

Ectomycorrhizal system of naturally established Norway spruce [*Picea abies* (L.) Karst] seedlings from different microhabitats – forest floor and coarse woody debris

Anna Lepšová

University of South Bohemia in České Budějovice, Faculty of Biological Sciences,
Department of Botany & Institute of Landscape Ecology, Academy of Science of the Czech Republic,
Na sádkách 7, CZ-370 05 České Budějovice, Czech Republic
Lepsova@uek.cas.cz

Abstract

Naturally established five years old Norway spruce seedlings from different microhabitats were compared with regard to the total root length; root, hypocotylus and shoot biomasses; and the frequency of ectomycorrhizal morphotypes. The seedlings from forest floor of healthy spruce forest exhibited significantly the shortest root system and the lowest biomass in comparison to the seedlings from decaying wood and mossy logs. Root systems of these seedlings were inhabited by the highest number of ectomycorrhizal species (from 11 to 14 morphotypes). Most of these ectomycorrhizal morphotypes belonged to common fungal species and genera characteristic for adult Norway spruce forest (i.e. *Russula ochroleuca*, *Cenococcium geophilum*, *Cortinarius* spp., *Lactarius* spp., *Russula* spp., *Dermocybe* spp.). The seedlings had the higher total root length and biomass in the forest stand, where adult trees were killed by bark beetle in 1997. Despite the fact that no fruitbodies of common ectomycorrhizal fungi occur in this stand, root systems were fully inhabited by several ectomycorrhizal morphotypes (from 3 to 4). The most common was ectomycorrhiza of *Tylospora fibrilosa*, a corticoid fungus forming negligible fruiting structures. Altogether 18 ectomycorrhizal morphotypes were distinguished within all stands. *Tylospora fibrilosa* was the prevalent species in all microhabitats under the study – forest floor, decaying wood and mossy logs. The microhabitat with grasses and ferns (*Calamagrostis villosa*, *Athyrium* spp.) has not been studied because of lack of established seedlings. The data suggested that the different microhabitats affect the development of seedlings in all parameters studied. The unfavourable effect of microclimate in closed Norway spruce canopy, allelopathic effect of spruce litter, and root competition are suggested to be the main causes of limited growth of spruce seedlings on the forest floor of healthy Norway spruce forest stands.

Key words: *Picea abies*, seedling, natural regeneration, ectomycorrhiza, coarse woody debris, root system

INTRODUCTION

The natural establishments of Norway spruce [*Picea abies* (L.) Karst.] in mountain forests of the Bohemian Forests are highly desirable. The conditions for natural spruce regeneration are generally known. The probable benefit of decaying wood for seedlings as a substrate in comparison to forest floor humus layers was confirmed by studies of VACEK (1981, 1982). The more indirect evidences suggest that the decaying wood (coarse woody debris, CWD) represents a microhabitat which provides the suitable niche for spruce seedlings to survive in some forest types as concluded by previous researchers (KNAPP & SMITH 1982, HARMON & FRANKLIN 1989, PONGE 1988, HARVEY & al. 1987, GENSAC 1990, BLASCHKE 1999, HÖRNBERG & al. 1997).

The emerging of Norway spruce seedlings and their survival is strongly suppressed in the canopy of grasses and ferns (*Calamagrostis villosa*, *Athyrium alpestre*). This may be attributed to the allelopathic effect of dominant plants, which was documented for *Vaccinium myrtillus*, *Pteridium aquilinum*, *Athyrium filix-femina* (see review PELLISSIER & SOUTO 1999). The negative effect of *Sphagnum* mosses for Norway spruce survival described OHLSON & ZACKRISSON (1992). Low survival values were also noted on mineral soil and on an organic layer of forest floor composed by spruce litter (VACEK 1982). PELLISSIER (1994) demonstrated the inhibitory effect of particular humus phenolics on germination and growth of Norway spruce.

The positive effect of decayed wood within the soil profile on the activity and lifetime of ectomycorrhizae was documented by HARVEY & al. (1978). The decayed wood supported survival of ectomycorrhizae during dry periods. As the feeder roots and ectomycorrhizae of forest trees occur in shallow depth of soil profile and are usually associated with organic soil layers, particularly in older stands, the structure and composition of organic soil layers are key factors for fine root and ectomycorrhizae development and survival (HARVEY & al. 1986, 1987).

The role of ectomycorrhizae for forest tree establishment was described many times and the connection of forest trees via mycelium of ectomycorrhizal fungi within soil humus has been documented (BROWNLEE & al. 1983, CARLETON & READ 1991, DUDDRIDGE & al. 1988, SIMARD & al. 1997). This is of special meaning for shaded seedlings and saplings, which are most probably supported by adults via ectomycorrhiza.

