrhiza are those from order Glomerales.

Some classes of plants are forming both VAM and ectomycorrhiza. For instance, the species, combining both type of mycorrhizas are these belonging to family Myrtaceae (incl. *Eucalyptus*) and also the widespread in temperate geographic zone genus *Salix* L.

Almost all species involved in ectomycorrhizas are trees but there are exceptions – some families composed of typical shrubs, and a very limited number of herbaceous plants (Table 1). However, most of genera are not studied in depth yet and we can expect more numbers, forming ectomycorrhiza.

Table 1. Genera of plants, with at least a species involved in ectomycorrhiza.

Family/Subfamily	Genus
Angiospermae	
Aceraceae	B <i>Acer</i> L.
Betulaceae	B <i>Alnus</i> Mill.
	B <i>Betula</i> L.
	B <i>Carpinus</i> L.
	B <i>Corylus</i> L.
	B <i>Ostrya</i> Scop.
	B <i>Ostryopsis</i> Decne.
Bignoniaceae	<i>Jacaranda</i> Juss.
Caprifoliaceae	B <i>Sambucus</i> L.
Casuarinaceae	B <i>Casuarina</i> L.
	B <i>Allocasuarina</i> L.A.S.Johnson
Cistaceae	B <i>Helianthemum</i> Mill.
	B <i>Cistus</i> L.
Compositae (Asteraceae)	B <i>Lactuca</i> * L. (incl. <i>Mycelis</i> Cass.)
Cyperaceae	B <i>Kobresia</i> * Willd.

Dipterocarpaceae	B <i>Anisoptera</i> Korth.		<i>Monopetalanthus</i> Harms
	B <i>Balanocarpus</i> Bedd.		<i>Paramacrolobium</i> J. Léonard
	B <i>Cotylelobium</i> Pierre		<i>Swartzia</i> Schreb.
	B <i>Dipterocarpus</i> C.F.Gaertn.		<i>Acacia</i> Mill.
	B <i>Dryobalanops</i> C.F.Gaertn.	Fabaceae – subfam.	<i>Chorizema</i> Sm.
	B <i>Hopea</i> Roxb.	Mimosoideae	<i>Daviesia</i> Sm.
	B <i>Monotes</i> A.DC.	Fabaceae – subfam.	<i>Dillwynia</i> Sm.
	B <i>Shorea</i> Roxb. ex C.F.Gaertn.	Papilionoideae	<i>Eutaxia</i> R. Br.
Elaeagnaceae	<i>Shepherdia</i> Nutt.		B <i>Gompholobium</i> Sm.
Epacridaceae	<i>Astroloma</i> R.Br.		B <i>Hardenbergia</i> Benth.
Ericaceae	<i>Arbutus</i> L.		<i>Jacksonia</i> R.Br. ex Sm.
	<i>Arctostaphylos</i> Adans.		<i>Kennedy</i> DC.
	<i>Chimaphila</i> Pursh		B <i>Mirbelia</i> Sm.
	<i>Gaultheria</i> Kalm ex L.		B <i>Oxylobium</i> Andrews
	<i>Kalmia</i> L.		<i>Platylobium</i> Sm.
	<i>Leucothoe</i> D.Don		<i>Pultenaea</i> Sm.
	<i>Rhododendron</i> L.		B <i>Robinia</i> L.
	<i>Vaccinium</i> L.		B <i>Vicia</i> L.
Euphorbiaceae	<i>Poranthera</i> Rudge		B <i>Viminaria</i> Sm.
	B <i>Uapaca</i> Baill.		<i>Comptonia</i> (L.) J. M.Coulter
Fagaceae	B <i>Castanea</i> Mill.		<i>Myrica</i> L.
	B <i>Castanopsis</i> (D. Don) Spach		B <i>Angophora</i> Cav.
	B <i>Fagus</i> L.	Myricaceae	B <i>Callistemon</i> R.Br.
	B <i>Lithocarpus</i> Blume		B <i>Campomanesia</i> Ruiz & Pav.
	B <i>Nothofagus</i> Blume	Myrtaceae	B <i>Eucalyptus</i> L'Hér.
	B <i>Pasania</i> Blume		B <i>Leptospermum</i> J.R. Forster & G. Forster
	B <i>Quercus</i> L.		B <i>Melaleuca</i> L.
	B <i>Trigonobalanus</i> Forman		B <i>Tristania</i> R.Br.
Gentianaceae	<i>Bartonia</i> * Muhl. ex Willd.		B <i>Neea</i> Ruiz & Pav.
Goodenaceae	B <i>Brunonia</i> * Sm. ex R. Br.	Nyctaginaceae	B <i>Torrubia</i> Vell.
	B <i>Goodenia</i> * Sm.		B <i>Pisonia</i> L.
Hamamelidaceae	<i>Parrotia</i> (DC.) C. A. Mey.		B <i>Fraxinus</i> L.
Juglandaceae	B <i>Carya</i> Nutt.	Oleaceae	B <i>Platanus</i> L.
	B <i>Juglans</i> L.	Platanaceae	<i>Coccoloba</i> P. Browne
	<i>Pterocarya</i> Nutt. ex Moq.	Polygalaceae	<i>Polygonum</i> * L.
Fabaceae – subfam.	<i>Azelia</i> Sm.	Polygonaceae	<i>Cryptandra</i> Sm.
Caesalpinioideae	<i>Aldina</i> Endl.	Rhamnaceae	<i>Pomaderris</i> Labill.
	<i>Anthonota</i> P. Beauv.		<i>Rhamnus</i> L.
	<i>Bauhinia</i> L.		<i>Spyridium</i> Fenzl
	<i>Brachystegia</i> Benth.		<i>Trymalium</i> Fenzl
	<i>Cassia</i> L.	Rosaceae	B <i>Crataegus</i> Tourn. ex L.
	<i>Eperua</i> Aubl.		B <i>Dryas</i> L.
	<i>Gastrolobium</i> R. Br.		B <i>Malus</i> Mill.
	<i>Gilbertiodendron</i> J. Léonard		B <i>Prunus</i> L.
	<i>Julbernardia</i> Pellegr.		

	B <i>Pyrus</i> L.
	B <i>Rosa</i> L.
	B <i>Sorbus</i> L.
Salicaceae	B <i>Populus</i> L.
	B <i>Salix</i> L.
Sapindaceae	<i>Allophylus</i> L.
	<i>Nephelium</i> L.
Sapotaceae	<i>Glycoxylon</i> Ducke
Sterculiaceae	B <i>Lasiopetalum</i> Sm.
	<i>Thomasia</i> J.Gay
Styliidiaceae	B <i>Styliidium</i> Sw.
Thymeleaceae	B <i>Pimelea</i> Banks & Sol. ex Gaertn.
Tiliaceae	B <i>Tilia</i> L.
Ulmaceae	B <i>Ulmus</i> L.
	<i>Celtis</i> L.
Vitaceae	B <i>Vitis</i> L.
Gymnospermae	
Cupresseceae	B <i>Cupressus</i> L.
	B <i>Juniperus</i> L.
Pinaceae	<i>Abies</i> Mill.
	<i>Cathaya</i> Chun & Kuang
	B <i>Cedrus</i> Trew
	<i>Keteleeria</i> Carrière
	<i>Larix</i> Mill.
	<i>Picea</i> A. Dietr.
	<i>Pinus</i> L.
	<i>Pseudolarix</i> Gordon
	<i>Pseudotsuga</i> Carrière
	<i>Tsuga</i> Carrière
Gnetaceae	<i>Gnetum</i> L.

