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Findings regarding ectotrophic stability of Norway spruce forest of the Krkonoše and Orlické Mountains based on mycorrhiza studies

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ABSTRACT: Analyses of root mycorrhizal samples and monitoring of fruiting bodies of macromycetes from Norway spruce stands at mountain and foothill localities in the Krkonoše and Orlické Mts. provided several data series allowing to assess and compare (albeit somewhat preliminarily) mycorrhizal conditions and macromycete incidence related to stand ages and location conditions. The overall mycorrhizal conditions in young (10 years old) and older (80 years old) stands on formerly agricultural soils are comparable to those in 80- and 90-year-old forests growing on standard forest soils, but young spruce stands in the Orlické Mts. replanted on forest soils do not surprisingly show any favourable mycorrhizal characteristics. The research documents a marked diversity of macromycete species composition in mountain spruce stands compared to foothill spruce stands on former agricultural soils. While in the younger mountain spruce stands of the Orlické Mts. the expansion of macromycete species composition began to develop in a similar fashion to that in the Krkonoše Mts., the comparison of older (80-year-old) stands on formerly agricultural soils in the Orlické Mts. foothills and Krkonoše Mts. implies that the macromycete species composition developed quite differently but with a similar success.

Keywords: forest ectotrophic stability; species spectrum of macromycetes; mycorrhizae; root; defoliation; *Picea abies*

Decline of forest stands can be caused by a number of stress factors, such as climate and weather conditions (recurring dry seasons, overall low precipitation or its uneven distribution over time, hard frost or, on the other hand, the absence of winter dormancy period, as well as rapid weather changes), together with changes in habitat conditions, whether in relation to climate (e.g. faster drainage and subsequent long-term soil water deficit, reduced groundwater levels), to anthropogenic influences (especially air pollution with all associated and subsequent influences, such as e.g. soil acidification, leaching of bases, changes in soil chemistry, accumulation of toxic substances) or to anthropic impacts (direct contamination and destruction of the natural environment, improper or insufficient forestry management, disrespect for the ecological requirements of trees and their needs for particular habitats). Additional unfavourable factors include, among others, the wildlife overpopulation and consequential damage to forest stands from browsing, gnawing and especially bark peeling.

Increased levels of ozone in the atmosphere have similar negative effects on the assimilation apparatus as did high levels of sulphur oxides. Epicuticular waxes also react to these harmful factors and thus provide some of the earliest signals of threats to stand health (Šrámek et al. 2009).

Damage to tree assimilation organs results in slower growth and reduces their capacity for self-recovery. A tree weakened by stress factors may not eventually be able to sustain the balance between the productive and degradation processes or to ensure the renewal of all its organs, and therefore it must begin to limit them. Conifers give precedence to younger organs, and thus older annual needle in-

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crements are lost prematurely. Cudlín (2002) set out to develop a method to determine the retrospective reaction of individual trees and of entire spruce stands to stress factors.

Weakened trees are consequently more sensitive to attacks by harmful insects or fungi, and the destruction or disruption of mycorrhizal associations can occur as well (Lepšová et al. 1987; Fellner, Pešková 1995). The process of tree damage can vary according to the type, intensity and duration of the stress factor, while interactions with soil, climatic and biotic factors also are influential (Cudlín et al. 1999).

Therefore, the stability and functionality of forest biotopes depend upon a number of biotic and abiotic factors. Fungi can be considered as suitable bioindicators for measuring the level of disruption of what can be called forest ectotrophic stability (FES), as given by ectomycorrhizal symbiosis. The level of disruption of the ectotrophic stability of a stand can be evaluated using data on the distribution of mycorrhizal fungus species and the proportions of active mycorrhizae collected from root probes (Pešková et al. 2007) or also from the data on the status of tree crowns (Cudlín et al. 2001).

