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## Ectomycorrhizae of *Populus alba* L. in South Hungary

By

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### Summary

JAKUCS E. 2002. Ectomycorrhizae of *Populus alba* L. in South Hungary. – *Phyton* (Horn, Austria) 42 (2): 199–210. – English with German summary.

Ectomycorrhizae (ECM) of needle and deciduous forests of North America and Northern, Southern and Western Europe have been investigated intensively, but only few data are available about the mycorrhizae characteristic of the forests in Central Europe under dry continental climate. That is why we aimed at studying the natural ECM communities in drought-adapted forests grown on sandy soils in the Hungarian Plain. The common ectomycorrhizae of *Populus alba* L., a native tree of the region, potentially suitable to be used also in the reforestation program in the lowlands, have been investigated. In the period of 1997–1999, from soil samples collected in two sampling plots, more than 70 morphotypes have been discerned and the 14 most common ECM-types of *P. alba* determined using morphological and molecular methods. Relative abundance of these dominating types and an identification key based on easily observable micro-morphological characteristics have been presented.

### Zusammenfassung

JAKUCS E. 2002. Ectomycorrhiza auf *Populus alba* L. in Südungarn. – *Phyton* (Horn, Austria) 42 (2): 199–210. – Englisch mit deutscher Zusammenfassung.

Die Ectomycorrhiza (ECM) der Nadel- und Laubbäume Nordamerikas sowie Nord-, Süd- und Westeuropas wurden bereits eingehend untersucht, hingegen sind nur wenige Daten über die Eigenschaften der Mycorrhiza der Wälder Mitteleuropas unter trockenem kontinentalen Klimaeinfluss vorhanden. Deshalb untersuchten wir die natürlichen ECM-Gesellschaften in an Trockenheit angepassten Wäldern, welche

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auf sandigen Böden der ungarischen Tiefebene stocken. Dabei wurde der Schwerpunkt auf die Ectomycorrhiza von *Populus alba* L., einem natürlich vorkommenden Baum dieser Gegend gelegt, welcher auch für die Wiederaufforstungen der Tieflagen geeignet ist. Von 1997 bis 1999 wurden über 70 Morphotypen aus Bodenproben zweier Probestellen unterschieden und die 14 häufigsten ECM-Typen von *P. alba* wurden mittels morphologischer und molekularbiologischer Methoden bestimmt. Die relative Menge dieser dominierenden Typen wurde abgeschätzt und ein Bestimmungsschlüssel, welcher sich nach leicht erkennbaren mikromorphologischen Kriterien richtet, vorgestellt.

## Introduction

Although the number of ectomycorrhizal (ECM) fungal species is estimated to be 5000–6000 world wide (MOLINA & al. 1992), only about 350 ectomycorrhizae have been described comprehensively (AGERER 1989–1998, AGERER & RAMBOLD 1998). ECM communities of needle woodlands in Northern, Southern and Western Europe and North America have been studied intensively in the last decade (GARDES & BRUNS 1996, COMANDINI & al. 1998, DAHLBERG & al. 1997, GEHRING & al. 1998, JONSSON & al. 1999, KERNAGHAN 2001, TAYLOR & BRUNS 1999), but ectomycorrhizae of deciduous forests have been less examined. Ectomycorrhizae of beech (BRAND 1991), birch (CUVELIER 1991) and alders (MILLER & KOO 1991, PRITSCH & al. 1997) have been investigated to some extent, but almost nothing is known about the natural ectomycorrhizal relationships of the poplar forests characteristic of Central Europe, nevertheless poplar species are frequently used in synthesized mycorrhizal experiments (GODBOUT & FORTIN 1985, BENCIVENGA & al. 1994).

*Populus alba* L. is distributed in the southern part of Eurasia between the 25<sup>th</sup> and 52<sup>nd</sup> degrees of latitude. It is a typical drought adapted lowland species with a great pretension of light and heat, but tolerating extreme seasonal changes of temperature (GENCSI & VANCURA 1992). In the wooded steppe zone, where the lowland territories of Hungary belong to, *P. alba* is dominant on sandy soils having originated from alluvial deposits and on sand dunes bordering the riverine gallery forests. As a native species of the territory and one of the potential trees suitable to be used in the reforestation program of the Hungarian Plain, *P. alba* is one of the most interesting trees for forestry.

