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# THE ROLE OF MYCORRHIZAE IN AFFORESTATION

(A REVIEW)

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Hungary is facing to perform intensive afforestation. New forests will be planted on dry, poorly fertile soils nonprofitable for agricultural use. Applying artificially mycorrhized seedlings may considerably increase the effectivity of afforestation and decrease costs.

**Keywords**: forest ecosystems, ectomycorrhizae

## Introduction

Hungary had always belong to the European forefront of quantitative afforestation. Therefore after the Second World War the forest area could be increased from 11% by the present to 18%. The extent of agricultural territories suitable for afforestation in the country is calculated to be between 700 thousand and 1 million hectares [1, 2, 3].

The agricultural territories possibly involved in afforestation belong partly to the very dry, sandy areas of Nyírség and the space between the rivers Duna and Tisza. In these places drougth is often raised by high lime content. The other part of land potentially usable for afforestation is the steeps of mountains and hills previously covered by woods. These areas have been cultivated for centuries but exhaustion and erosion degraded the soils, so their agricultural use is nonprofitable.The humus content of these soils is very low, usually below 1%. In addition agricultural soils miss the normal microbiota of forest soils the trees are adapted to and contain highly different microbe communities disadvantageous for the development of planted seedlings.

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It can be stated that the roots of tree seedlings planted into agricultural soils get into a hostile environment which a part of the plantlets cannot cope with. That is one reason why new forests must be planted in average 1.6–1.7 times or even twice.

According to our results merely in Bács-Kiskun County there are more than 300 thousand hectares of land where no profitable agriculture can be proceeded. In such case afforestation seems to be the most reasonable use of land [4].

Rapid ecological changes of the last years (e.g. warming up, drying and sink of underground water level) warn us to look for new ways of afforestation successful also in disadvantageous circumstances. Establishing artificial mycorrhizae on the roots of plantlets is such a new and in addition a natural method.

## The role of ectomycorrhizae in forest ecosystems

Mycorrhiza, a symbiotic relationship between roots and fungi, is widespread all over the world. Different types of mycorrhizae, characteristic to plant communities having evolved in different geographical and climatical zones, exist. In the deciduous and needle woods under temperate climate trees typically form ectomycorrhizal connections mainly with basidiomycetes, less with some ascomycetes.

Diversity of fungal communities in forest ecosystems is determined by the following factors:

- age of the trees in the stand
- composition of natural plant association, the occurrence of host plants, specificity of host-symbiont connection
- soil factors (pH, chemical composition, organic contents, etc.)
- climatic and microclimatic factors

### *Spatial and temporal changes of fungal communities correlated to the age of tree stand*

In balanced forest ecosystems the composition of fungal community changes spatially and temporally [5, 6, 7, 8, 9]. In other terms, biodiversity is modifying in all forest ecosystems during aging, causing irreversible changes in fungal community structure being expressed in succession.

It is easy to observe that fungal species are continuously rearranged quanitatively and qualitatively in a given forest shown by the change of spatial distribution of the fruitbodies. Ectomycorrhizal fungi starting from the trunks mainly follow the radial lines of the roots. The primarily appearing so-called “early stage fungi” are continuously pushed away from the trunk in the direction of the margin of the canopy’s shade. In contrast, “late stage fungi” appear near the trunk in the line of old roots. *Hebeloma, Laccaria, Thelephora, Suillus, Pisolithus, Scleroderma, Rhizopogon, Melanogaster, Tuber,* etc. species are considered as early stage fungi while the members of the genera *Amanita, Russula, Cortinarius* and *Boletus* are late stage species.

Members of both groups may occur in the same stand but some scientists observed the contrary. The investigations of Bendiksen in Norway [cited in 10] showed that fungal fruitbodies of an old *Pinus sylvestris* stand were not different from that of a 20-year-old one. In contrast, Vries [cited in 10] observed much more mycorrhizal fungi in 50–80-year-old *Pinus* stands than Termorshuizen & Schaffers [11] in young, 50–10-year-old stands.

This phenomenon is very important in the respect of artificial mycorrhization. For establishing mycorrhizae on plantation seedlings obviously early stage fungi are recommended.