In 2005–2007, in the framework of various grant-funded projects, detailed studies on mycorrhiza were conducted in parallel in mountain spruce stands at elevations of about 1,000 m a.s.l. in the Krkonoše Mts. and Orlické Mts. and in the foothills of the latter (Pešková et al. 2007, 2009; Šrámek et al. 2009). Within these projects several data series from higher-elevation spruce stands were acquired and analyzed which allow, albeit somewhat preliminarily, assessing mycorrhizal proportions in stands of different age and elevation. The material is compared with the incidences of macromycetes and completed with data on the stand health status and basic soil characteristics.

MATERIAL AND METHODS

Localities and plots selection

For research purposes, several mountain localities (Table 1) were selected in the Orlické Mts. (Anenský vrch 1 – 50°13.1'N 16°30.6'E, Anenský vrch 2 – 50°12.4'N 16°29.9'E), foothill localities in the Orlické Mts. (Bystré 10 – 50°19.7'N 16°15.1'E, Bystré 80 – 50°19.7'N 16°15.1'E), and in the Krkonoše Mts. (Sněžka – 50°44.0'N 15°4'E, Jelení vrch – 50°40.0'N 15°42.0'E), where long-term research was conducted as reported previously (e.g. Fellner et al. 1991, 1995; Soukup et al. 2008; Pešková, Soukup 2009; Šrámek et al. 2009). All selected plots are Norway spruce (*Picea abies* [L.] Karst.) stands with only negligible representation of other tree species. The size of the plots in all localities was 2,500 m^2. Each plot was divided into subplots (100 m^2 each) for more detailed evaluations of the frequency of fungi incidence.

Evaluation of fungi incidence

The method of monitoring and evaluation of macromycetes was similar in all plots. From June to November in 2005–2007, the composition of macromycete species, as well as their abundance and frequency (presence of a species on the 100 m^2 subplots) were evaluated once per month based on the sporocarps found. Detected macromycete species were classified according their trophic category (M – mycorrhizal, Sl – lignicolous saprotrophic or saproparasitic, S – other saprotrophic, especially terricolous and humicolous or sporadically muscicolous, fungicolous or fimicolous, Pm – parasitic muscicolous).

The macromycete species spectrum was evaluated over the period of 3 years. During this period,

Table 1. Description of selected study plots of Norway spruce with their age in 2005 on the beginning of study period (in bracettes abbreviated names use in following tables)

Mountains	Site (code)	Study period	Age	Elevation (m a.s.l.)
Krkonoše	Sněžka (KR 80)	2005–2007	80	980
Krkonoše	Jelení vrch (KR 90)	2005–2007	90	1,040
Orlické	Anenský vrch 1 (OH 15)	2005–2007	15	950
Orlické	Anenský vrch 2 (OH 20)	2005–2007	20	900
Orlické	Bystré 10 (B10)	2005–2007	10	510
Orlické	Bystré 80 (B 80)	2005–2007	80	510

given roughly normal weather conditions in some years, up to about 90% of represented fungal species could be determined, which is adequate for the needs of evaluating FES (Pešková, Soukup 2006).

It was further assumed that the procedure for evaluating FES (Fellner, Pešková 1995; Soukup 1996) was fully applicable in stands at 50 years of age and older. Worse degrees of FES (acute and lethal) indicating its disruption are connected with a decline in the ectomycorrhizal macromycete percentage below 40%, while the lignicolous macromycete percentage tends to rise above 30% of the total number from all species. A sharp decrease in fructification of mycorrhizal species is evident, as a rise in the abundance of lignicolous mycocoenoses is associated with the stimulation of wood-rotting fungi fructification. An acute degree of FES disruption is characterized by a constantly low percentage of mycorrhizal fungal species (below 40%), while the ratio of wood-rotting fungal species is usually above 40%. Pronounced depletion of the ectomycorrhizal mycocoenoses, along with a rise in the species diversity of lignicolous mycocoenoses and their increased fructification are evident. A lethal degree of FES degradation is the final, practically irreversible stage; the percentage of ectomycorrhizal fungal species remains below 20% of the total macromycete count, while the wood-rotting macromycete proportion is above 50%.