Ectomycorrhizal fungi may be negatively influenced by phenolic acids, which naturally occur in mor-humus layer of soil (PELLISSIER 1993, SUOTO & al. 2000). Herb understory also influences the ectomycorrhiza development. There are some evidences on negative effect of plants from the family *Ericaceae* on the development of ectomycorrhizae in tree root systems (WALKER & al. 1999).

The natural colonization of tree roots by symbiotic fungi has been studied in a variety of conditions. The most studies on the development of ectomycorrhizae on seedlings were performed in forest nurseries (e.g. URSIC & al. 1997). Some investigators used outplanting of not mycorrhizal tree seedlings to the forest and searched for "trapped" ectomycorrhizae later (SMITH & al. 1995). Other investigators brought the intact blocs of the forest soil into the greenhouse to test the effect of different man-managed factors (clearcut, burning, disturbance) on the ectomycorrhizal status of seedlings (e.g. SCHOENBERGER & PERRY 1982, PILZ & PERRY 1984). The part of the study of the latter authors and another studies (HORTON & al. 1998, FLYNN & al. 1998) are focused on the ectomycorrhizal colonization under natural conditions (of bishop pine after burning, or of Sitka spruce in plantation forest, respectively).

This study is focused on ectomycorrhizal root systems of naturally established Norway spruce seedlings, which were found at different microhabitats available in highly protected areas of Šumava National Park in the Czech Republic. The aim is to evaluate the decaying wood as a substrate for natural regeneration and to support the proper management of wood in forest ecosystems of the Bohemian Forest.

STUDY SITES, MATERIAL AND METHODS

Three study sites were chosen in the area of the natural occurrence of Norway spruce in the central Bohemian Forest. The sites represent mature forest close to the type of old-grown mountain spruce forest stands. They differ in elevation, forest type, intensity of bark-beetle attack in the past, and coarse woody debris (CWD) management (Table 1).

The microhabitats selected for this study were (a) forest floor covered by litter layer (consists mostly of spruce needles, bark, and small twigs), and (b) decaying wood of spruce rep-

Table 1. – Localities under study in the Bohemian Forest, Czech Republic.

Locality	Area	Elevation Latitude N Longitude E	Forest type Zone of protection	Forest health status: bark-beetle damage
Častá	Strážný	920 m 48°55'40" 13°40'20"	<i>Bazzanio-Piceetum</i> 1 st zone	only in gaps from 1984 to 1999
Ztracená	Modrava	1100 m 48°59'15" 13°29'50"	<i>Calamagrostio villosae</i> – <i>Piceetum/Bazzanio</i> – <i>Piceetum</i> 1 st zone	only in gaps from 1997 to 1999
Roklanská	Modrava	1200 m 48°59'15" 13°25'35"	<i>Calamagrostio villosae</i> – <i>Piceetum</i> 1 st zone	dead adult trees from 1995 and 1997

resented by logs lying on the ground (up to 10 cm in diameter in this study). Mossy logs were covered by bark and moss vegetation, predominantly with *Dicranum scoparium*, sometimes with herbs – *Oxalis acetosella*, *Vaccinium myrtillus*. Other logs are without any vegetation and without bark. Mossy logs were the only logs presented at the locality Častá, both types of logs were at the locality Ztracená, and there were only logs and decayed wood stumps, which were included to the category of logs at the locality Roklanská.

The year 1995 brought the last high production of Norway spruce seeds, which has resulted in natural regeneration.

The ectomycorrhizal root system of the established seedlings (those more than one year old up to 1.3 m height, as defined by HÖRNBERG & al. 1997) represents the whole root system with the main root, lateral roots and short roots for the purpose of this study. Short roots are mostly inhabited by symbiotic fungi and are called ectomycorrhizae. Ectomycorrhizae are morphologically different and are described as (ectomycorrhizal) morphotypes.

Ectomycorrhizal root systems were checked for the diversity of ectomycorrhizae, length and biomass of roots. The study was performed at three study sites in 2000. Predominantly, seedlings, which were of 5 and 8 years old were found. The experimental design followed this scheme: at least 5 seedlings (mainly about 10 seedlings), which were 5 years old, were collected to compose one sample. Five to ten samples (according to seedling availability at the stand) were collected in a particular microhabitat at each study site. The individual seedling samples (of 5 to 10 seedlings) from the litter microhabitat were picked at least 10 m apart to exclude the possibility of collective sharing of the same mycorrhizal fungus. The individual seedling sample from logs and CWD was composed of seedlings from one or several places, according to the availability of seedlings.

The seedling samples were stored in refrigerator until the next preparation. The whole seedling was handled during the roots were carefully washed off the attached litter and wood.