### *Effect of the tree species of forests on fungal community structure*

It is widely known that mycorrhizal fungi are much more influenced by biotic factors than saprotrophic species with broader metabolic tolerance. Many examples of ubiquist saprothrophic, nitrophilic fungi exist that can colonize both nitrophilic forests and ruderal areas rich in nitrogen as well as composts of plant and animal origin. In contrary, selectivity of mycorrhizal fungi is much stronger even if much of them are able to be connected to several host plants. However, some fungi are connected to a single host, e.g. some species of the genera *Suillus, Lactarius, Rhizopogon* and *Leccinum* [12].This specificity is also characteristic to a few tree species like the members of *Alnus* and *Larix* and some *Pinus*-species (*P. cembra, P. strobus*) [10].

Otherwise, it would not be easy to set a range of forest trees based upon the number of partner fungi. Within the family Fagaceae probably beech and oaks, in mountain forests the *Abies* stands are the richest in fungal partners. However, the quantitative richness in fruitbodies may cover a medium or weak diversity of species, like in atlantic *Pinus* stands. In *Qercus ilex* and *Q. suber* forests the presence of shrubs cause a significant variability of fungal community. The complexity of plant species of forests is resulting in a more diverse mycobiota.

Mycological investigations have detected 104 basidiomycete and 4 ascomycete species in sandy poplar forests of Kiskunság, mainly connected to *Populus alba* [13]. Characteristic fungal species, represented by fruitbodies, are *Xerocomus bubalinus, Cortinarius paracephalixus, Hebeloma ochroalbidum, H. ammophilum, Inocybe aeruginascens, I. javorkae, Laccaria tortilis, Lactarius controversus, Russula clariana, R. pelargonia, R. medullata, Tricholoma populinum* and *T. inocybeoides*. However, mycorrhizal community is dominated by different species (e.g. *Xerocomus armeniacus, Thelephora* and *Tomentella* species, *Tuber rapaeodorum* and *Russula amoenolens*). Five new ectomycorrhizae (*Tomentella subtestacea, T. pilosa, Russula amoenolens, Lactarius controversus* and *Scleroderma bovista*) have been described from *P. alba* forests of the southern region of the Kiskunság [14, 15, 16, 17, 18].

Guinberteau et al. [19] demonstrated strong intraspecific inhibition between *Suillus granulatus* and *S. bovinus* in *Pinus pinaster* plantations. While these two species push out each other, e.g. *S. granulatus* is able to colonize the same tree together with *Lactarius deliciosus*. A similar feature has been found by Murakami with *Russula* species [cited in 10].

Forests growing under different climatic and soil conditions are dominated not only by characteristic tree species but also by their typical mycorrhizal community [20]. Nevertheless, the rate of mycorrhization is correlated with the change of environmental factors. Decreasing of mycorrhizae indicates pollution or other degradation processes of environment [21, 22, 23, 24, 25, 26].

### *Effect of soil on fungi*

Although mycorrhizae are mainly determined by the species of plant association in forest ecosystems, soil characteristics also play an outstanding role in fungal community structure. Certain fungi are connected to certain soils. E.g. the mycobiota of calcareous beech woods is quite different from that of acidophilous beech woods. Some authors use the term soil specificity for the demand of fungal species. Tyler [cited in 10] investigated the distribution of 150 mushroom species in different forest associations according to soil types, measuring pH and organic content. The occurrence of the majority of species was correlated with soil characteristics. Only one third of the species showed wide soil tolerance. It has been clearly demonstrated that diversity of mycorrhizae is much higher on acidic soils, e.g. on podsols containing moor-humus, than on other types. In contrary, *Xerocomus badius* and *Russula ochroleuca* are strongly connected to organic soils while other fungi prefer alcalic soils with mull-humus.

From truffle cultivation experiences it is evident that *Tuber melanosporum* and *T. uncinatum* (*T. aestivum*) both prefer calcareous, humus-rich soils, although they are able to cooperate with several tree species (*Quercus ilex, Q. robur, Q. pubescens, Q. cerris, Tilia platyphyllos, Carpinus betulus, Corylus avellana, C. colurna, Pinus nigra, Cedrus atlantica*). The previously unknown mycorrhizal relationship between black locust (*Robinia pseudoacacia*) and the white truffle *Terfezia terfezioides* being present only in locust strands on calcareous soils can also be mentioned here [27]. As this fungus is an edible one, it may have importance in accessory use of locust plantations.