In accordance with the initial assumption that the FES evaluation procedure is fully applicable to stands over 50 years of age, when evaluating young and very young stands by a similar method (proportion of ectomycorrhizal macromycetes) it must be taken into account that the numbers of represented mycorrhizal species are always initially lower (in the first two decades) and the established ratio of their occurrence is not as reliable a figure.

The fungi were designated mostly according to the Index Fungorum nomenclature.

Root sampling, extraction and evaluation of mycorrhizae

Mycorrhizal samples were collected on selected plots in spring (from 17 May to 3 July) and autumn (from 8 September to 10 October) in 2005–2007. Sampling was done in approximately the same (in no case identical) location at approximately the same distance from the trunks of trees selected in 2005. On each plot probed, five root samples were collected.

Root samples were temporarily stored in a refrigerator and then processed and evaluated in a laboratory. All the roots from a soil probe were prepared by hand using tweezers and teasing needles and categorized into root classes according to their diameters. Subsequently, the roots were thoroughly washed in water to remove the maximum of mineral impurities. Roots of 1 mm or less in diameter were immersed into a glutaraldehyde fixing solution for the actual determination.

Among the main criteria analyzed were the absolute numbers of active (AM) and non-active mycorrhizae (NM) on roots of 1 mm or less in diameter, as they are the most adaptable and at the same time the most active parts of root systems (Mejstřík 1988; Gryndler et al. 2004).

The main unit used to determine the number of mycorrhizae was a root segment 5 cm long, of 1 mm or less in diameter, complete with the fine lateral roots. Twenty basic root segments were evaluated from each probe extracted. The numbers of individual types of mycorrhizal tips were determined under a binocular loupe at 40× magnification and according to the following diagnostic features (Peterson et al. 2004): tips with developed fungal mantle, Hartig net, high turgidity, lacking root hair, smooth on the surface, of a lighter colour were grouped into AM. On the other hand, tips with significant loss of turgidity, wrinkled on the surface, without the fungal mantle and Hartig net were grouped under NM. Some AM can be wrinkled and appear as partly withered but can still retain their physiological function. Such ambiguous cases were re-examined on thin slices under a light microscope.

The level of mycorrhizal association was determined using two parameters: mycorrhiza density and their proportions measured in percentage. The densities of active and non-active mycorrhizae were quantified as the average value of the determined number of mycorrhizae per 1 cm of root length.

Determination of soil pH

The pH level determined in soil suspension (the standard ISO 10390 Soil Quality – pH Determination) was the main soil characteristic used. The method consists in the soil sample measurement in a soil–water (pH–H_2O) suspension at a 1:5 ratio by volume after 5 min of horizontal agitation and then standing for a period of at least 2 h but not longer than 24 h. The pH was measured potentiometrical-

ly with a suitable pH meter with the glass combined electrode with available pH range 2–9.

Evaluation of tree defoliation

The health status of trees is visually characterized by the level of defoliation, which is defined as the relative loss of assimilation apparatus in the treetops compared to a healthy tree growing in the same stand and location conditions. Such a loss is primarily caused by unfavourable abiotic factors. Treetop defoliation is therefore a non-specific damage symptom and is usually caused by multiple factors. These may act separately or collectively and also interact mutually. It is very difficult to determine the priority and proportional contribution of each factor (Rösel, Reuther 1995; Fabiánek et al. 2004; UNECE 2006).

Defoliation was evaluated regularly once per year (during August and September) and expressed as a percent with 5 percentage point intervals. To minimize a subjective influence on the evaluation, each tree was evaluated by three evaluators and the average values were used.