Note: * – herbaceous plants; B – representatives of the genera forming simultaneously ectomycorrhiza and VAM (modified from Smith and Read 2008).

Origin of Ectomycorrhizal fungi

The most ancient evidence for ectomycorrhiza was found in British Columbia (Canada) in fossil roots of *Pinus* species, and dates more than 50 million years ago (LePage et al. 1997). It is believed, however, that ectomycorrhizal relationships have more ancient origins and appeared

roughly about 130 ± 1 million years ago (Smith and Read 2008).

Phylogenetic studies (Hibbett et al. 2000) have shown that it is very possible the formation of ectomycorrhiza derives independently from several clades of fungi and has evolved into a long period of time from different fungal organisms – saprotrophs forming subsequently symbiotic relationship with autotrophic plants. Since the dynamics of the process is different in various latitudes, structurally there is a wide range of ectomycorrhizal variety. The question, however, is still discussible about the reversibility of the process of changing the trophic level. It is believed that for only one species of genus *Lentaria* Corner (Bruns and Steffarson 2004) there is enough evidence to argue that the fungus has moved from ectomycorrhizal to saprotrophic life style and this can be considered more as an exception than as a rule. It is possible, however, for other fungi to have covered the same evolutionary paths.

Compared to VAM, ectomycorrhiza is younger with approximately 250 million years. The oldest fossils of mycorrhizal sample found up to date appear to be those of VAM and were dated 400 million years ago (Remy et al. 1994).

Fungi Involved in Ectomycorrhizal Associations (Mycobionts)

According to Molina et al. (1992), the fungi forming ectomycorrhiza are between 5000 and 5500. However, the current number is estimated between 20 000 and 25 000 species, bearing in mind that the known fungi represent not more than 20 % of all existing, and even 5–10 % according to some authors (Deacon 2006). Various comparisons between the floristic richness

studied in detail and the fungal diversity on a particular territory revealed the regularity, which indicates that they are 5 to 6 times more than the tracheophytes species. About 80 % of ectomycorrhizal fungi have epigeous spore-forming structures, and in others, they are formed below the surface and are called hypogeous. Both types are characterized by a great variety of morphological and ecological features. The latter are hard to locate. The overall assessment of the global species richness of ectomycorrhizal fungi varies considerably according to different authors, and there is a trend of increasing their number in the most recent studies. Many families were considered as ectomycorrhizal, but in many studies there is no clear evidence of their current trophic status. For instance, up to date, there is no proof for the ectotrophic status of *Morchella* Dill. ex Pers. species. Their cultivation trials failed in most cases. Its saprotrophic status is either well understood and leads to the conclusion that some species can form a very complex life style, which follows their specific ontogenetic pathway.

Rinaldi et al. (2008) analyzed the trophic status of the most fungal genera by reviewing a large amount of publications and the conclusion was that of over 300 fungal genera known to be ectomycorrhizal, the published data till now is incomplete or the status is unconfirmed and completely hypothetical for about one third of them. As an evidence of the latter, they analyzed the scientific proofs, dividing them into: morpho-anatomical characteristics of naturally occurring ectomycorrhizal fungi; synthesis of pure cultures, molecular, isotopic analysis and phylogenetic studies. Due to the limitations of each method, the authors proposed to clarify the trophic status for the combina-

tions of them, and the more methods are available, the evidences of the ectomycorrhizal status of the species are more obvious and indisputable. According to the estimation, based on various publications, total number of ectomycorrhizal fungi (modified) is about 8334, which significantly exceeds the number proposed by Molina et al. (1992). However, here it should be noted that authors use different methodologies to assess, and different views of taxonomists exist, who often raise a taxon within a species based on certain criterion, mainly morphological, often not clear enough and not well-established. The modern molecular methods are an excellent tool to solve the existing uncertainties and taxonomic problems.

It can be assumed that the current knowledge on the diversity of ectomycorrhizal fungi supported by experimental evidence is only partially complete and that the inclusion of many fungal families in the trophic and ecological category should be done only after thorough studies, supported by appropriate scientific evidence.

The fungi involved in ectomycorrhiza are associated with a particular plant taxon, most often family or families and usually the occurrence of sporophores are indicators of the habitat in similar conditions. Different studies have shown that the most common spatial distribution of ectomycorrhizal communities is a patchy type (Peter 2000).

The formation of spore-forming structures does not always, however, give a clear picture of species composition and population size because it often varies and depends on the combinations of different factors. It happens frequently, that the species forming these structures often inhabit a relatively small proportion of physiologically active root system, while

others, which do not form or does it rarely, occupy a large part of it. All this leads to the conclusion that in most cases there is little or no obvious correlation between the aboveground and belowground spatial distribution of ectomycorrhizal communities. Several studies confirmed this, and revealed also that a significant part of ectomycorrhizal fungi can form sporophores rarely and sporadically. The results from the long-term observation plots performed by Egli (2009) for a sporocarps inventory showed clearly two tendencies – only very small number of mycorrhizal fungi produce sporophores regularly, i.e. to discover the most fungal diversity we need a long term observations, and a trend of decline in ectomycorrhizal fungal diversity.

It has been found that the number of different fungi, associated with a particular host plant is much fewer than the fungal species, forming ectomycorrhiza with the same plant. It is possible for the individual plant (photobiont) to participate in ectomycorrhiza with 20 or more species at the same time. Modern molecular methods, particularly DNA analysis, have proven the last fact using microsatellites (Saari et al. 2005). It is more likely to be a result of different evolutionary pathways for development on one hand, and of a different strategy for the propagation and life cycle, on the other. The most important factors for occupying the ecological niches by ectomycorrhizal fungi in their natural habitats are tree age, soil, climatic conditions and availability of ectomycorrhizal inoculum.

Due to the relative ecologically equal value of most ectomycorrhizal fungi, they compete each other. As a result, the species occupy different ecological niches and complement themselves. For instance, it is not unusual to observe several species on the same rootlet, neigh-

boring each other. The main reasons are: different growing optimum parameters (temperature/humidity) of each ectomycorrhizal fungus; the surrounding micro- and mesofauna, flora and mycota at the impact on the formation and distribution of specific microenvironment. The last is mainly composed of different bacteria and endophytes and some of them play a positive role and form a complex relationship with the plant and/or the ectomycorrhizal fungus or fungi. This is true especially for the dark septate endophytes, which are located in the root system and have a neutral to slightly positive effect, and for some types of bacteria (Jumpponen and Trappe 1998). Most endophytes, located in the root are septate fungi with dark color, from phylum Ascomycota but some of them are colorless (O'Dell et al. 1993). For example, very often on/in the roots of woody plants are found species of *Mortierella* Coem., an ecologically important group which are the first organisms colonizing roots. Salt (1977) reported, that they occur much more often on the root surface of genus *Picea* species, compared to species of the genera *Fusarium* Link, *Pythium* Pringsh, which are pathogens. The role of *Mortierella* species for the plants is not enough understood, but two things are known presently – they did not impact negatively on the trees' health status, and even more – occupying the roots, they blocked the access of some nutrient resources used by the pathogens. Some of *Mortierella* species grow rapidly on FDA and MN¹ medium and make the isolation of axenic culture of ectomycor-

¹FDA (Ferry medium) and MN (Melin-Norkans medium) – modified or original recipes are one of the most common media for isolation of ectomycorrhizal fungi.

rhizal fungi from ectomycorrhizal rootlets difficult and even impossible.