The analysis of the structure of natural ECM communities may give useful hints to apply artificial inoculation with ECM fungi in silviculture of the region. Therefore we aimed at a study of the most common ectomycorrhizae formed on *P. alba* in drought-adapted forests grown on sandy soils in the southern part of Hungary.

Up to now only few *Populus*-ectomycorrhizae have been thoroughly studied. There are detailed descriptions of the *Tricholoma scalpturatum* (Fr.) Quél. and *Inocybe lacera* (Fr.) Kumm. mycorrhizae from *P. tremuloides*

Michx. (CRIPPS 1997a, b), of *Inocybe fuscomarginata* Kühn. from *Salix* sp. and *P. nigra* L. (BEENKEN & al. 1996) and of "*Populirhiza pustulosa*" from *P. tremula* L. (MLECZKO 1997). As a result of our three-year-investigations, the morphological-anatomical descriptions of five new ectomycorrhizae from *P. alba*, i.e. *Lactarius controversus* Pers., *Russula amoenolens* Romagn., *Scleroderma bovista* Fr., *Tomentella pilosa* (Burt.) Bourdot & Galzin and *T. subtestacea* Bourdot & Galzin have been or are going to be published in Descriptions of Ectomycorrhizae (JAKUCS & al. 2001, JAKUCS & BEENKEN 1999, JAKUCS & AGERER 1999a, b, JAKUCS & AGERER 2001).

This paper gives an overview of the most common ectomycorrhizae and their relative abundance found on *P. alba* in South Hungary. An identification key including the dominating ectomycorrhizal types has been compiled

## Material and Methods

### Sampling

Two sampling plots in *P. alba* stands grown on sandy soils in the southern part of the Hungarian Plain near the Serbian border, at 120–130 m altitude, have been chosen. The average rainfall in the region is 550 mm/year. The two plots, one near Tompa, the other near Kelebia, are about 10 km far from each other. The forest near Tompa is a *P. alba* stand with 35–40 m high trees mixed with *Acer negundo* L., *Celtis occidentalis* L., *Fraxinus excelsior* L., *Robinia pseudoacacia* L., *Sambucus nigra* L. and *Ulmus laevis* Pall. The forest has been established replacing an autochthon zonal Convallario-Quercetum roburis stand. The soil is sandy, mixed with loess and with a quite thick (about 10 cm) moder-type humus layer containing 0.4% lime, pH 7.1–7.6. The forest near Kelebia is a younger plantation with no higher than 30 m *P. alba* trees mixed with *Celtis occidentalis*, *Crataegus monogyna* Jacq., *Robinia pseudoacacia* and *Prunus* sp. The soil is sandy with a thinner (about 4 cm) moder type humus layer than in the Tompa stand, containing 2.4–2.6% lime, pH 7.9.

At both sites, mycorrhizal soil samples were taken five times during the period of 1997–1999. At each time 3–5 soil samples (as repetitions) were taken randomly from the same 25 × 25 m square in both territories. 20 × 20 × 20 cm soil cubes were cut with a sharp knife from the upper layer of the forest soil. During our three-year investigation, altogether 21 soil samples have been elaborated from 'Tompa' and 15 from 'Kelebia'. After taking to the laboratory, soil samples had been stored in a refrigerator at 4 °C for not more than one week. Roots were washed out from the soil cubes with tapwater over a sieve and ectomycorrhizae were separated and selected in water under a stereomicroscope. Ectomycorrhizal tips were partly used alive for some microscopical observations and histochemical reactions, partly they were fixed in FEA for further microscopy (AGERER 1991). For DNA-analysis three ectomycorrhizal tips from each type were fixed in CTAB buffer and elaborated following the protocol of AGERER & al. 1996a, b. Vaucher specimens fixed in FEA had been preserved in the collection of E. Jakucs and the identified new morphotypes deposited in the Hungarian Natural History Museum, Budapest.