*Effect of mycorrhizae on growth, water and mineral uptake of host plants*

Drastical drying of soil is followed by defensive regulatory processes of the plant. The main steps of these are:

*closing the stomata*
*osmotic control*
*decreasing foliar growth*
*defoliation*
*intensive growth of fine roots*
*embolia*

In ectomycorrhizae, a dense sheet of fungal mycelium, the so called mantle, is covering the root tips. Emanating hyphae, growing from the mantle into the soil, multiply the surface of root active in water uptake. This is the main reason of the advantageous effect of mycorrhizae to plants. The mantle and the adjoining hyphal network supply the root also with substances (certain metallic ions, phosphorous compounds) which the root itself is unable to take up [28]. At the same time the fungal partner gets assimilates (sugars) from the plant.

The advantage of mycorrhizae compared to non-mycorrhizated plants is more distinctly manifested in dry soils, poorly supplied with phosphorous and nitrogen. Mycorrhization increases growth (Table I) as well as P and N content of plants (Table II.)

The experiment was carried out in partly controlled conditions. The 3,5-months-old seedlings have been inoculated partly by artificially cultivated mycelia, partly by extract of naturally mycorrhized roots.

Soil samples originated from horizon A.

1. podsol with humus; 2. sandy adobe soil; 3a. sandy soil poor in humus (humus < 0,55 %); 3b. sandy soil poor in organic componds (humus = 0,08 %).

Mycorrhizae help plants to survive dry periods and adapt to limy [29, 30, 31, 32]. Mycorrhizal seedlings can tolerate higher soil temperature and lower pH conditions. Mycorrhizae increase tolerance of plants against inorganic and organic toxic substances, protecting them from heavy metal stress [33, 34, 35, 36]. This is extremely significant economically in afforestation and reafforestation of dry, poor and polluted areas (Table III).

**Table I**

*Effect of ectomycotrrhiza on overfround growth of Pinus pinaster seedlings 10 months after planting out (fresh plant mass in g)*
*MOUSSAIN et al [cited in 10]*

| Soil | Non-mycorrhized control | Natural mycorrhiza | Pisolithus tinctorius | Hebeloma cylindrosporum |
|---|---|---|---|---|
| 1. | 1.4 ± 0.2 | 3.5 ± 1.2 | 10.6 ± 1.6 | 13.5 ± 2.1 |
| 2. | 0.6 ± 0.3 | | 7.3 ± 2.6 | 8.9 ± 1.7 |
| 3a. | 1.4 ± 0.7 | 3.5 ± 0.5 | 7.7 ± 0.7 | 6.9 ± 1.1 |
| 3b. | 1.5 ± 0.3 | | 4.6 ± 0.8 | 3.8 ± 0.4 |

**Table II**

*Effect of mycorrhization on the N and P content of overground parts of Pinus pinaster seedlings 10 months after planting out*
*(expressed as % of dry mass)*
*Moussain et al. [cited in 28]*

| Content | Non-mycorrhized control | Natural mycorrhiza | Pisolithus tinctorius | Hebeloma cylindrosporum |
|---|---|---|---|---|
| Total P | 0.09 | 0.17 | 0.21 | 0.32 |
| Total N | 1.79 | 2.19 | 2.10 | 2.77 |

## Possibilities of application of mycorrhizae

### *The role of mycorrhizae in plant protection of nurseries*

In natural woods seedlings are mycorrhized from the beginning of their life. However, the so-called mycorrhizosphere includes also other microorganisms, advantageous, disadvantageous or neutral for the roots.

Mycorrhizae seldom develop in nurseries where seedlings get everything they need (nutrients, water). As partly desinfected soils often contain microorganisms harmful to root development, seedligs can be damaged by pathogenic fungi, especially in monocultures and in the case of surplus irrigation. Fungicides applied against pathogens not only kill these but also mycorrhizal fungi. Therefore, “integrated plant protection”, using much less pesticides and preferring biological control methods by spreading beneficial microbes, is getting more and more perspective.