The seedlings were scanned using CorelScan program for the root length determination and the shape documentation. Later, the shoot and hypocotylus were cut off the root system and all parts were air-dried prior biomass evaluation.

The root systems were searched for ectomycorrhizal morphotypes using the dissecting microscope (magnification 20×). Different morphotypes were separated for determination and documentation. The presence of at least one particular mycorrhiza within the root system was scored as a presence in the whole sample. No quantitative assessment of mycorrhiza occurrence within the single root system was made. The root systems were then air-dried.

Detached morphotypes and emanating structures were documented following the Checklist of AGERER (1987–1998). The morphotypes were stored in glutaraldehyde buffer

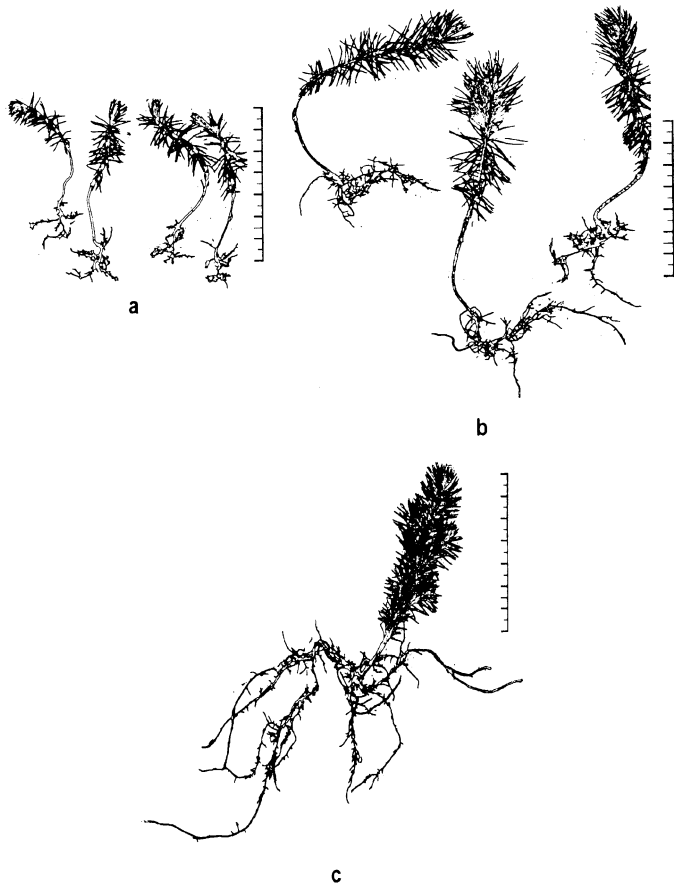


Fig. 1. – The root architecture of 5 years old Norway spruce seedlings: a – locality Ztracená, microhabitat litter; b – locality Častá, microhabitat mossy log; c – locality Roklanská, microhabitat log (CWD). The scale represents 7 cm.

(ALEXANDER & BIG 1981) for the microscopic examination. The determination keys of AGERER (1987–1998) and GRONBACH (1988) were used to identify individual morphotypes.

The root, shoot and hypocotylus biomasses were determined after 24 h drying at 90°C (using Sartorius analytical tool, accuracy 10^{-3} g).

The basic description statistics and Kruskal-Wallis one-way ANOVA has been used to evaluate the biomass and root length from different microhabitats and localities.

RESULTS

The roots of spruce seedlings in humus layers of the forest floor in the healthy forest stand (namely Častá and Ztracená) are in physical contact with fine roots of adult spruce trees. They are relatively short and occupy a small volume (Fig. 1a). They are spread in the fermentation layer of the aboveground humus. Both the root length and biomass are lower ($P < 0.01$) than in other microhabitats (Figs. 2 and 3; Tables 2 and 3). The number of ectomycorrhizal types is generally higher in root systems from forest floor (up to 11 and 14 types at the localities Častá and Ztracená, respectively) in comparison to the ectomycorrhizal diversity in mossy logs and coarse woody debris (Table 4, Fig. 4).

The most frequent ectomycorrhizal types found in the root systems of seedlings of forest floor microhabitat at Častá and Ztracená were two morphotypes, E and F, which represent short roots with very thin hyphal mantle and visible cortical cells. These morphotypes correspond to the ectomycorrhiza of *Tylospora fibrilosa*. They differ in beaded shape of F one, caused by repeated growth periods disturbed by drought. Morphotypes C, D, G, I, and J are the next common types, and correspond to ectomycorrhizal genera or species *Lactarius* spp., *Dermocybe* sp., *Cenococcum geophilum*, *Russula ochroleuca*, and *Cortinarius* spp., respectively. These ectomycorrhizae are common within the fine root system of Norway spruce trees.