## Morphological characterization

Ectomycorrhizae have been characterized and described using the widely accepted morphological, anatomical and histochemical methods introduced by AGERER 1991. The mycorrhizal system was examined by stereomicroscopy. The mantle structure, the hyphal and rhizomorphal characteristics were studied by DIC (Nomarski) microscopy, including microscopic drawings and photodocumentation. Identification on morphological and anatomical basis was carried out using the Colour Atlas of Ectomycorrhizae (AGERER 1989–1998) and the DEEMY CD-ROM (AGERER & RAMBOLD 1998). In some cases rhizomorphal connections between the ectomycorrhizae and the fruitbodies helped to identify ectomycorrhizae.

## DNA-analysis

Molecular taxonomical investigations were used only for identification purposes in the case of a few frequent ECM-types. DNA from sporocarps and mycorrhizal tips were extracted in CTAB and PCR amplification was performed according to GARDES & BRUNS 1993.

To prove the supposed identity of the *Lactarius controversus* and *Russula amoenolens* ectomycorrhizae with their fruitbodies, RFLP analysis of the ITS region of the ribosomal DNA amplified by PCR using ITS1 and ITS4 primers has been performed as described elsewhere (JAKUCS & BEENKEN 1999, JAKUCS & al. 2001).

The three *Tomentella*-ectomycorrhizae had been identified comparing ITS-sequences of the rDNA of ECMs and fruitbodies, as published by KÖLJALG & al. 2001.

## Estimation of relative abundance

The relative abundance of the most common 14 ECM-types within each soil sample was calculated by visual estimation of the mycorrhizal tips using the semi-quantitative method of GARDES & BRUNS 1996. The relative abundance of ECM morphotypes means the percentage of tips of the individual morphotypes within the total mycorrhizal root tips in the soil cube. Abundance values were ranked as: A, minor component (<10% of the total tips in the soil cube), B, minority codominant (10–50%), C majority codominant (50–90%), D, dominant (>90%).

## Results

The ECMs collected in the two sampling plots represent more than 70 different morphotypes. Only the most abundant 14 types have been identified to species or genus level. Identification of the taxa included in Table 1 was carried out in different ways. In the case of *Scleroderma bovista* and *Xerocomus armeniacus* fruitbodies were determined and rhizomorphal connections with the ectomycorrhizae supported the identity, which was also suggested anatomically. The determination of the *Tuber rapaeodorum* ectomycorrhiza was based on identification of fruitbodies near mycorrhizae, and by its similarity with the ectomycorrhizae of *Tuber borchii* (RAUSCHER & al. 1996), a very close species to the former. This determination needs further confirmation using molecular methods.

Table 1.  
The most common ectomycorrhizae of *P. alba* in the southern part of the Hungarian Plain (listed alphabetically).

ECM-type/species	Colour	Mantle	Morphological characteristics			Characteristic feature
			Rhizomorph	Cystidia	Clamps	
<sup>1</sup> <i>Cortinarius</i> spp.	W	PL	UNDIFF	-	+	Woolly surface
<sup>1</sup> <i>Genea</i> sp.	BR (Y)	PS-A	-	Globular	-	Thick em. hyphae
<sup>1</sup> <i>Hebeloma</i> spp.	O	PL	-	-	+	Cottony surface
<sup>2</sup> <i>Lactarius controversus</i> Pers.	W (Y)	PL	-	-	-	Rosette crystals
<sup>1</sup> <i>Lactarius</i> spp	W (Y)	PL	-	-	-	Smooth surface
<sup>2</sup> <i>Russula amoenolens</i> Romagn.	W (Y)	PL	-	Bottle-shaped	-	Short spiny surface
<sup>1</sup> <i>Russula</i> spp.	W (Y)	PL	-	Bottle-shaped	-	Short spiny surface
<sup>3</sup> <i>Scleroderma bovista</i> Fr.	W	PL	DIFF	-	+	Cottony surface
<sup>1</sup> <i>Tomentelloid-types</i>	BR, BL	PS-A	-	-	+ or -	Em. hyphae + or -
<sup>4</sup> <i>Tomentella galzinii</i> Bourd.	O, Y	PS-A	UNDIFF	Clamped	+	Tort. hyphae -
<sup>4</sup> <i>Tomentella pilosa</i> Bourd. & Galz.	O, Y	PS-A	DIFF	Clamped	+	Tort. hyphae +
<sup>4</sup> <i>Tomentella subtestacea</i> Bourd. & Galz.	O, Y	PS-A	DIFF	Clamped	+	Tort. hyphae +
<sup>1</sup> <i>Tuber rapaeodorum</i> Tul.	O, Y	PS-E	-	Awl-shaped	-	Long spiny surface
<sup>3</sup> <i>Xerocomus armeniacus</i> Quél.	W (Y)	PL	DIFF	-	-	Mantle silvery