Statistical evaluation and its difficulties

Soil probes were taken with the intention of sampling the root system in a representative manner on an examined plot having a homogeneous stand. The samples were usually extracted ca. 1 m from the tree trunk. Practical experience has shown that the sampled root systems on the whole well represent the characteristic mycorrhizal conditions for a given plot. However, not all probes collect a sufficient number of relevant roots because of their size or, more seriously, exceptionally roots with a completely untypical number of mycorrhizae appear. We do not know the exact causes. This can be due e.g. to a local anomaly in the soil chemical composition, hyphal node, inhibition or activation effect (of another fungus or plant), or perhaps to a locally unfavourable water balance. Because these anomalous samples are significantly different from

Table 2. Average density values and percentages of active (AM) or non-active (NM) mycorrhizae and pH

Site	Year	AM-s	NM-s	%AM-s	%NM-s	AM-a	NM-a	%AM-a	%NM-a	pH
	2005	0.87	0.92	49	51	1.90	0.94	67	33	3.74
Sněžka	2006	1.22	0.68	64	36	1.11	0.89	55	45	3.85
	2007	1.44	0.63	70	30	2.70	0.70	79	21	3.78
	2005	1.71	0.75	70	30	1.39	1.11	56	44	3.84
Jelení vrch	2006	0.98	0.73	57	43	0.47	0.46	51	49	3.87
	2007	0.49	0.43	53	47	0.88	0.55	62	38	3.76
	2005	1.10	0.27	80	20	0.56	0.14	79	21	3.99
Anenský vrch 1	2006	0.57	0.4	59	41	0.96	0.68	58	42	4.30
	2007	0.58	0.51	53	47	1.08	0.67	62	38	4.28
	2005	1.22	0.32	79	51	0.84	0.38	69	31	3.91
Anenský vrch 2	2006	0.57	0.74	44	56	2.15	0.48	82	18	4.21
	2007	0.74	0.88	46	54	0.60	1.56	28	72	4.02
	2005	0.88	0.25	78	22	1.01	0.30	77	23	4.76
Bystré 10	2006	1.15	0.13	89	11	0.81	0.11	88	12	4.86
	2007	1.39	0.17	89	11	2.41	0.57	81	19	5.06
	2005	0.88	1.08	45	55	0.72	1.68	30	70	3.80
Bystré 80	2006	0.89	0.31	74	26	0.60	0.42	59	41	3.90
	2007	1.29	0.77	63	37	1.71	0.98	64	36	3.97

s – spring; a – autumn

the majority of the others, for statistically justified reasons we decided to exclude such outlying values. That was the case of maximally 10% of the measurements deviating both in negative and positive directions. After this adjustment, the data from the probes (if their numbers are sufficiently high) show a nearly normal distribution around the mean value and can be then validly tested using usual statistical methods.

The three data sets were analyzed using the QC Expert 3.1 and STATISTICA 8.0 software, the main aim being to test for significance of differences and to determine statistically different mean values (goodness of fit test, pairwise comparison).

RESULTS AND DISCUSSION

Evaluation of mycorrhizae

Average density values and percentage proportions of mycorrhizae in the five samples extracted in spring and autumn of each year are summarized in Table 2. Higher yearly AM density values (spring, autumn) were reported in the Krkonoše Mts. (1.27 cm^{-1}), Bystré 10 (1.28 cm^{-1}) and Bystré 80 (1.02 cm^{-1}) localities, in contrast to the Orlické Mts. (0.92 cm^{-1}). NM densities were also higher on Bystré 80 (0.88 cm^{-1}) and Krkonoše Mts. (0.74 cm^{-1}) plots than in the Orlické Mts. (0.59 cm^{-1}), the lowest being at Bystré 10 (0.26 cm^{-1}). When compared, the AM percentage was the highest at Bystré 10 (84%), identical in the Krkonoše and Orlické Mts. (61%), while Bystré 80 had the lowest percentage (56%).