*Effect of root pathogenic fungi*

Underground parts of plants are damaged as frequently by pathogens as green parts but these infections may be much more serious because they can be hardly observed and protected against. In most cases development of plants stops, plants became chlorotic followed by fading and decay.

The most frequent root pathogenic fungi belong to the following genera:

*Phytophthora*
*Pythium*
*Fusarium*
*Rhizoctonia*
*Cylindrocarpon*

Several other facultative parasitic fungi are normal components of the communities of nursery soils. These cause diseases when plants are stressed (alcalic pH, bad water draining, too low/high temperature or irradiation). In contrary, *Phytophthora*, *Fusarium* and *Rhizoctonia* are agressive pathogens damaging also healthy plantlets. These are difficult to eliminate because they can survive even the strongest soil desinfection. The different pathogenic fungi are difficult to determine as they cause similar symptoms. For identification laboratory methods are needed.

*Methods of biological control*

The most simple controlling methods are crop-rotation and soil desinfection. The sensibility of phytopathogenic fungi against chemicals are highly different. Many pathogenic fungi (e.g. *Phytophthora*, *Pythium*) are resistant to commonly used fungicides .Worldwide tendency of decreasing the use of pesticides from human health and environmental protection reasons helps biological control methods to expand.

In forestry elaborated biological methods exist and “biopesticides” against insects (e.g. *Bacillus thüringiensis* against different larvae) are in commercial use. Fungal antagonism is a phenomenon also potenially suitable to apply against root pathogens (e.g. *Trichoderma*). The problem is that some phytopathogens are connected to the rhizosphere much stronger than their antagonists.

The plant protection effect of ectomycorrhizal fungi have been demonstrated. These fungi may play a significant role in the biological control of nurseries. The main advantage is that, in contrast of pesticides which must be applied repeatedly, mycorrhizae have to be applied only once. However, it is important that inocula get into the soil before phytopathogens can spread.

*The protection mechanism of ectomycorrhizal fungi*

Fungal mantle itself serves as a mechanical defense barrier to root. Besides this, the fungus protects the plant also by physiologic processes, by degrading toxins and enzymes of the pathogens. Some fungi also produce acids and antibiotics inhibiting the enemy. Ectomycorrhizal fungi compete with pathogenic species for the use of root carbohydrates. The rhizosphere of mycorrhized roots are about ten times richer in other microorganisms than non-mycorrhized ones. Some microbes enhance mycorrhization (MHB=Mycorrhiza Helper Bacteria) and some of them show an additional inhibition against pathogens. It has been already demonstrated in 1969 that seedlings mycorrhized with different fungi were protected against the damage of *Phytophthora cinnamomi. Pinus sylvestris* seedlings mycorrhized with *Laccaria laccata* were resistant to *Cylindrocarpon* infection which often causes disease as facultative parasite [37]. The main damages caused by fungi in *Pinus* and *Picea* nurseries are fusarioses. Mycorrhized by *Laccaria laccata Pseudotsuga* and *Picea* seedlings were able to resist *Fusarium oxysporum*. The mechanism of inhibition had been explained as incresed synthesis of phenolic substances in mycorrhizal roots.

## The role of mycorrhizae in afforestation

The experiences mentioned above indicate that establishing mycorrhizal symbioses could play an outstanding role in successful afforestation. While beeing planted out, seedlings must survive a strong stress mainly caused by water loss and pathogenic soil fungi. Natural way of developing ectomycorrhizae is often impossible, because mycorrhizal fungi may be absent from the soil, especially in the case of previously agricultural areas. Several experiments carried out in the U.S., in France and Australia proved that planting out mycorrhized seedlings enhance their chance of surviving planting stress. These plantlets grow faster and are more resistant to drought (Table III).

According to experiments carried out in Germany the sheat volume of beech seedling mycorrhized with *Pisolithus tinctorius* was by 72% higher than that of the non-mycorrhized control. The same value in the case of *Paxillus involutus* was 58% [38].