The fungus *Suillus tomentosus* (Kauffman) Singer produces specialized structures with *Pinus contorta* Dougl. ex Loud. var. *latifolia* Engelm, known as tuberculate ectomycorrhiza. The possible reasons are probably within the mechanism of protection from insects and pathogens in addition to becoming a host of nitrogen fixing bacteria, which allows the plant to grow in/on poor in organic nitrogen soils (Paul et al. 2007). This is an example of mutualistic association wherein among plant and fungal species are involved other organisms – bacteria.

One of the most active directions in the study of an ectomycorrhizal symbiosis is the relationship between fungal diversity and establishment of mycocoenological association. There is an increasing support for the hypothesis that ectomycorrhizal fungal species diversity affects the structure of plant communities, which in turn influences them. It is formed by complex combinations of abiotic and biotic factors. One of the most important biotic factors in this respect is the photobiont specificity or selectivity (Zhou and Hyde 2001). The latter phenomenon may be considered from 'phytcentric' or 'myco-centric' terms, i.e., to emphasize the influence of fungal symbiotrophs that combine with particular plant taxon or a variety of plant species.

Studies of the ectomycorrhizal diversity and selectivity of particular plants can be made only when the basic information on the trophic status of fungi in an ecosystem is known. Such knowledge is also necessary to study the habitats in wider aspects where fungi play an important role in biochemical cycles. It is important to know which of them are ectomycorrhizal and which have saprotrophic or mixo-

trophic (dual) life style (Taylor and Alexander 2005, Koide et al. 2005).

Fungi involved in ectomycorrhiza belong to three major groups. These are from the phyla: Basidiomycota, Ascomycota, and family Endogonaceae (Table 2). The species of last group have an aseptate coenocytic mycelium, unlike the first two fungal groups which belong to the higher fungi, many of which are macromycetes.

Despite the functional similarity of ectomycorrhiza, the number of fungi involved in it is quite large and unevenly represented among the three groups above. The species from phylum Basidiomycota are the most numerous among them. Table 2 presents the majority of fungal families forming ectomycorrhiza.

The total number of ectomycorrhizal species is about 8328. In Europe to date 1518 macroscopically ectomycorrhizal fungi from phylum Basidiomycota are established (Moser 1983, Jülich 1984).

Modern molecular methods open new opportunities in the investigation and determining of fungi trophic status in which data interpretation plays a key role. For first time in 2006, the entire genome of epigeous fungus *Laccaria bicolor* (Maire) P.D.Orton was completely read (Martin et al. 2007). This fungus was chosen among many other ectomycorrhizal fungi because it is widespread and has a large phytobiontic spectrum. Its genome is 65 Mbp and is much larger than of other fungi from Basidiomycota, which have been completely sequenced. The results show that more than 50 % of genes are with unknown function. Over 20,000 proteins are found and have been identified. Among them, the most important are protease, lipase, phytase and about 315 others involved in carbohydrate metabolism,

Table 2. Fungal genera, forming ectomycorrhiza (by Rinaldi et al. 2008 – modified).

Fungal genera	Number of known species		
Ascomycota Caval.-Sm.		<i>Labyrinthomyces</i> Boedijn	7
<i>Amylascus</i> Trappe	2	<i>Leptodontidium</i> de Hoog	1
<i>Balsamia</i> Vittad.	6	<i>Loculotuber</i> Trappe, Parladé & I.F. Alvarez	1
<i>Barssia</i> Gilkey	2	<i>Meliniomyces</i> Hambl. & Sigler	3
<i>Cazia</i> Trappe	1	<i>Muciturbo</i> P.H.B. Talbot	3
<i>Cenococcum</i> Moug. & Fr.	1	<i>Mycoclelandia</i> Trappe & G.W. Beaton	2
<i>Chloridium</i> Link	1	<i>Neocudoniella</i> S. Imai	2
<i>Choiromyces</i> Vittad.	5	<i>Nothojafnea</i> Rifai	2
<i>Delastria</i> Tul. & C. Tul.	1	<i>Otidea</i> (Pers.) Bonord.	15
<i>Dingleya</i> Trappe	7	<i>Pachyphloeus</i> Tul. & C. Tul.	6
<i>Elaphomyces</i> Nees	20	<i>Paradoxa</i> Mattir.	1
<i>Eremiomyces</i> Trappe & Kagan-Zur	1	<i>Paurocotylis</i> Berk.	1
<i>Fischerula</i> Mattir	2	<i>Peziza</i> Dill. ex Fr.	84
<i>Genea</i> Vittad.	35	<i>Phaeangium</i> Pat.	1
<i>Geopora</i> Harkn.	12	<i>Phialocephala</i> W.B. Kendr.	6
<i>Geopyxis</i> (Pers.) Sacc.	6	<i>Phialophora</i> Medlar	1
<i>Gilkeya</i> M.E. Sm., Trappe & Rizzo	1	<i>Picoa</i> Vittad.	4
<i>Glischroderma</i> Fuckel	1	<i>Plicaria</i> Fuckel	1
<i>Gymnohydnotrya</i> B.C. Zhang & Minter	3	<i>Pseudaleuria</i> Lusk	1
<i>Gyromitra</i> Fr.	15	<i>Pseudotulostoma</i> O.K. Mill. & T.W. Henkel	1
<i>Helvella</i> L.	40	<i>Pulvinula</i> Boud.	24
<i>Humaria</i> Fuckel	15	<i>Reddellomyces</i> Trappe, Castellano & Malajczuk	4
<i>Hydnobolites</i> Tul. & C. Tul.	2	<i>Ruhlandiella</i> Henn.	1
<i>Hydnocystis</i> Tul. & C. Tul.	2	<i>Sarcosphaera</i> Auersw.	1
<i>Hydnotrya</i> Berk. & Broome	12	<i>Sowerbyella</i> Nannf.	14
<i>Hydnotryopsis</i> Gilkey	2	<i>Sphaerosoma</i> Klotzsch	3
<i>Kalaharituber</i> Trappe & Kagan-Zur	1	<i>Sphaerosporella</i> (Svrček) Svrček & Kubička	2
		<i>Sphaerozone</i> Zobel	4
		<i>Stephensia</i> Tul. & C. Tul.	6
		<i>Tarzetta</i> (Cooke) Lambotte	8
		<i>Terfezia</i> (Tul. & C. Tul.) Tul. & C. Tul.	12