The root system of spruce seedlings from the microhabitat mossy logs is more extensive

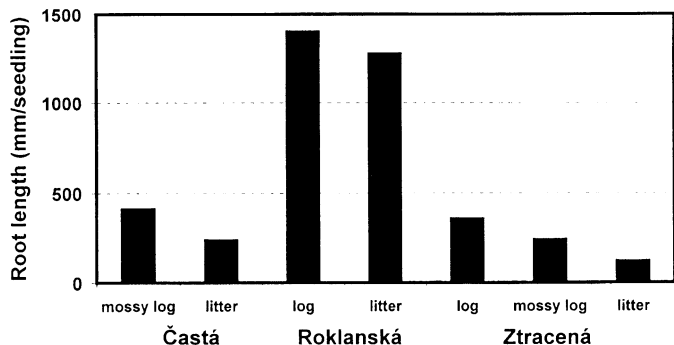


Fig. 2. – Root length of an average 5 years old Norway spruce seedling in different microhabitats of three study sites in the Bohemian Forest.

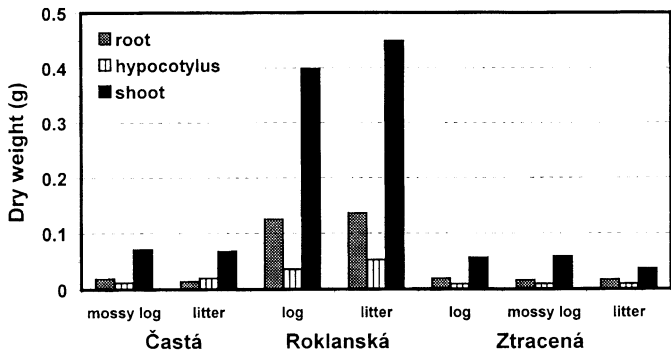


Fig. 3. – Biomass (dry weight) of root, hypocotylus, and shoot of an average 5 years old Norway spruce seedling in different study sites and microhabitats in the Bohemian Forest.

and flat (Fig. 1b), and roots are spreading under the bark, which is covered by mosses (namely *Dicranum scoparium*).

The total root length and biomass of seedlings from mossy logs are higher than those from forest floor both at the locality Častá and the locality Ztracená. Both total root length and biomass are comparable for mossy logs at the locality Častá and Ztracená (Tables 2 and 3; Figs. 2 and 3).

Ectomycorrhizal types are lower in the number (up to 7 and 9 at Častá and Ztracená, respectively; Table 4, Fig. 4). Root systems of seedlings on mossy logs are not in contact with those of adult trees.

The prevailing morphotype is E. Some root systems are occupied by the well differentiated morphotype D, which corresponds to *Dermocybe* sp.

The roots of spruce seedlings growing in decayed wood (logs), especially in those moderately decayed, are growing longitudinally following the more decayed space, and do not penetrate to the harder wood. The values of root length and biomass were higher than those in the root systems from “litter” at the plot Ztracená (Tables 2 and 3; Figs. 2 and 3).

Altogether 9 ectomycorrhizal morphotypes were found in root systems of this microhabitat at the site Ztracená (Table 4; Fig. 4). Besides morphotypes E and F, *Cenococcum geophilum* (G) and *Cortinarius* spp. (J) were the other frequent ectomycorrhizal morphotypes in this microhabitat.

The root systems of established Norway spruce at the locality Roklanská (5 years old dead forest after the bark-beetle outbreak) in litter layer and coarse woody debris (CWD; logs and stumps without any vegetation) were extensive, and occupy a large volume (Fig. 1c). The roots of seedlings in forest floor grew both in fermentation and in humification layers. The most CWD was highly decayed. Due to the lack of the trees alive, the roots of spruce seedlings were not in contact with the roots of adult spruce.

The total root length is remarkably the highest of the whole data set ($P < 0.01$; Table 2, Fig. 3). The biomass of roots and shoots follows the same pattern as root length (Table 3, Fig. 3). But the values of the total root length and biomass do not considerably differ between samples from CWD and forest floor ($P > 0.05$).

Table 2. – Root length of an average 5-years old Norway spruce seedling from different study sites and microhabitats

Study site microhabitat	Root length [mm]				
	number of samples	average	stanard deviation	minimum	maximum
Častá litter	6	239	70.6	169	369
Častá mossy log	10	414	94.6	306	612
Roklanská litter	5	1280	556	595	2091
Roklanská CWD	5	1373	206	1145	1629
Ztracená litter	12	122.1	35.7	197	581
Ztracená mossy log	8	243.8	79	152	381
Ztracená CWD	10	331	117	198	581

Table 3. – Dry weight of root, hypocotylus, and shoot of an average 5-years old Norway spruce seedling from different study sites and microhabitats.