When comparing the mountain ranges, older stands (Sněžka in the Krkonoše Mts. and Anenský vrch 2 in the Orlické Mts.) always displayed higher AM densities. On the other hand, higher average AM density was found in the young locality Bystré 10. All these localities also had higher NM densities, with only Bystré 80 displaying higher NM densities than Bystré 10.

When comparing the mountain ranges, a significant difference in AM density can be seen in the Orlické Mts. compared to Bystré 80 and Krkonoše Mts. plots. NM density was found to be significantly different in the Orlické Mts. compared to the Krkonoše Mts. and Bystré 10. AM densities in the Krkonoše Mts. are comparable to non-forest soil plots in Bystré 80 and Bystré 10, with both plots showing higher values than did forest soils in the Orlické Mts. The Krkonoše and Orlické Mts. are comparable in AM density values with the non-forest soil of Bystré 10. In NM density, the Krkonoše Mts. values are higher than at Bystré 10, which, however, shows lower NM values than the Orlické Mts. plots (Table 3).

The aforementioned analyses show that the mycorrhizal conditions of the young, formerly agricultural soils are comparable even with those of forests 80 years old, and by contrast, young spruce stands do not show any good mycorrhizal characteristics. That would indicate that even non-forest soils are favourable for mycorrhizae development after some time. For example in Bartoš et al. (2010), where the wood characteristics of spruce stands were compared between former agricultural soils and long-term forest stands, most of the monitored parameters did not differ.

In the Orlické Mts. areas, significant air pollution in the 80s induced the present higher sensitivity of spruce growth increment to stress condi-

Table 3. Pairwise comparison of active mycorrhizae and non-active mycorrhizae

Non-active mycorrhizae	Active mycorrhizae			
	Krkonoše Mts.	Orlické Mts.	Bystré 80	Bystré 10
	n = 56, average = 0.92	n = 55, average = 0.66	n = 29, average = 0.96	n = 29, average = 0.99
Krkonoše Mts. n = 58, average = 0.74		** t = 2.75, P = 0.007	– t = 0.38, P = 0.700	– t = 0.48, P = 0.630
Orlické Mts. n = 56, average = 0.46	*** t = 3.79, P = 0.000		** t = 3.04, P = 0.003	– t = 1.93, P = 0.062
Bystré 80 n = 30, average = 0.86	– t = 1.14, P = 0.260	*** t = 4.25, P = 0.000		– t = 0.17, P = 0.870
Bystré 10 n = 28, average = 0.18	*** t =11.47, P = 0.000	*** t = 5.84, P = 0.000	*** t = 6.67, P = 0.000	

Statistically significant differences for levels: * for $P < 0.05$; ** for $P < 0.01$; *** for $P < 0.001$;– not significant

Table 4. List of macromycetes found on plots. Figures represent numbers of fruiting bodies/number of positive subplots. Only maximum values found during a visit in the entire study period 2005–2007 are presented