<i>Tirmania</i> Chatin	3	<i>Boletochaete</i> Singer	3
<i>Tricharina</i> Eckblad	12	<i>Boletopsis</i> Henn.	5
<i>Trichophaea</i> Boud.	20	<i>Boletus</i> Dill. ex Gray	300
<i>Tuber</i> P. Micheli ex F.H. Wigg.	63	<i>Bothia</i> Halling, T.J. Baroni & Manfr.	1
<i>Underwoodia</i> Peck	1	<i>Boughera</i> Vernes, Johnson & Castellano	1
<i>Wilcoxina</i> Chin S. Yang & Korf	3	<i>Byssocorticium</i> Bondartsev & Singer	9
<i>Wynnella</i> Boud.	1	<i>Byssoporia</i> M.J. Larsen & Zak	1
Basidiomycota R.T. Moore		<i>Calostoma</i> Desv.	15
<i>Afroboletus</i> Pegler & T.W.K. Young	5	<i>Cantharellus</i> Adans. ex Fr.	65
<i>Albatrellus</i> Gray	12	<i>Castoreum</i> Cooke & Massee	2
<i>Alnicola</i> Kühner	~30	<i>Chalciporus</i> Bataille	15
<i>Alpova</i> C.W. Dodge	20	<i>Chamonixia</i> Rolland	8
<i>Amanita</i> Pers.	~500	<i>Chlorogaster</i> Læssøe & Jalink	1
<i>Amarrendia</i> Bougher & T. Lebel	1	<i>Chondrogaster</i> Maire	1
<i>Amaurodon</i> J. Schröt.	6	<i>Chroogomphus</i> (Singer) O.K. Mill.	15
<i>Amogaster</i> Castellano	1	<i>Clavariadelphus</i> Donk	18
<i>Amphinema</i> P. Karst.	4	<i>Clavulina</i> J. Schröt.	32
<i>Anamika</i> K.A. Thomas, Peintner, M.M. Moser & Manim.	3	<i>Coltricia</i> Gray	13
<i>Andebbia</i> Trappe, Castellano & Amar.	1	<i>Coltriciella</i> Murrill	7
<i>Arcangeliella</i> Cavara	12	<i>Corditubera</i> Henn.	5
<i>Aroramycetes</i> Castellano & Verbeken	2	<i>Cortinarius</i> (Pers.) Gray	~2000
<i>Astraeus</i> Morgan	2	<i>Craterocola</i> Bref.	2
<i>Aureoboletus</i> Pouzar	5	<i>Craterellus</i> Pers.	20
<i>Auritella</i> Matheny & Bougher	7	<i>Cribbea</i> A.H. Sm. & D.A. Reid	4
<i>Austroboletus</i> (Corner) Wolfe	30	<i>Cystangium</i> Singer & A.H. Sm.	7
<i>Austrogaster</i> Singer	3	<i>Cystogomphus</i> Singer	1
<i>Austrogautieria</i> E.L. Stewart & Trappe	6	<i>Dermocybe</i> (Fr.) Wünsche	15
<i>Austropaxillus</i> Bresinsky & Jarosch	9	<i>Descolea</i> Singer	10
<i>Bankera</i> Coker & Beers	2	<i>Descomyces</i> Bougher & Castellano	3
<i>Boletellus</i> Murrill	50	<i>Destuntzia</i> Fogel & Trappe	5
		<i>Diplocystis</i> Berk. & M.A. Curtis	1

<i>Efibulobasidium</i> K. Wells	2	<i>Inocybe</i> (Fr.) Fr.	500
<i>Entoloma</i> (Fr. ex Rabenh.) P. Kumm.	~100	<i>Laccaria</i> Berk. & Broome	25
<i>Fevansia</i> Trappe & Castellano	1	<i>Lactarius</i> Pers.	~400
<i>Fistulinella</i> Henn.	15	<i>Leccinellum</i> Bresinsky & Manfr.	~5
<i>Gallacea</i> Lloyd	5	<i>Leccinum</i> Gray	75
<i>Gastroboletus</i> Lohwag	10	<i>Lenzitopsis</i> Malençon & Bertault	1
<i>Gastroleccinum</i> Thiers	1	<i>Leucogaster</i> R. Hesse	20
<i>Gastrotylophilus</i> T.H. Li & Watling	1	<i>Leucopaxillus</i> Boursier	15
<i>Gautieria</i> Vittad.	25	<i>Leucophleps</i> Harkn.	5
<i>Gigasperma</i> E. Horak	2	<i>Lindtneria</i> Pilát	11
<i>Gomphidium</i> Fr.	10h	<i>Lyophyllum</i> P. Karst.	50
<i>Gomphogaster</i> O.K. Mill.	1	<i>Maccagnia</i> Mattir.	1
<i>Gomphus</i> Pers.	10	<i>Mackintoshia</i> Pacioni & Sharp	1
<i>Gummiglobus</i> Trappe, Castellano & Amar	2	<i>Macowanites</i> Kalchbr.	30
<i>Gummivena</i> Trappe & Bougher	1	<i>Malajczukia</i> Trappe & Castellano	8
<i>Gymnogaster</i> J.W. Cribb	1	<i>Mayamontana</i> Castellano, Trappe & Lodge	1
<i>Gymnomyces</i> Masee & Rodway	37	<i>Melanogaster</i> Corda	25
<i>Gymnopaxillus</i> E. Horak	2	<i>Membranomyces</i> Jülich	3
<i>Gyrodon</i> Opat.	10	<i>Mesophellia</i> Berk.	4
<i>Gyroporus</i> Qué!l	10	<i>Multifurca</i> Buyck & V. Hofstetter	5
<i>Hallingea</i> Castellano	3	<i>Mycoamaranthus</i> Castellano, Trappe & Malajczuk	1
<i>Hebeloma</i> (Fr.) P. Kumm.	~150	<i>Mycolevis</i> A.H. Sm.	1
<i>Heimioporus</i> E. Horak	16	<i>Naucoria</i> (Fr.) P. Kumm.	~30
<i>Hoehnelogaster</i> Lohwag	1	<i>Nothocastoreum</i> G.W. Beaton	1
<i>Horakiella</i> Castellano & Trappe	1	<i>Octaviania</i> Vittad.	15
<i>Hydnangium</i> Wallr.	3	<i>Paragyrodon</i> (Singer) Singer	1
<i>Hydnellum</i> P. Karst	38	<i>Paxillus</i> Fr.	15
<i>Hydnum</i> Pers.	120	<i>Paxillogaster</i> E. Horak	1
<i>Hygrophorus</i> Fr.	~100	<i>Phellodon</i> P. Karst.	16
<i>Hymenogaster</i> Vittad.	~100	<i>Phylloporus</i> Qué!l.	~50
<i>Hysterangium</i> Vittad.	50	<i>Piloderma</i> Jülich	6