Abbreviations: Colours: W (white), BR (brown), Y (yellow), O (ochre), BL (black); Mantle: PL (plectenchymatous, PS (pseudoparenchymatous), A (angular), E (epidermoid); Rhizomorphs: UNDIFF (undifferentiated), DIFF (differentiated); Characteristic: Em. hyphae (emanating hyphae), Tort. hyphae (tortuous hyphae in rhizomorphs); Designation of identification method: <sup>1</sup>identified on morphological basis, <sup>2</sup>identified by ITS -RFLP, <sup>3</sup>identified by rhizomorph connection with fruitbody, <sup>4</sup>identified by ITS- sequencing; + (present), - (not present).

Comparing DNA-ITS RFLP patterns of traditionally identified fruitbodies and ectomycorrhizae supposed to belong to them was successful in the case of *Lactarius controversus* and *Russula amoenolens*. RFLP patterns of these two species have been published within the comprehensive description of these ECMs and are not repeated here (JAKUCS & BEENKEN 1999, JAKUCS & al. 2001).

The three *Tomentella*-ectomycorrhizae (*T. galzinii*, *T. pilosa* and *T. subtestacea*) were identified comparing their rDNA ITS-sequences with those included in the *Tomentella*-fruitbody DNA-database of Kõljalg (KÕLJALG & al. 2001). Determination of other taxa, mainly to the genus level (*Cortinarius*, *Genea*, *Hebeloma*, *Lactarius*, *Russula* and tomentelloid morphotypes), was based on anatomical evidence (AGERER & RAMBOLD 1998).

The main morphological characteristics of the most common 14 ECMs of both sites, which can be regarded as the dominant types of the *P. alba* forests in this region, are listed in Table 1. Detailed anatomical characterization of the five new ectomycorrhizae described first from these samples has been published already (JAKUCS & al. 1997, JAKUCS & AGERER 1999a, JAKUCS & AGERER 1999b, JAKUCS & al. 2001, JAKUCS & AGERER 2001).

Table 2 shows the occurrence and relative abundance of ECMs in the 'Tompá' and in the 'Kelebia' samples.

Based on the common ectomycorrhizal types summarized in Table 1 an identification key has been compiled using easily observable anatomical characteristics to get support for further examinations of ECMs in this region including quantitative studies and ecological investigations (Table 3).

## Discussion

From Central Europe, limited ECM-data are available although regular ECM investigations have been carried out on spruce stands in Slovenia (TROŠT & al. 2000) and on oak forests in Austria (KOVACS & al. 1999). Nevertheless, ECM relationships of drought-adapted poplar forest have not been investigated up to now.

The presented data are insufficient to draw any conclusion on seasonal changes and spatial distribution of ECM communities. However, it is clear that the same ECM-types occur repeatedly in the samples taken from the same plots during the three years, while a higher variability between the two sampling plots could be observed. Although the two sites are quite close together and ecologically similar, a significant difference in their ECM-types could be detected. The observed differences may be related to the lime content of soil of the Kelebia stand and to the influence of co-occurring non-ECM tree species on spatial distribution of *P. alba* trees in the two territories.



Table 2.