**Table III**

*The percentage of survival and growth of forest tree seedlings mycorrhized by Pisolithus tinctorius at different biotopes in the U.S.*
*Marx & Cordell [31]*

| TREE SPECIES | NUMBER OF PLACES INVESTIGATED | AGE OF THE STAND (year) | PLUS PERCENTAGE OF SURVIVAL (%) | PLUS GROWTH (%) |
|---|---|---|---|---|
| *Pinus clausa var. immuginata* | 2 | 2 | 96 – 169 | 270 – 274 |
| *Pinus clausa var. immuginata* | 1 | 7 | 11 | 35 |
| *Pinus echinata* | 1 | 4 | 0 | 41 – 89 |
| *Pinus echinata* | 2 | 2 | 34 – 39 | 96 – 141 |
| *Pinus elliottii var. elliottii* | 3 | 2 | 5 – 22 | 6 – 175 |
| *Pinus palustris* | 1 | 2 | 6 – 55 | 11 – 99 |
| *Pinus palustris* | 1 | | 116 | |
| *Pinus palustris* | 2 | 3 | 7 – 38 | 100 – 180 |
| *Pinus palustris* | 1 | 7 | 22 | 58 |
| *Pinus strobus* | 1 | 2 | 8 | 500 |
| *Pinus taeda* | 4 | 2 | 0 | 21 – 63 |
| *Pinus taeda* | 8 | 3 | 5 | 25 |
| *Pinus taeda* | 1 | 3 | 0 | 28 |
| *Pinus taeda* | 1 | 2 | | 38 |
| *Pinus taeda* | 1 | 2 | 20 | 54 |
| *Pinus taeda* | 1 | 2 | 0 | 18 |
| *Pinus virginiana* | 2 | 2 | 2 – 4 | 29 – 55 |
| *Quercus acutissima* | 1 | 2 | 73 | 53 |
| *Quercus palustris* | 1 | 2 | 3 | 39 |

Calculations exist that in Hungary every hectare afforestation must be repeated 1.6–1.7 times to survive the seedlings optimally. Therefore all trials enhancing the effectiveness of planting out must be considered significant.

Between 1990 and 1994, which was the weakest period of afforestation of the last ten years, yearly 221 million seedlings were planted out in average in Hungary.

Although the area of the plantation decreased, no significant decrease in the number of seedlings used can be demonstrated. One reason of this is the very dry weather of these years causing a great loss in plantlets.

*The average costs of seedlings (at 1994 cost-level):*

| | | |
|---|---|---|
| oaks | 5.5–8.0 | HUF/piece |
| beech | 6.0–8.0 | “ |
| *Q. cerris* and other hard–leaved | 3.5–6.0 | “ |
| locust | 2.5–3.0 | “ |
| average broad–leaved | 5.3 | “ |
| poplar | 12.0–20.0 | “ |
| Pinaceae | 3.5–12.0 | “ |

Counting by the values above, the yearly average cost of the 221 million seedlings is 1 353.4 million HUF. The 50–60% of the seedlings were used for replacing the losts. That is a significant waste which means only for the seedlings 726.7–872.0 million HUF. In addition the high costs of surplus work, e.g. wages must be mentioned. Altogether, the replacement of seedlings caused about 1 billion HUF plus cost yearly.

In 1999–2000 the afforested area again increased to 6–8000 hectares yearly. It is planned that the forest area in Hungary reach 700 thousand to 1.2 million hectares within 40 years. That means afforestation of 15-20 thousand hectares yearly, so the planted area should be increased threefold. As the costs of seedlings have doubled since 1994, that means that six times higher costs (about 6 billion HUF/year) should be calculated for afforestation now.

A significant proportion of this high sum could be saved up by using mycorrhized seedlings. A part of the spared money would cover the costs of elaboration and adaptation mycorrhizal technology to Hungarian relations and introduce this economic and environment protecting method in forestrial application.

According to experiments carried out in Germany the sheat volume of beech seedling mycorrhized with *Pisolithus tinctorius* was by 72% higher than that of the non-mycorrhized control. The same value in the case of *Paxillus involutus* was 58% [38].

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