<i>Pisolithus</i> Alb. & Schwein.	~12	<i>Stephanopus</i> M.M. Moser & E. Horak	5
<i>Podohydangium</i> G.W. Beaton, Pegler & T.W.K. Young	1	<i>Stephanospora</i> Pat.	4
<i>Polyzellus</i> Murrill	1	<i>Strobilomyces</i> Berk.	20
<i>Polyporoletus</i> Snell	1	<i>Suillus</i> Gray	50
<i>Pseudotomentella</i> Svrček	9	<i>Thelephora</i> Fr.	49
<i>Psiloboletinus</i> Singer	1	<i>Timgrovea</i> Bougher & Castellano	5
<i>Pterygellus</i> Corner	5	<i>Tomentella</i> Pers. ex Pat.	75
<i>Pulveroboletus</i> Murrill	25	<i>Tomentellopsis</i> Hjortstam	5
<i>Ramaria</i> Fr. ex Bonord.	~60	<i>Torrendia</i> Boidin & Gilles	2
<i>Retiboletus</i> Manfr. Binder & Bresinsky	6	<i>Trechispora</i> P. Karst.	46
<i>Rhizopogon</i> Fr.	~150	<i>Tremellodendron</i> G.F. Atk.	8
<i>Rhodactina</i> Pegler & T.W.K. Young	2	<i>Tremelloscypha</i> D.A. Reid	1
<i>Rhodogaster</i> E. Horak	2	<i>Tricholoma</i> (Fr.) Staude	~200
<i>Rhopalogaster</i> J.R. Johnst.	1	<i>Truncocolumella</i> Zeller	3
<i>Richoniella</i> Costantin & L.M. Dufour	5	<i>Tubosaeta</i> E. Horak	5
<i>Riessia</i> Fresen.	4	<i>Tulasnella</i> J. Schröt.	46
<i>Riessiella</i> Jülich	2	<i>Turbinellus</i> Earle	5
<i>Royoungia</i> Castellano, Trappe & Malajczuk	1	<i>Tylopilus</i> P. Karst.	~75
<i>Rozites</i> P. Karst.	20	<i>Tylospora</i> Donk	2
<i>Rubinoboletus</i> Pilát & Dermek	10	<i>Veloporphyrellus</i> L.D. Gómez & Singer	2
<i>Russula</i> Pers.	~1300	<i>Xanthoconium</i> Singer	7
<i>Sarcodon</i> Quéél. ex P. Karst.	36	<i>Zelleromyces</i> Singer & A.H. Sm.	17
<i>Scleroderma</i> Pers.	25	Zygomycota Moreau	
<i>Scutigera</i> Paulet	1	<i>Endogone</i> Link	~20
<i>Sebacina</i> Tul. & C. Tul.	6	<i>Peridiospora</i> C.G. Wu & Suh J. Lin	4
<i>Setchelliogaster</i> Pouzar	6	<i>Sclerogone</i> Warcup	1
<i>Setogyroporus</i> Heinem. & Rammeloo	1	<i>Youngiomyces</i> Y.J. Yao	2
<i>Sinoboletus</i> M. Zang	5		
<i>Sistotrema</i> Fr.	4		

Note: The number to the right shows the number of known species belonging to the corresponding genus.

including glucanase, cellulase, chitinase, which are indicators of the large and diverse metabolic processes. It is believed

that there are possibilities of degradation of polymers found in the upper soil horizons which are rich in organic matter. This means that *L. bicolor* is particular saprotroph, i.e. it is optional mixotroph and this is a proof, which is already found in ecological studies, that there is no clear distinctive border in the way of nutrition. At some stage of their life cycle they can adapt to the habitat, according to their underlying genetic mechanism that largely determines the amplitude of the ecological plasticity.

Three years later, the genome of *Tuber melanosporum*, another ectomycorrhizal fungi, but hypogeous and from phylum Ascomycota, was entirely sequenced. The result shows very different characteristics comparing to *L. bicolor* sequencing, i.e. the particular behavior exhibited by *Tuber* may be a symptom of a not yet perfected system of mutual symbiosis in some ectomycorrhizal ascomycetes (Martin et al 2010). From the comparison of the genomes of the basidiomycete *L. bicolor* and of the ascomycete *T. melanosporum*, it is evident that the mycorrhizal symbiosis

has evolved in divergent pathways in these totally different systematic groups.

For a long time it has been known that among the most common of ectomycorrhizal fungi are these from phylum Ascomycota and the most common among them is *Cenococcum geophilum* Fr. It is found in large geographical areas (Fig. 4 and Fig. 5), especially in temperate latitudes, and it is associated with a large number of autotrophic plants (Gymnospermae and Angiospermae) from sea level to high altitudes (2000 m a. s. l.), but not in overmoisturized soils (Nedelin, unpublished data). A lot of studies demonstrated its dominant role in various ectomycorrhizal communities. This fungus does not form true sporophores as it belongs to the anamorphic (imperfect) fungi but black oval structures with a size of about 1 mm, which are densely intact with interwoven hyphae (microsclerotium, Fig. 5.1, black arrow head with white outline). Despite its high prevalence its ecological role is not yet fully understood, or as Mikola (1948) wrote (Smith and Read 2008) in his monograph on ecology and physiology of *Ceno-*

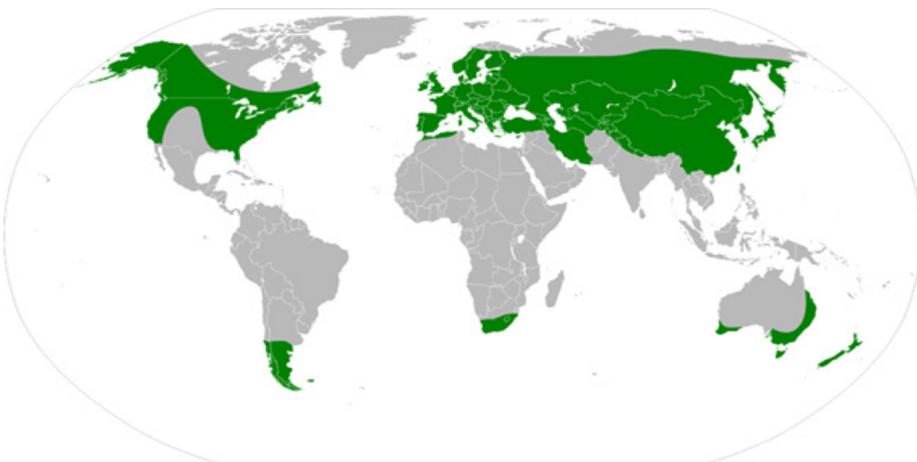


Fig. 4. Worldwide distribution of *Cenococcum geophilum* (Anonymous 2014).

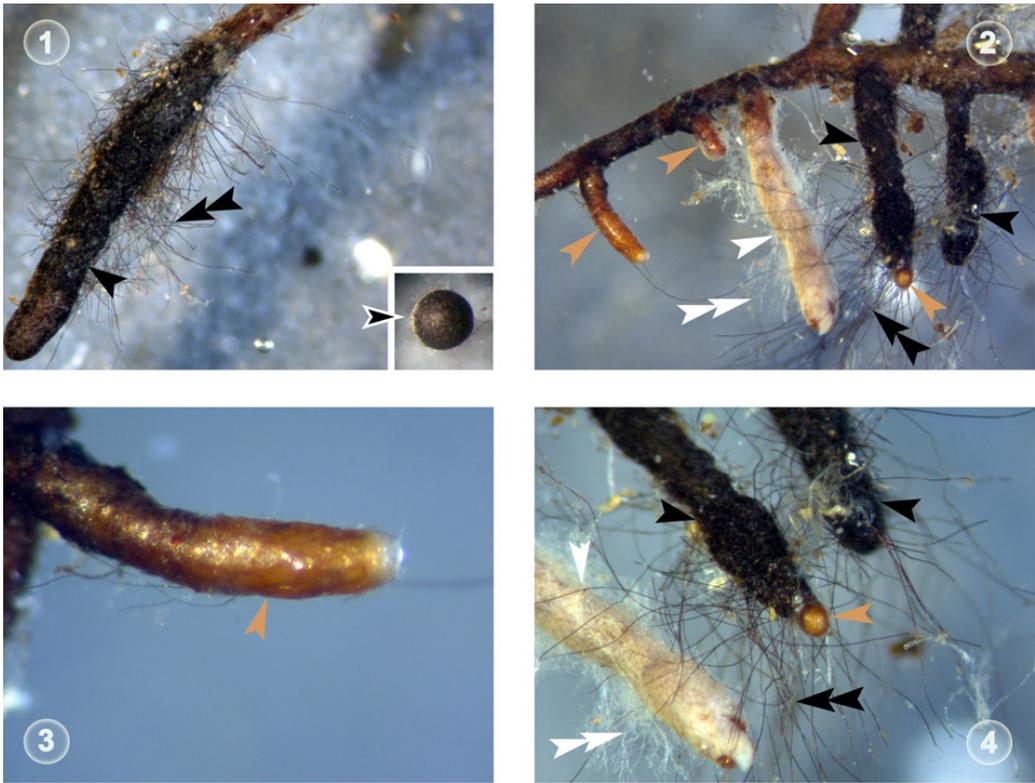


Fig. 5. Ectomycorrhiza on the roots of *Picea abies* with *Cenococcum geophilum*.

coccum geophilum: 'Whether *Cenococcum* is useful to its plant – symbiotroph as other useful ectomycorrhizal fungi is not clear'. It has been found that *C. geophilum* is extremely drought tolerant (Orcutt and Nilsen 2000) and this raises a hypothesis that the partnership in the formation of ectomycorrhizal mycocoenosis at dry conditions will increase. This gives us reason to believe that its role in the global climate change, particularly in the global warming, will be significant.