Relative abundance of the most common ECM types within the soil samples collected from *Populus alba* at different dates in Tompa and Kelebia

Mycorrhiza	Sampling date Sampling plot	July 1997		April 1998		June 1998		Oct. 1998		Juni 1999	
		Tompa	Kelebia	Tompa	Kelebia	Tompa	Kelebia	Tompa	Kelebia	Tompa	Kelebia
<i>Cortinarius</i> spp.		-	-	-	-	-	-	B	B	-	-
<i>Genea</i> spp.		-	-	-	-	-	-	A	B	-	-
<i>Hebeloma</i> spp.		-	B	-	-	-	-	-	-	-	-
<i>Lactarius controversus</i>		-	-	-	-	-	-	B	-	-	-
<i>Lactarius</i> spp.		-	C	C	-	A	-	A	-	B	-
<i>Russula amoenolens</i>		-	-	-	-	-	-	-	B	-	-
<i>Russula</i> spp.		A	-	-	-	-	-	A	-	-	-
<i>Scleroderma bovista</i>		A	-	-	-	-	-	A	-	B	-
<i>Tomentelloid-types</i>		B	B	A	B	B	A	B	B	B	A
<i>Tomentella galzinii</i>		A	-	-	-	-	-	-	-	-	-
<i>Tomentella pilosa</i>		-	-	A	-	B	-	-	-	B	-
<i>Tomentella subtestacea</i>		-	-	-	-	-	-	A	-	-	-
<i>Tuber rapaeodorum</i>		B	-	A	-	B	A	B	B	-	B
<i>Xerocomus armeniacus</i>		-	B	-	C	-	C	-	B	-	C

Ranks: A , minor component ( <10%); B, minority codominant (10–50%); C, majority codominant (50–90%); D, dominant (>90%); – (not detected)

Table 3.

Identification key of the most common ectomycorrhizae of *Populus alba* L.

1a Ectomycorrhizae black-brown or brown	2
b Ectomycorrhizae not black-brown or brown	3
2a Emanating hyphae present, broad, brown, thick-walled, septate and partly warty without clamps. Mantle pseudoparenchymatous-angular. Rhizomorphs lacking	<i>Genea</i> spp.
b Emanating hyphae present or lacking, if present, then not warty, walls brown or colourless, with or without clamps. Mantle pseudoparenchymatous-angular or -epidermoid; rhizomorphs may be present or lacking	tomentelloid-types
3a Cystidia lacking; mantle plectenchymatous	4
b Cystidia present; mantle plectenchymatous or pseudoparenchymatous	8
4a Ectomycorrhizae with more or less cottony surface; emanating hyphae clamped	5
b Surface of the ectomycorrhizae smooth; emanating hyphae clamped or simple septate	7
5a Rhizomorphs are lacking; colour of ectomycorrhizae ochre; cottony emanating hyphae bind many soil particles; hyphae in the outer mantle layer clamped	<i>Hebeloma</i> spp.
b Rhizomorphs present; ectomycorrhizae silvery-white	6
6a Rhizomorphs undifferentiated; ectomycorrhizal systems dense, consisting of tortuous, worm-like ends	<i>Cortinarius</i> spp.
b Rhizomorphs highly differentiated, some thick central hyphae with yellow contents and with partly dissolved septa; mycorrhizal ends not tortuous, worm-like	<i>Scleroderma bovista</i>
7a Laticifers present ( <i>Lactarius</i> spp.). Rhizomorphs lacking; mantle plectenchymatous, gelatinized, with laticifers in the middle layer; surface covered by white Ca-oxalate rosette crystals; ectomycorrhizae yellowish white	<i>Lactarius controversus</i>
b Laticifers lacking. Rhizomorphs present, highly differentiated, nodulate; rosette crystals lacking; colour of ectomycorrhizae white to yellowish with brown dots	<i>Xerocomus armeniacus</i>
8a Cystidia without clamps	9
b Cystidia clamped and in addition with simple septa ( <i>Tomentella</i> spp.)	10
9a Cystidia bottle-shaped ( <i>Russula</i> spp.), some of them ending in a knob; mantle plectenchymatous, yellowish white	<i>Russula amoenolens</i>
b Cystidia awl-shaped, without clamps; mantle pseudoparenchymatous-epidermoid; ectomycorrhizae ochre to brown	<i>Tuber rapaeodorum</i>
10a Cystidia thick-walled at base; rhizomorphs undifferentiated	<i>Tomentella galzinii</i>
b Cystidia thin-walled; rhizomorphs differentiated, with narrow, clamped, tortuous marginal hyphae	11
11a Cystidia 25–30 µm long, containing a dark blue or black-brown pigment	<i>Tomentella subtestacea</i>
b Cystidia 55–60 µm long, with somewhat clavate tips	<i>Tomentella pilosa</i>