Study site microhabitat – seedling part	Dry weight [g]				
	number of samples	average	standard deviation	minimum	maximum
Častá litter – root	6	0.014	0.004	0.009	0.02
Častá litter – hypocotylus	6	0.021	0.001	0.011	0.039
Častá litter – shoot	6	0.068	0.002	0.042	0.089
Častá mossy log – root	10	0.022	0.006	0.015	0.032
Častá mossy log – hypocotylus	10	0.016	0.003	0.012	0.019
Častá mossy log – shoot	10	0.105	0.031	0.007	0.174
Roklanská litter – root	5	0.136	0.075	0.043	0.253
Roklanská litter – hypocotylus	5	0.053	0.013	0.034	0.067
Roklanská litter – shoot	5	0.449	0.213	0.151	0.744
Roklanská CWD – root	5	0.125	0.027	0.100	0.116
Roklanská CWD – hypocotylus	5	0.036	0.006	0.028	0.043
Roklanská CWD – shoot	5	0.399	0.117	0.287	0.581
Ztracená litter – root	12	0.017	0.025	0.005	0.023
Ztracená litter – hypocotylus	12	0.010	0.003	0.007	0.016
Ztracená litter – shoot	12	0.037	0.14	0.022	0.075
Ztracená mossy log – root	9	0.016	0.008	0.006	0.027
Ztracená mossy log – hypocotylus	9	0.010	0.004	0.003	0.018
Ztracená mossy log – shoot	9	0.059	0.026	0.025	0.117
Ztracená CWD – root	8	0.020	0.007	0.011	0.028
Ztracená CWD – hypocotylus	8	0.010	0.003	0.005	0.014
Ztracená CWD – shoot	8	0.057	0.024	0.016	0.097

Only three ectomycorrhizal types were found within the extensive root system of 5 years old seedlings at this locality in both microhabitats (Table 4). The most common morphotypes found were E and F (*Tylospora fibrilosa*). They occur along the long roots with root hairs. The next morphotype was rare and represents the ectomycorrhiza *Piceirhiza bicolorata* (type R).

Table 4. – Frequency of ectomycorrhizal types found in root systems of 5-year old Norway spruce seedlings from different sites and microhabitats.

Study site and microhabitat	number of samples	Ectomycorrhizal types frequency in microhabitats [%]																	
		A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R	
Častá litter	7	57	29	43	57	57	71	29		57	41	14		14					
Častá mossy log	12	17	17		67	100	100				33			8					
Roklanská litter	5	20				100	20											20	
Roklanská CWD	5					100	40		20										
Ztracená litter	14	21	14	29	7	71	86	79	29	43	36		7		29	7	7		
Ztracená CWD	9	22	22	22	22	89	100	33			33		11						
Ztracená mossy log	8		13	13	13	100	100	13	13	25								25	

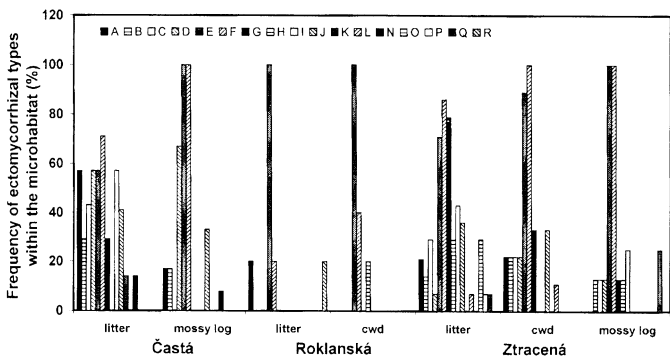


Fig. 4. – Distribution of ectomycorrhizal types within different sites and microhabitats in root systems of 5 years old Norway spruce seedlings (cwd – coarse woody debris). Letters from A to R represent different morphotypes of ectomycorrhizae; the possible fungal partner is described in the text.

The total number of 18 ectomycorrhizal morphotypes were distinguished altogether within the root systems of 5 years old Norway spruce seedlings from all plots and microhabitats (Tab. 4, Fig. 4) under the dissecting microscope.

The mycotrophy of morphotypes E and F, which was not obvious under the dissecting microscope, was proved by light microscopy. Both of the morphotypes E and F involve the fungus *Tylospora fibrilosa* (family Corticiaceae). The hyphal mantle is compact, thin (about 20 mm) and translucent. The cortical cells are easy to distinguish. The Hartig net is sufficiently developed within the layer of primary cortex of the short roots. The emanating hyphae are with clamp connections and bear warts on the surface. Morphotype F does not differ under the microscope, but is beaded like, most probably affected by drought periods during the growth. Mycorrhizae of *Tylospora fibrilosa* are the most frequent within all microhabitats.