Figure 5 presents results of our own studies. The fine black mycelia threads (double black arrow heads) of *Cenococcum geophilum* ectomycorrhiza (black

arrow head) out of the mantle compact soil near ectomycorrhiza and protect both plant and fungus from drought. These threads are visible with naked eye and can serve also as an inoculum (Fig 5.1). Very often there are more species on the tree and even on particular root as we can observe directly but mostly in different stage of development (some of them are not functioning and even dead, but the fungal inoculum is very often active). On the root of this *Picea abies* we can find at least three different ectomycorrhizal morphotypes, referring to three different ectomycorrhizas (Fig 5.2 and Fig. 5.4). The white one in the center (Fig. 5.2, white ar-

row head) has clearly visible extraradical mycelium (white double arrow heads) and is well developed and fully functional. The right next to it (*Cenococcum geophilum* with the ochraceous orange tip) is old, the mantle is probably inactive, but the root is alive. From the tip of this ectomycorrhiza a new one emerge (Fig. 5.4, orange arrow head). It is unusual from *Cenococcum geophilum* ectomycorrhizas to grow another ones, the opposite in most cases is more often but as we see this sometimes happens and the most important factors are the availability of other active ectomycorrhizas near root tips or root buds (Fig 5.3) and the favorable conditions for development of particular species.

Among the most popular and studied ectomycorrhizal fungi from Ascomycota are these of genus *Tuber*, taxonomically in order Pezizales. They are about 60 species hypogeous fungi, mainly distributed in the Northern Hemisphere. At the end of 20th century attempts have been made, partly successful, to be introduced in New Zealand (Hall et al. 1994). Truffles belong to three different geographical regions – Southeast Asia, North America and Europe, with a major center of distribution – the Mediterranean region. The most valuable species in Europe is the white truffle from Piedmont – *Tuber magnatum* Pico and the black truffle from Périgord – *Tuber melanosporum* Vittad. Despite the progress of science and incredible efforts of experts, cultivation of the first is still almost impossible. This is due to the very specific ecological niche (Mediterranean climate and specific macro- and microstructure of the soil, pH 7–8), and probably the surrounding microbial environment that influences further development and preservation of ectomycorrhiza and for formation of ascomata. Studies

showed for *T. melanosporum* (Hall et al. 1994), that after inoculation, the photobionts increase their dry weight and size compared to the uninfected plants. The herbaceous vegetation around them dries and circles of dead vegetation called 'brulé' can be observed. This is most as a result of a phytotoxic action produced by the truffle mycelium, which affects the soil environment by killing some microorganisms (Pacioni 1991).

Structural Elements of the Ectomycorrhiza

Hartig net

Hartig net is made by hyphae that lead to its development from the inner layer of the mantle and grows between the cells of the cortical layer of root tips, without penetrating the cell walls. The main function of Hartig net is the bidirectional exchange of water with solutes and carbohydrates from the plant. This is achieved by complex, labyrinth-like branching hyphae, wherein the contact area is increased significantly and with the aid of enzymes that partially dissolve the outer layer of the cell wall, wherein the hyphae are 'wedge' between the cells of the plant roots. The presence of cytoplasm, rich in organelles is an evidence of dynamic metabolic processes. Hartig net can be observed by colorization with Chlorazol black E stain and Nomarsky DIC microscopy.

Mantle

The largest relatively constant structure is the mantle (mycoclena). It covers the outer part of ectomycorrhizal root tips and

constitutes 20–40 % of the biomass of ectomycorrhiza and its morphological structure is defined by mycobionts, photobionts and to a lesser extent by other factors like soil and climatic conditions (Harley and McCready 1952). In most of the ectomycorrhizal fungi, three layers are well distinguished: outer, middle and inner. Usually, the inner layer is compact, and branches intensively toward the root cortex in order to better exchange of nutrients, such as the hyphae are densely intertwined. The outermost one is often looser. Very often in the empty space between the hyphae are accumulated phenolic compounds and polysaccharides that serve subsequently for various metabolic processes (Peterson et al. 2004). Mantle cells envelope the root of its outer surface, and



Fig. 6. Semi-transparent mantle in dichotomously branched mycorrhiza in *Pinus sylvestris*.

come in direct interaction with the soil. Functional properties of the mantle are the result of its different structure. The mantle of the ectomycorrhizal fungi barred a root from the soil environment. In this way it acts as a physical barrier, but because morphologically and physiologically the fungal cell differs from the plant, all the advantages of the first are used by the plant. For instance, an important one is to restrict the downward flow of synthesized carbohydrates, located in the cells of the plant to the ground, where there is a drying in depth in a drought season. In general, the movement of water, mineral salts and carbohydrates is according to a physical law of the fluids – osmosis/diffusion, i.e., the direction of movement is from a higher to a lower concentration. This can be detrimental to the plant sometimes. Reduction of losses regulated by mantle fungal hyphae that exhibit selective bidirectional permeability. They are able to absorb glucose and fructose from the root cells by converting them through the enzymatic processes into digestible for the plants carbohydrates and glycogen. The latter can be stored for long periods in the mantle hyphae and can serve as nutritive reserve.

The mantle morphological and anatomical features are the basis for 'Colour Atlas of ectomycorrhizae' (Agerer 1986–2008). It was built on the ability of a fungus to form unique ectomycorrhizal patterns with one plant genus with a constant structure. Important morphological features for the differentiation of the mantle are: visibility of outer mantle layer (Fig. 6), cell organization type, presence of milky substances, cystidia and others. Agerer (1991, 1995) distinguished nine types plectenchymatous and seven types pseudoparenchymateous structure of the

mantle. The study can be done at different levels (Brundrett et al. 1994) – via dissecting and compound microscope, by a specialized microscopy – polarizing, Nomarski differential interference contrast microscopy (DIC), fluorescent and confocal, transmission – electron microscope (TEM) and scanning electron microscope (SEM). The last clearly differentiate the topography of external hyphae of the mantle. For a more detailed study it is necessary to combine more methods of microscopy. Another reason is that only the most thorough study of the ectomycorrhiza can make possible a reliable identification under natural conditions.

