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Black Types of Ectomycorrhizae on Six - Month Old Norway Spruce Seedlings

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Summary

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The mycorrhizal potential of differently polluted forest research plots in the emission area of the Thermal Power Plant in Šoštanj (Zavodnje and Mislinja) was analysed. Types of ectomycorrhizae on short roots of six - months old Norway spruce seedlings were briefly described, from which the "black and dark brown" types are presented here. We compared their anatomical and morphological characteristics to other known black types of ectomycorrhizae. In the first series of analyses two black types were identified: *Piceirhiza horti - atrata* (A93) and a type with characteristics, similar to the type *Quercirhiza atrata* (C93). In the second series, two more types of ectomycorrhizae were identified: the type H94 and the type *Cenococcum geophilum* (I94). In contrast to some previous reports, we could not correlate the occurrence of black types of ectomycorrhizae to the more polluted soils.

Introduction

Mycorrhiza serves as the primary network in nutrient and energy cycling in the forest ecosystems (SIMARD 1996) and can therefore present the key element of stability in these highly interactive environments (AMARANTHUS & PERRY 1994). The reaction of a certain mycorrhizal community on pollution depends predominantly on species structure, whereby the presence of sensitive and insensitive or more tolerant species in the mycocoenosis is of highest importance. The functional compatibility of different myco- and macro-symbionts is species or even strain specific (GIANINAZZI - PEARSON 1984), while at the same time some

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symbionts are better adapted to different stress factors than the others (TAYLOR 1995). The method of mycobioindication of forest site pollution has been developed in Slovenia in the emission area of the Thermal Power Plant in Šoštanj. Ectomycorrhiza of *Hydnum rufescens* on Norway spruce has been proposed as a sensitive type, while the insensitive one was proposed to be *Paxillus involutus* (KRAIGHER & al. 1996). In this study we have focused on the anatomical characterization of black and dark brown types of ectomycorrhizae, which have previously as a group been reported as insensitive (KOWALSKI 1987), their resemblance to other types of ectomycorrhizae and abundance on six months old spruce seedlings, used for the estimation of the mycorrhizal potential of two differently polluted forest soil substrates.

Material and Methods

The mycorrhizal potential of forest soils is defined as the capability of propagules of naturally occurring fungi in forest soils to colonize roots of spruce seedlings. It is expressed as the percentage of mycorrhizal short roots of the total number of short roots in the sample (KROPAČEK & al. 1988). A modified method of a pot experiment for determination of the mycorrhizal potential of forest soils has been used (AL SAYEGH PETKOVŠEK 1997). Types of ectomycorrhizae and mycorrhizal potential were determined on six months old Norway spruce seedlings, grown in sieved soil substrates (2 mm sieve) from two differently polluted forest research plots in the emission zone of the Thermal Power Plant in Šoštanj. The two plots (850m a.s.l., distric cambisol, *Luzulo - Fagetum*, predominant tree species *Picea abies*, ca 80 - 100 years old) were as similar regarding site characteristics as it was possible to select, but differently polluted: the plot in Mislinja is relatively unpolluted and the plot in Zavodnje is the polluted one. For the first series of analyses (march-June 1993), two subplots were chosen on each site regarding the light regime (plot no. 5 on each site with 80% of canopy closure, the other two plots with 50% of canopy closure). In the second series (february-june 1994), only soil substrates from plots no. 5 were chosen. Soil substrates have been taken predominantly from OhAh soil horizons and used in pots, in which three weeks old seedlings were planted and grown in growth-cabinets (T= 22-25° C, photosynthetic photon flux = 90 mikromol/m^2s) for approximately six months. The mycorrhizal potential of the more and the less polluted substrates were analysed in pairs. Four (first series) or five (second series) pots per plot were chosen and five seedlings per pot were analysed. All roots of seedlings, irrespective of their length, were counted and the percentage of mycorrhizal roots was estimated. From the total number of mycorrhizal roots, all unidentifiable types (too old or too young for identification) were subtracted. The identifiable types of ectomycorrhizae were characterized and compared according to the Color Atlas of Ectomycorrhizae (AGERER 1987 - 1998) and other primary sources of identification.