Morphotypes C, D, G, I, J, and P correspond to the ectomycorrhizae of adult Norway spruce trees. The fungi involved in this ectomycorrhizae are common in spruce forests: *Lactarius* (namely *L. rufus*, *L. helvius*, *L. tabidus*), *Dermocybe* (different species, *D. cinnamomea*, *D. bahtailei*, and *D. croceoconia* are the most common), *Cenococcum geophilum*, *Russula ochroleuca*, *Cortinarius* (namely *C. brunneus*), and *Lactarius deterrimus*, respectively.

Some morphotypes had been already described and are known from spruce forests, but the fungal component has not been determined yet: *Piceirrhiza gelatinosa* (morphotype B), *Piceirrhiza bicolorata* (R), and *Piceirrhiza obscura* (N).

Other morphotypes left undetermined in this study. Morphotypes A, L, O, and Q represents family Russulaceae (the most common within the locality are *Lactarius* species mentioned above and different species of *Russula*: *R. emetica*, *R. paludosa*, *R. consobrina*, and *R. decolorans*). Morphotype K represents another species of *Dermocybe*. Fungus involved in morphotype H may be broadly included in Agaricales.

DISCUSSION

The growth pattern, biomass and length of ectomycorrhizal system differ among microhabitats under the study. All short roots within the root system of Norway spruce seedlings examined were ectomycorrhizal. Our observation confirmed also differences in the diversity of ectomycorrhizal morphotypes, i.e. ectomycorrhizal fungi involved.

VACEK (1981) resumed major factors indispensable for successful regeneration of Norway spruce in autochthonous mountain forests of the Giant Mts. in the north-east Bohemia: besides the sufficient quantity and quality of seeds, the first are favourable humidity and temperature for emerging and survival of seedlings and suitable microhabitat. He found the best survival of seedlings to the next year after emerging in the moss cover (*Dicranum scoparium* and *Polypodium* sp.), on decaying wood (both logs and stumps) and on other elevated sites. Elevated microhabitats generally provide more favourable temperature regime due to shorter snow cover with longer time of vegetation period, and the protection against the melting snow and running water.

Decaying wood belongs to the most important microhabitats for natural spruce regeneration in mountain areas. It provides elevated microhabitats with suitable temperature and humidity regime. The humidity of decaying wood depends on the contact with the ground and the type of decay, which is caused by fungi. HARVEY & al. (1986) noticed more steady and more favourable water content in decaying wood. VACEK (1982) suppose the brown rotted Norway spruce wood to be the better water holder than white rotted wood due to the different mode of fungal activity towards the wood component. He found the regeneration of spruce on the decaying logs after about 20 years of the tree death.

The total length of roots of Norway spruce seedlings and limited and differently shaped space, which they occupy in the microhabitat forest floor of the healthy spruce forest, suggested that mor-humus layer ultimately provides unfavourable conditions for root growth. The growth and survival of seedlings is affected probably by a low nutrient pool, temperature and light intensity, and water content fluctuation with pronounced periods of drought. The humus layers are densely rooted by trees (LEPŠOVÁ & OBORNÍK 1992) and herbs in the spruce forests of the Bohemian Forest. Therefore, the strong competition for nutrients and for space may be expected here. All these factors may cause that the seedlings develop the short root system limited in space. Moreover, the development of seedlings is also negatively affected by phenolic compounds (PELLISSIER 1993, PELLISSIER & SUOTO 1999). The phenolics also affect the growth of some ectomycorrhizal fungi (SOUTO & al. 2000). They proved, that an artificial mixture of defined phenolics, which corresponded to those found in humus layer of Nor-

way spruce forest, inhibited or suppressed the respiration and growth of the two mycorrhizal fungi.

The ectomycorrhizal status of seedlings from forest floor in the sound forest revealed that seedlings are infected with numerous ectomycorrhizal fungi, which commonly occur in the mountain Norway spruce forests in central Europe (*Lactarius* spp., *Russula ochroleuca*, *Cortinarius* spp.). Most probably these ectomycorrhizal fungi are shared with mature trees. The persistence of spruce seedlings may be supported by site selected ectomycorrhizal fungi, which provide the connection with mature trees and facilitate the nutrient and water input to seedlings as suggested by several experiments (BROWNLEE & al. 1983, DUDDRIDGE & al. 1988, CARLETON & READ 1991).