One of the most difficult to study processes is ectomycorrhizal morphogenesis. In past two decades due to the rapid development of molecular methods and efforts of researchers, many of phases are clarified enough to outline pathway from embryonic root stage to well-developed ectomycorrhiza. It is known that first, the precolonization events occurred in molecular level, then microclena is formed, hyphae are placed the intracellular space and, at last, extraradical mycelium develop (Smith and Read 2008). From this time the ectomycorrhiza begin to function in full capacity and its longevity was estimated to be about 4 months in the *Paxillus involutus* (Batsch ex Fr.) Fr. or *Tylospora fibrillosa* (Burt) Donk *Picea sitchensis* (Bong.) Carr. systems (Downes et al. 1992). One of the most interesting phenomena is cell death during ectomycorrhizal morphogenesis. The recent studies show, that during the pre-symbiotic phase in *Tuber* ectomycorrhiza formation, progressive accumulation of polyphenol deposits developed in epidermal and cortical cells, leading to the cell death (Ragnelli et al. 2014). This unexpected cell death, observed in healthy

mycorrhiza (both in fugal and plant partner) need to be further investigated and will be one of the key points for human influence on forest ecosystems.

Extraradical mycelium and common mycelial network

For the greatest importance of ectomycorrhiza is the extraradical mycelium, which usually varies or changes seasonally, and the emanating from mantle structures – rhizomorphs, sclerotia and others. In various ectomycorrhizal fungi the extraradical mycelium differs, sometimes considerably, according to the fungus – plant combinations and soil conditions, but the most important thing they have in common is the crucial role in the functioning of forest ecosystems.

Hyphae, developing from the outside of the mantle into the surrounding soil are forming the extraradical (extramatrical)² mycelium. Although studies under natural conditions are difficult, the experiments in Plexiglas containers, rhizotrones or root windows with natural or artificial substrate can provide useful information about its growth and development (Egli 1991). There is already sufficient evidence showing that the extraradical mycelium grows and colonizes substrates most commonly forming a spatial fan-shaped frame of the hyphae in the soil, extending to tens of centimeters from the plant root. Moreover, it connects them to each other, and makes a network between the roots of individual plants of one or of different species, most frequently of the same family (Kennedy 2005). This depends on the phytobiontic spectrum for the particular

²According to Agerer there is a difference between extraradical and extramatrical mycelium.

ectomycorrhizal fungus. When several compatible mycelium unite together, they form a common mycelial network that is capable to carry out transmission of water and minerals in different directions, but most often, to the plant, which suffers from deficiency (Read and Boyd 1986). Sometimes the hyphae produced sticky substances, the product of their metabolism, which have different functions as the most important of these is the adherence of soil particles to each other and to the hyphae. This is especially important for improving the structure of sandy soils and the increase of water retention ability. They are also able to penetrate into the soil colloidal particles, affecting favorably the soil structure. For instance – in clay and heavy soils they separate them while in sandy soils act compacting. Read (1992) has estimated that in 1 cm³ of soil, the length of these hyphae in *Pisolithus arhizus* (Scop.) Rauschert exceeds 200 m, as is often their thickness are comparable to the cross-section of a cell. When the rock below the upper soil horizons is reached, the extraradical mycelium is able to affect it destructively. It is because of small cracks that are in the rock formed by the secretions of organic acids and the penetration of hyphae inside. Some of the minerals then become available for absorption by the plant to perform its vital functions (Peterson 2004).

The natural environment of extraradical mycelium is a part of the soil, which in turn is also a natural habitat for various soil microorganisms. That is why there is a complex effect on the soil as they enhance or decline its conditions to a certain degree.

Very often it has been observed on the surface of the extraradical mycelium various aggregations of bacteria, fungi

and other saprotrophs in different layers of the soil. Studies show, that their role in the formation and functioning of ectomycorrhiza are not fully understood, but the experiments raise a hypothesis, that some of them help in the formation of mycorrhiza, others for their functioning and third have negative effects (Tarkka and Frey-Klett 2008).

The extraradical mycelium grows most often seasonally, but some authors confirmed that it can develop slowly even in winter in evergreen species – 0.44 mm per day for *Thelephora terrestris* Ehrh. (Coutts and Nicoll 1990). The growth rate per day in the active season can reach up to 4 mm, and in some combination of fungus-plant even more. The different seasonal growing trends of each ectomycorrhizal fungus affect to the ectomycorrhizal composition below and above ground. In this dynamic process the extraradical mycelium serves as a primary inoculum in the soil for colonizing new rootlets. For revealing of the functional role ectomycorrhizal fungi play, it is critical to compare the ratio of the hyphae length, which sets up the extraradical mycelium, compared to the total root system length. This can serve as an indicator of soil colonization degree and the potential for absorption by the plant. In different species, the results vary quite widely and sometimes are very impressive – from 200 to 8000 m of hyphae·m⁻¹ roots (Read and Boyd 1986). There is an attempt done by Weigt et al. (2012) to standardize the values of spatial distribution of extraradical mycelium in soil, according to different types suggested by Agerer (2001). Some terms, quantifying the extraradical mycelium are accepted as a standard: Specific Potential Mycelial Space Occupation (sPMSO) – the exploration type specific complete area that is covered

by the ectomycorrhizal mushroom systems ($\text{mm}^2 \cdot \text{cm}^{-1} \cdot \text{ECM}^{-1}$), Specific Actual Mycelial Space Occupation (sAMSO) – the projection area of mycelial systems ($\text{mm}^2 \cdot \text{cm}^{-1} \cdot \text{ECM}^{-1}$), Specific Extramatrical Mycelial Length (sEML) ($\text{m} \cdot \text{cm}^{-1} \cdot \text{ECM}^{-1}$), and Specific Extramatrical Mycelial Bio-mass (sEMB) ($\mu\text{g} \cdot \text{cm}^{-1} \cdot \text{ECM}^{-1}$).

Morphological structures of the extraradical mycelium

The extraradical mycelium forms different structures in natural habitats. The most important of them are: rhizomorphs (hyphal treads), sclerotia, asexual and sexual reproductive organs – fruiting bodies (sporophores – ascomata and basidiomata).

The term 'rhizomorph' is usually primarily used to describe the linear aggregations of parallel-oriented hyphae in wood-decomposed fungi (e.g. *Armillaria melea* (Vahl) P. Kumm., *Serpula lacrymans* (Wulfen) P. Karst., etc.). Unlike them, in the ectomycorrhizal fungi, these structures are not expressed so clearly in a morphological and structural manner and are not typical rhizomorphs but resembling them. Not every cord can be named rhizomorph and in ectomycorrhizal fungi, they are placed in several categories, according to their anatomical and functional structure. Morphology, color, internal composition and the type of ornamentation of calcium oxalate crystals are the most important features for their determination (Agerer 1986–2008). The study of rhizomorphs and cords shows that the interwoven of the hyphae are different in the species in a cross-sectional view. In more complex of them, one or more central hyphae are enlarged and extended up to the central cavity, which function is the transportation of water and minerals. External hyphae

are thickened with pigmented walls and perform mostly two functions – providing sufficient strength and flexibility, and prevent water loss during the transport.

Unlike the rhizomorphs, the thicker hyphal mycelial cords take an intermediate position according to the terms of morphology – between rhizomorphs and sucking hyphae. In most of the ectomycorrhizal fungi, sclerotia are rare and only appeared in certain species. It is a compact mass of hardened fungal mycelium containing food reserves. It is almost formed from the extraradical mycelium, but in some cases originated from rhizomorphs or emanating hyphae grown from the mantle. At a later stage of development, when it reaches maturity, the outer surface of sclerotium is covered by densely intertwined cells similar to a cortex. In the central hyphae of the sclerotia are deposited reserve substances such as polysaccharides, lipids, and polyphosphates. Almost always in favorable or unfavorable (under the stress) conditions from sclerotia, where they are available, according to the genetics of species, are developed spore-forming epigeous or hypogenous structures, as well as hyphae, which may subsequently form mycorrhizas. Their relative long durability is making them a suitable source for the preservation of the fungal cultures of the species under laboratory conditions and can be used for further research or other purposes (Brundrett et al. 1996).