The tomentelloid ectomycorrhizae, representing about nine different morphotypes, occurred in all samples from spring to autumn both in 'Tompá' and 'Kelebia'. These mycorrhizae are known to be widespread and important members of ECM communities in needle forests (BRADBURY & al. 1998, KRANABETTER & WYLIE 1998). KÖLJALG & al. 2000 demonstrated that tomentelloid mycobionts colonize 1–8% of the total root tips of conifers in Sweden. Our results show that black-brown tomentelloid ECMs, as minor or minority codominant components, are permanently present also in the poplar forests of South Hungary. However, it was surprising, that among the black ECMs *Cenococcum geophilum*, common in all needle and broad leaved forests (LOßUGLIÓ 1999), has not been found at all on *P. alba* roots during the three-year program, although it is present on oak and pine in the same region of the Hungarian Plain (personal observations).

In 'Kelebia' the *Xerocomus armeniacus* ectomycorrhiza was a dominating type (abundance B or C) during all seasons. It can be considered as relative abundant both above and below ground. Nevertheless, neither this species nor other Boletaceae-type ECMs could be found in 'Tompá'. On the contrary, *Scleroderma bovista*, represented by ECMs and also by a great number of fruitbodies, was present in 'Tompá' but lacking in 'Kelebia'. We would like to propose for consideration that both mycobionts play a similar ecological role in the two plots, as they show a high fruitbody biomass production and have highly differentiated rhizomorphs with thick central hyphae which indicates long-distance transportation processes (RAIDL 1997).

The presence of the hypogeous fruitbodies of *Tuber rapaeodorum* is characteristic of poplar forests on sandy soils. However, its ectomycorrhizae were surprisingly abundant on *Populus alba* roots. *Genea*-type ECMs, although no fruitbodies could be detected, were also common at both sampling plots indicating that ascomycetous ECMs may play a significant role in poplar forests. Also the *Cortinarius* species were abundant in the soil samples of both sites. In contrast, *Hebeloma*-type ECMs could be found only in 'Kelebia'.

Russulaceae were represented by several, up to now unidentified *Lactarius* and *Russula*-type ECMs. Only *R. amoenolens* from 'Kelebia' and *L. controversus* from 'Tompá' could be identified to the species level. *Lactarius controversus* is known as a characteristic fungus of drought-adapted poplar forests of the region (RIMÓCZI & VETTER 1990). The majority of the *Russula*-types belong to the group *Foetentinae* characterized by gelatinous mantles (ROMAGNESI 1967), indicating drought-adaptation (JAKUCS & MAGYAR 1999). The *R. amoenolens* ECM has been reported to be abundant also in bishop pine forests of California, although fruitbodies were rare in the region (GARDES & BRUNS 1996). Our observations on this species support the theory that *R. amoenolens* may have low efficiency in carbon

transfer from roots to mycelium resulting in a low biomass in fruitbody production (GARDES & BRUNS 1996).

Although *Tomentella*-species are regarded as widespread and their ECMs as important components in forest communities of the temperate northern hemisphere (JÜLICH & STALPERS 1980, GARDES & BRUNS 1996), these ectomycorrhizae have been poorly studied so far. One of our most interesting findings was the presence of the three, until now undescribed ochre coloured *Tomentella* ectomycorrhizae (*T. galzinii*, *T. pilosa* and *T. subtestacea*) forming rhizomorphs and clamped cystidia. They have been found only in the 'Tompá' samples at one place, within the same square meter. Only *T. pilosa* occurred repeatedly. Fruitbodies have not been observed.

Our results demonstrate that ECM communities of drought-adapted poplar forests show a high diversity of mycobionts playing an essential role in nutrient and energy transfer in these ecosystems. Studies on natural ectomycorrhizal relationships are not only important to get basic knowledge about the community structure of these forests but can also support the reforestation program of the Hungarian lowland.

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