The ectomycorrhizal diversity in the root system of spruce growing on mossy logs and CWD in sound forest is slightly lower than in forest floor. The root systems may occupy the space in which the competition of mature trees root systems is excluded. Most probably nutrients and water are more available in CWD, namely in the mossy log microhabitat. PONGE (1988) found that roots of Norway spruce seedlings followed holes within the wood and cracks caused by brown rot fungi and insects. He supposed that these microhabitats are rich in nutrients due to insect excrements and activity of wood decaying fungi.

The outstanding and different conditions of the "dead forest" after the bark-beetle outbreak are very supportive for the growth of Norway spruce seedlings both in the microhabitat of forest floor and CWD. The diversity of ectomycorrhizal types spruce seedlings is considerably lower than in other microhabitats. The mineralisation processes in humus layer are generally faster than under the closed forest canopy. Favourable nutrient pool, sufficient light intensity and humidity thus may provide better condition for Norway spruce seedlings growth.

Results presented in this study support the findings that the decaying wood provides favourable conditions for early growth and establishment of seedlings in sound forest. Seedlings occupying this microhabitat are protected from the competition of roots of adult trees to some extent. The development of root system within the forest floor in the healthy spruce forest is limited, but the causes were not tested. We may consider the allelopathic effect of row-humus and tree root competition among others. This difference between two microhabitats examined, the forest floor and decaying wood, for the Norway spruce natural regeneration is not pronounced in the forest after the heavy bark beetle attack 4 and 5 years ago. The competition for light and rooting space is excluded in these forest stands because of the death of adult trees. Due to the fast decomposition of aboveground humus in these sites the seedlings obtain sufficient nutrient input. Thus, the role of decaying wood in the "dead forest" is comparable with elevated forest floor microhabitats.

The discussion on the proper growth of seedlings, their ectomycorrhizal status and the diversity of ectomycorrhizae would be more precise with the knowledge of specific microhabitat environmental conditions, including available nutrient pool, acidity, water capacity properties and water retention abilities of different substrates available for spruce seedlings.

Acknowledgements. This study was supported partly by the project 342/2000, G4 of the Ministry of Education and Youth of the Czech Republic and by project CEC EU, Brussels, PL 97/1169. I am grateful for discussion and comments to P. Cudlín and to R. Gronský, I. Mikyšková, A. Lepšová jr. and V. Pouska for technical support.

REFERENCES

- AGERER R., 1987–1998: Colour Atlas of Ectomycorrhizae with Glossary. *Einhorn Verlag*.
ALEXANDER I.J. & BIG W.L., 1981: Light microscopy of ectomycorrhizas using glycol metacrylate. *Transactions of the British Mycological Society*, 77: 425–429.