Usually, under the occurrence of combination of a favorable conditions – suitable temperature, soil and air humidity, CO_2 concentration and the availability of nutrients, obtained from the host plant, the hyphae of extraradical mycelium grow and intersect at certain places just below the surface or below the upper soil horizon and form primordia. They

give the beginning of the special spore-forming structures – sporophores or most commonly known – fruiting bodies, which are very diverse in terms of morphology. Since last name is associated with the development of flowers and fruit in plants and is used in botanical sense, it is conceptually wrong, but it became more popular (Courtecuisse and Duhem 1995). Their proper name is ascoma (pl. ascomata) when they belong to Ascomycetes (Ascomycota) and basidioma (pl. basidiomata) if they belong to Basidiomycetes (Basidiomycota).

Ecological and Physiological Aspects of Ectomycorrhiza and Their Functional Role in Ecosystems

Fungal ectomycorrhizal component, typically act advantageously to the plant, especially in a poor soil conditions, where a deficit of one or more vital elements exists. The hyphae of the extraradical mycelium can reach considerably greater distance comparing to the fine sucking roots. They are able to overcome the zone of deficit for a particular macro- and microelement (Read, 1991) and supply it to the plant in the form of aqueous salt ions. In return, the plant give to ectomycorrhizal fungus ready to digest carbohydrates (sucrose or glucose+fructose depending on the ability to produce autonomously invertase by fungus) that are products of the photosynthesis. The effect for the plant is a result from the balance of synthesized by plant products on one hand, and the quantity of the basic components used for photosynthesis, on the other (Agerer et al. 2012). It is obvious that this problem is a challenging and a complex task because it depends on many factors variable in space and time.

Because of the diversity and different ecological niches occupied by both plants and fungi involved in ectomycorrhiza, there is a significant difference in the transport capacity of nutrients as well as different effects on growth of tree species involved in it (Burgess et al. 1994). This applies not only to the extent of colonization of the roots, but also for the development of hyphae of the species in the soil.

Agerer (2001) has classified the exploration types of ectomycorrhizal fungi, according to their structural elements and development differences based on the extraradical mycelium and now several exploration types are well recognized:

1) contact exploration type – only a few emanating hyphae occurs;

2) medium-distance exploration type – with three subtypes:

2.1) fringe subtype, which form fans of emanating hyphae and rhizomorphs,

2.2) mat subtype with the undifferentiated rhizomorphs or slightly differentiated,

2.3) smooth subtype with rhizomorphs with a central core of thick hyphae;

3) long distance exploration type – this type has a few but highly differentiated rhizomorphs of type F³;

4) pick-a-back.

In the evolutionary aspect, the first

³According to Agerer, 1986–2008, there are six type of ectomycorrhizal rhizomorphs:

a) Undifferentiated rhizomorphs with a loosely woven hyphae with several hyphae grow not parallel;

b) Undifferentiated rhizomorphs with compact arranged hyphae, growing parallel;

c) Slightly differentiated rhizomorphs with a little enlarged central hyphae;

d) Differentiated rhizomorphs with randomly distributed thick hyphae;

e) Highly differentiated rhizomorphs with a central core of very thick septate hyphae;

f) Highly differentiated rhizomorphs with a central core of very thick partially or completely hyphae.

types are more primitive, while long distance exploration type of the soil is the most complete. Pick-a-back type is not so widespread. Typically it often produces haustoria in the cortical cells of the root, and grows in association with other ectomycorrhizal types. The proposed classification is used by a number of authors in their mycocoenological studies. The presence of several types of exploration ectomycorrhiza with one plant allows a greater utilization of the available resources on one hand and on the other – a more efficient cover of the ecological niches of ectomycorrhizal fungi. Establishment of the exploration types made by Agerer (2001) has a great practical role for the quantifying and qualifying the ability of ectomycorrhiza to improve tree growth.

Extraradical mycelium, which spreads into the soil, can colonize it in considerable distances. Rhizomorphs of *Pisolithus arhizus* can connect saplings of *Pinus* at distance of about 42 cm and in long distance exploration type – more than 70 cm (Schramm 1966). In a study of the transfer of radioactive carbon isotopes *in situ*, there is an evidence of bidirectional nutrients exchange between *Betula papyrifera* Marshall and *Pseudotsuga menziesii* (Mirb.) Franco, through a common mycelial network composed of several extraradical mycelia belonging to one fungal species, forming ectomycorrhiza with both plants (Simard et al. 1997). In this experiment, there was a variable amount of transfer of ^{14}C in the direction from *Betula papyrifera* to *Pseudotsuga menziesii*, that vary from 6 % to 23 %, depending on the percentage of exposition to light 100 % (full) or partially. In the first case, the transfer is the greatest, and this experiment shows clearly that the plant species are able to adapt better and are more stable if they

grow as a community than as single individuals.

The function of extraradical mycelium for nutrients transportation has been established by a number of authors, who demonstrated the correlation between development stage, rhizomorphs type and quantity of transported phosphates in them. Due to its limited surface area and the small contact with the surrounding mycorrhizosphere the mantle plays a relatively limited role in obtaining nutrients, so the emanating hyphae and rhizomorphs significantly increase the volume of explored soil and the nutrients uptake takes a place mostly thanks to them.

The fundamental ecological importance of ectomycorrhiza is mainly for supporting of the plant with water and dissolved mineral salts by higher absorptive capacity of hyphae and the much larger area compared to this of fine roots; protection from pathogens; soil improved structure; degradation of the main rocks by organic acids released from the hyphae and reduction of adverse pollution effect by decomposition and transformation of toxic molecules for humans to non-toxic compounds.

Conclusion

The ectomycorrhizosphere plays a key role for plants, especially those in forest ecosystems, which grow under physiological stress due to various abiotic and biotic factors. Ectomycorrhiza is actively involved in the carbon cycle. According to classical studies from Read (1991) at least 10 % of it is used by ectomycorrhizal fungi for their growth and development. The dynamics of the distribution of ectomycorrhizal ecological niches in time and

space significantly affects the appearance of plant communities in the boreal region.

The studying of ectomycorrhiza is almost 130 years old. For the past two decades it reached a new quality stage and the modern molecular methods shed new light on the distribution, structure and size of various populations in different habitats and phylogenetic relation between species.

The benefits from ectomycorrhiza are proven experimentally and are no longer matter of debate. For several decades in a lot of countries, for afforestation purpose, on nutrient limited soils are used specially inoculated (often with several ectomycorrhizal species) seedlings. Disadvantageous effects of pollution, mainly from heavy metals in soils with anthropogenic impacts can be reduced and the vitality of weakened woody plants with conservation significance enhanced by the introduction of additional ectomycorrhizal inoculum. The synthesis of ectomycorrhiza and subsequent success can be achieved only on the basis of deep knowledge of ectomycorrhizal fungi biology, their photobiotic spectrum and appropriate soil and climatic characteristics of the terrain.

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