Taxon	Tr	KR90		KR80		OH20		OH15		B80		B10	
Amanita battarrae	M	***	6/7	*	3/2								
Amanita excelsa var. *spissa*	M									*	2/2		
Amanita fulva	M									* *	1/1		
Amanita pantherina	M									*	1/1		
Amanita porphyria	M									*	4/2		
Amanita rubescens	M	* *	8/5							* *	17/7		
Ampulloclitocybe clavipes	S					**	2/1						
Armillaria bulbosa	P					*	14/2						
Bjerkandera adusta	Sl									**	20/1		
Calocera viscosa	Sl	***	46/6	***	43/13	**	2/1	*	20/1	***	5/3		
Calocybe persicolor	S							*	1/1				
Clavulina coralloides	S									*	4/1		
Clitocybe costata	S											*	5/1
Clitocybe ditopa	S							*	9/1				
Clitocybe metachroa	S									*	4/1		
Clitocybe phaeophthalma	S			*	1/1								
Clitocybe phyllophila	S					*	2/1						
Clitocybe vibecina	S	*	7/2	* *	89/8	**	6/2	*	17/2				
Collybia cirrhata	S	*	2/1										
Collybia cookei	S							*	10/1				
Conocybe aporos	S							* *	17/2				
Coprinellus cf. *ephemerus*	S											*	2/1
Cortinarius (Lepr.) *gentilis*	M	*	1/1										
Cortinarius (Phleg.) cf. *glaucopus*	M					*	4/1						
Cortinarius (Seric.) *anomalus*	M	*	11/2	*	1/1							*	8/3
*Cortinarius (*Seric.) aff. *anomalus*	M	*	3/1										
Cortinarius (Seric.) *azureus*	M	**	9/2										
Cortinarius (Seric.) *eburneus*	M	*	7/2										
Cortinarius (Telam.) *acutus*	M					*	1/1						
Cortinarius (Telam.) *biformis*	M	*	6/2										
*Cortinarius (*Telam.) *brunneus*	M	***	26/5	**	5/1								
Cortinarius (Telam.) cf. *ceraceus*	M			*	1/1								
Cortinarius (Telam.) *decipiens*	M	**	58/5	* *	7/1								
Cortinarius (Telam.) *evernius*	M	**	3/4	*	11/1								
Cortinarius (Telam.) aff. *hinnuleus*	M	*	12/1										
Cortinarius (Telam.) cf. *jubarinus*	M	*	8/1										
Cortinarius (Telam.) aff. l*eucopus*	M	**	11/1										
Cortinarius (Telam.) cf. *leucopus*	M	*	1/1										

Table 4 to be continued

Taxon	Tr		KR90		KR80		OH20		OH15		B80		B10
Cortinarius (Telam.) cf. *miniatopus*	M	*	1/1										
Cortinarius (Telam.) cf. *paleiferus*	M	*	108/5										
Cortinarius (Telam.) aff. *rigidus*	M	*	16/1										
Cortinarius (Telam.) cf. *rigidus*	M	* *	207/9	* *	66/5								
Cortinarius (Telam.) cf. *umidicola*	M	*	6/1										
Cortinarius (Telam.) sp.	M	*	1/1						*	1/1			
Cortinarius (Telam.) sp. 1	M			*	5/1								
Cortinarius (Telam.) sp.2	M			*	21/4								
Cystoderma amianthinum	S	* *	8/6	***	14/5								
Dacrymyces stillatus	Sl								**	50/2	*	50/1	
Dermocybe bataillei	M			*	3/1								
Dermocybe cinnamomea	M					* *	19/6						
Dermocybe cf. *cinnamomea*	M	*	1/1										
Dermocybe cinnamomeolutea	M	*	1/1										
Dermocybe crocea	M	*	23/8	***	16/2	***	15/4						
Dermocybe crocea v.*porphyreovelata*	M	*	1/1	***	38/9								
Dermocybe holoxantha	M					*	2/1						
Dermocybe pallidipes	M			*	9/1								
Dermocybe semisanguinea	M	* *	4/2	***	16/5								
Dermocybe sommerfeltii	M	*	1/1	* *	36/4	***	8/3						
Entoloma (Nolanea) sp.	S	*	1/1										
Entoloma cetratum	S			***	11/8								
Entoloma venosum	M	*	2/1	**	8/5	*	2/1						
Fomitopsis pinicola	P	***	15/11			***	4/1	* *	2/1				
Galerina badipes	Sl			***	14/5	***	3/1						
Galerina cf. *cephalotricha*	Pm			*	3/3								
Galerina cf. *laevis*	Pm			*	1/1								
Galerina mniophila	Pm	***	1/1	* *	3/2	*	1/1	*	1/1				
Galerina sp.	Pm			*	8/2								
Galerina sp. 1	Pm	**	2/1										
Galerina sp. 2	Sl	*	1/1										
Galerina sphagnorum	Pm			*	3/1								
Galerina cf. *tibiicystis*	Pm			*	1/1								
Ganoderma carnosum	Sl					*	1/1						
Gymnopilus penetrans	Sl	***	40/7	*	4/3			*	8/1				
Gymnopilus sapineus	Sl					***	14/3	**	36/2				
Gymnopilus cf. *sapineus*	Sl			*	14/3								
Gymnopus dryophilus	S					*	1/1	*	2/1			*	4/1