- BLASCHKE M., 1999: Waldverjüngung in montanen und subalpinen Fichtenwäldern Mitteleuropas. *Forst und Holz*, 54: 457–459.
- BRANG P., 1998: Early seedling establishment of *Picea abies* in small forest gaps in the Swiss Alps. *Canadian Journal of Forest Research*, 28: 626–639.
- BROWNLEE C., DUDDRIDGE J.A., MALIBARI A. & READ D.J., 1983: The structure and function of mycelial systems of ectomycorrhizal roots with special reference to their role in forming inter-plant connections and providing pathways for assimilate and water transport. *Plant and Soil*, 71: 433–443.
- CARLETON T.J. & READ D.J., 1991: Ectomycorrhizas and nutrient transfer in conifer-feather moss ecosystem. *Canadian Journal of Botany*, 69: 778–785.
- DUDDRIDGE J.A., FINLAY R.D., READ D.J. & SÖDERSTRÖM B., 1988: The structure and function of the vegetative mycelium of ectomycorrhizal plants. III. Ultrastructural and autoradiographic analysis of inter-plant carbon distribution through intact mycelial systems. *The New Phytologist*, 108: 183–188.
- FLYN D., NEWTON A.C. & INGLEBY K., 1998: Ectomycorrhizal colonisation of Sitka spruce [*Picea sitchensis* (Bong.) Carr] seedlings in a Scottish plantation forest. *Mycorrhiza*, 7: 313–317.
- GENSAC P., 1990: Régénération en altitude de l'épicéa (*Picea abies* (L.) Karst.) sur les souches dans les Alpes françaises. *Annales des Sciences Forestières* 47: 173–182.
- GRONBACH E., 1988: Charakterisierung und Identifizierung von Ektomykorrhizen in einem Fichtenbestand mit Untersuchungen zur Merkmalsvariabilität in sauer berechneten Flächen. *Bibliotheca Mycologica*, 125. 216 pp., 37 pl.
- HARMON M.E. & FRANKLIN J.F., 1989: Tree seedlings on logs in *Picea-Tsuga* forests of Oregon and Washington. *Ecology*, 70: 48–59.
- Harvey A.E., Jurgensen M.F., Larsen M.J. & Schlieter A.J., 1986: Distribution of active ectomycorrhizal short roots in forest soils of the inland Northwest: Effects of site and disturbance. United States Department of Agriculture, Forest Service, Research Paper ITN-374.
- Harvey A. E., Jurgensen M.F., Larsen M.J. & Graham R.T., 1987: Relationships among soil microsite, ectomycorrhizae, and natural conifer regeneration of old-growth forests in western Montana. *Canadian Journal of Forest Research*, 17: 58–62.
- Hörnberg G., Ohlson M. & Zackrisson O., 1997: Influence of bryophytes and microrelief conditions on *Picea abies* seed regeneration patterns in boreal old-growth swamp forest. *Canadian Journal of Forest Research*, 27: 1015–1023.
- HORTON T.R., CAZARES E. & BRUNS T.D., 1998: Ectomycorrhizal, vesicular-arbuscular and dark septate fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 month of growth after wildfire. *Mycorrhiza*, 8: 11–18.
- KNAPP A.K. & SMITH W.K., 1982: Factors influencing understory seedling establishment of Engelmann spruce (*Picea engelmannii*) and subalpine fir (*Abies lasiocarpa*) in southeast Wyoming. *Canadian Journal of Botany*, 60: 2753–2761.
- LEPSONA A. & OBORNİK M., 1992: Fine root distribution in Norway spruce forest stands (*Picea abies* (L.) Karst.) in Šumava Mts. with regard to liming. In: *Root ecology and its practical application*. 3rd ISRR Symposium, Wien, KUTSCHERA L. & al. (eds), Univ. Bodenkultur, Sept. 1991, pp. 529–533.
- OHLSON M. & ZACKRISSON O., 1992: Tree establishment and microhabitat relationships in north Swedish peatland. *Canadian Journal of Forest Research*, 22: 1869–1877.
- OSWALD B.P. & NEUENSCHWANDER L.F., 1993: Microsite variability and safe site description for western larch germination and establishment. *Bulletin of the Torrey Botanical Club*, 120: 148–156.
- PELLISSIER F., 1993: Allelopathic effect of phenolic acids from humic solutions on two spruce mycorrhizal fungi: *Cenococcium graniforme* and *Laccaria laccata*. *Journal of Chemical Ecology*, 19: 2105–2114.
- PELLISSIER F., 1994: Effects of phenolic compounds in humus on the natural regeneration of spruce. *Phytochemistry*, 36: 865–867.
- PELLISSIER F. & SOUTO X.C., 1999: Allelopathy in northern temperate and boreal semi-natural woodland. *Critical Reviews in Plant Sciences*, 18: 637–652.
- PILZ D.P. & PERRY D.A., 1984: Impact of clearcutting and slash burning on ectomycorrhizal association of Douglas-fir seedlings. *Canadian Journal of Forest Research*, 14: 94–100.
- PONGE J. & al., 1998: The Forest Regeneration Puzzle. *BioScience* 48: 523–530.
- SCHOENBERGER M.M. & PERRY D.A., 1982: The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay. *Canadian Journal of Forest Research*, 12: 343–353.
- SIMARD S., PERRY D.A., JONES M.D., MYROLD D.D., DURRAL D.M. & MOLINA R., 1997: Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature*, 388: 579–582.
- SMITH J.A., MOLINA R. & PERRY D.A., 1995: Occurrence of ectomycorrhizas on ericaceous and coniferous seedlings grown in soils from the Oregon Coast Range. *The New Phytologist*, 129: 73–81.
- SOUTO C., PELLISSIER F. & CHAPUSIO G., 2000: Allelopathic effects of humus phenolics on growth and respiration of mycorrhizal fungi. *Journal of Chemical Ecology*, 26: 2015–2023.
- URŠIČ M., PETERSON R.L. & HUSBAND B., 1997: Relative abundance of mycorrhizal fungi and frequency of root rot on

- Pinus strobus* seedlings in a southern Ontario nursery. *Canadian Journal of Forest Research*, 27: 54–62.
- VACEK S., 1981: Perspectives of the successful natural regeneration in protective mountain forests of Giant Mts. *Lesnická práce, Praha, 1981: 118–124 (in Czech)*.
- VACEK S., 1982: Ecological aspects of biomass decomposition in autochthonous protective spruce forests. *Zprávy lesnického výzkumu, Praha, 27: 4–11 (in Czech)*.
- WALKER J.F., MILLER O.K. Jr., LEI T., SEMONES S., MILSEN E. & CLINTON B.D., 1999: Suppression of ectomycorrhizae on canopy tree seedlings in *Rhododendron maximum* L. (Ericaceae) thickets in the southern Appalachians. *Mycorrhiza*, 9: 49–56.