Results and Discussion

Three or four types of ectomycorrhizae (on different soil substrates) have been identified on short roots of young spruce seedlings, mostly belonging to the 'black' types (Table 1). From these, two could be identified as *Piceirhiza horti - atrata* and *Cenococcum geophilum*, while two did not correspond to any other published descriptions. We presume that the type with the reference number C93

could be an as yet undescribed type of ectomycorrhizae, while the type number H94 could belong to a broader group of *Cenococcum geophilum.*

Table 1. Similarities of the 'black types' with other descriptions of types of ectomycorrhizae and their average percentages in total mycorrhizal short roots of spruce seedlings (* data for H94 and I94 combined, ECM – ectomycorrhizae, *C.g.* - *Cenococcum geophilum*).

Type no.	A93	C93	H94	I94
SIMILARITY to other ECM types	adequate to *Piceirhiza horti-atrata*	resembles to *Quercirhiza atrata* (on oaks)	macro-, not microscopically, resembles to *C.g.*	adequate to *Cenococcum geophilum*
% ECM				
Zavodnje p. 4	14.6	17.1	/	
Zavodnje p. 5	4.2	0	35.8*	
Mislinja p. 2	4.6	40.9	/	
Mislinja p. 5	0	5.3	62.4*	

Table 2 shows morphological and anatomical differences among the individual types in order to better discriminate between this group of ectomycorrhizal types. We have also tried to correlate the 'black' types, which have been in some other studies recorded as a complex *Cenococcum geophilum* group, to the polluted soil substrates, as suggested by KOWALSKI 1987. From our studies these observations could not be confirmed, in contrary, types H94 and *Cenococcum geophilum* have occurred in higher percentages on short roots of seedlings, grown on substrates from the unpolluted site. Similar conclusions were done previously in studies of types of ectomycorrhizae in standard volumes of soils taken from the same two sites in which *Cenococcum geophilum* occurred on both sites (KRAIGHER & al. 1996). Similarly KROPAČEK & al. 1989 found no correlation between the occurrence of the black types and pollution. Therefore, the type *Cenococcum geophilum* and the other black to dark brown types of ectomycorrhizae from our studies can not be ranked among the bioindicators of the pollution of forest soils.

Table 2. An overview of morphological and anatomical characteristics of the "black types of ectomycorrhizae" (ecm).

Type no. / feature	A93	C93	H94	I94
MORPHOLOGY				
color of ecm	black	dark brown	black	black
ramification	monopodial	monopodial to mon.-piramidal	monopodial	monopodial
length of axes	0.8 to 1mm	to 5.5mm	0.3 to 1mm	0.3 to 1mm
diameter of axes	0.3mm	0.3-0.5 mm	0.4 to 0.6 mm	0.4 to 0.6 mm
surface of ecm	smooth with veil of hyphae	shiny with soil particles	shiny with soil particles	shiny with soil particles
emanating HYPHAE	woolly, no rhizomorphs	few hyphae, no rhizomorphs	woolly, no rhizomorphs	woolly, no rhizomorphs
color of hyphae	young bright, later brown	bright brown to dark brown	brown	dark brown or brown
surface of hyphae	smooth or grainy	smooth	smooth or often grainy	smooth or a grainy
length of hyphae	30-50 µm	20-45 µm	40-57 µm	10-60 µm
diam. of hyphae	2-4 µm	5-7 µm	4-5 µm	6-8 µm
color of hyphal walls	dark brown to black	dark brown to black	brown to dark brown	Dark brown to black
septa	simple	simple	simple	Simple
anastomoses	rare	none observed	open (type D) or with clamps (type E)	Open (type D)
OUTER MANTLE SURFACE	plectenchym	plectenchym, stellate pattern of mantle (type G)	plectenchym with hyphae in star- like arrangements	plectenchym, stellate patterned mantle (type G)
length of hyphae	3-19(30) µm	5.5-16.5 µm	3.5-53.5 µm	20-70 µm
diam. of hyphae	3.6-11 µm	3.5 -7.5 µm	3-7 µm	6-10 µm
INNER MANTLE SURFACE	plectenchym	pseudoparenchym with globular and angular cells	pseudoparenchym with epidermoid and angular cells	plectenchym
length of hyphae / no. of cells in a square (20 x 20 µm)	1.8-16.5 µm	less then one cell to 3 cells	middle layer 1-3 cells, inner surface 5-10 cells	4-50 µm
diam. of hyphae	1-4.5 µm	—	—	3-4 µm

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