Table 4 to be continued

Taxon	Tr	KR90		KR80		OH20		OH15		B80		B10	
Hebeloma crustuliniforme	M	*	2/1	* *	18/4			*	2/1			*	5/2
Hygrophorus olivaceoalbus	M	***	49/12	***	13/8	*	1/1						
Hygrophorus pustulatus	M			* *	1/1			*	4/1				
Hypholoma capnoides	Sl	**	4/2	* *	2/2	**	92/4						
Hypholoma epixanthum	Sl					*	1/1						
Hypholoma marginatum	Sl	* *	9/1	*	5/2			*	8/1				
Hypholoma radicosum	Sl			**	5/1								
Inocybe napipes	M			*	1/1	***	9/3	*	2/1				
Inocybe nitidiuscula	M					*	2/1						
Inocybe sp.	M					*	8/1						
Ischnoderma benzoinum	Sl					*	6/1						
Laccaria amethystina	M			***	8/1	*	8/1						
Laccaria laccata s.l.	M					***	51/4	*	4/1	*	4/1	* *	4/2
Laccaria proxima	M					*	3/1					**	8/3
Lactarius camphoratus	M			*	19/3								
Lactarius helvus	M			***	9/3								
Lactarius lignyotus	M	* *	28/9	* *	19/6								
Lactarius necator	M	* *	30/2	* *	66/9					*	1/1		
Lactarius rufus	M	***	199/7	***	45/7					*	13/3		
Lactarius tabidus	M					*	2/1			*	30/3		
Lepista nebularis	S									*	3/1		
Lepista nuda	S					*	1/1						
Lycoperdon foetidum	S					***	117/4	**	10/3	*	2/1		
Lycoperdon molle	S					*	1/1						
Lycoperdon perlatum	S					***	63/1	*	23/5				
Lycoperdon pyriforme	S					*	20/1						
Lycoperdon utriforme	S							*	1/1				
Marasmius androsaceus	Sl	***	190/11	***	200/17	**	10/1	**	10/2	**	107/6		
Marasmius graminum	S											*	1/1
Megacollybia platyphylla	S					*	1/1						
Micromphale perforans	S	***	250/17	***	20,000/17	***	1,000/10	**	145/6				
Mycena cf. *citrinomaginata*	S											*	1/1
Mycena epipterygia	S			*	1/1	* *	29/3	* *	14/4	*	12/1		
Mycena filopes	S					* *	25/1	* *	15/2				
Mycena galericulata	Sl			*	1/1								
Mycena galopus	S	***	47/11	***	320/16	*	2/2			*	2/1		
Mycena maculata	Sl			**	16/2								
Mycena rorida	S			*	6/1								
Mycena sp.	S											*	1/1

Table 4 to be continued

Taxon	Tr	KR90		KR80		OH20		OH15		B80		B10	
Mycena viridimarginata	Sl	*	3/1			**	2/1						
Mycena vulgaris	S							*	1/1				
Panellus stipticus	Sl									*	20/1		
Paxillus involutus	M			***	14/4	*	1/1			*	2/2		
Phallus impudicus	S									**	7/3		
Phellinus viticola	Sl	*	6/1										
Pholiota astragalina	Sl			*	5/1								
Pluteus cervinus	Sl					*	1/1			*	1/1		
Polyporaceae sp.	Sl					*	2/2						
Postia caesia	Sl	*	1/1	**	9/2					*	2/1		
Postia fragilis	Sl							*	3/1				
Psathyrella cf. *gracilis*	S							**	8/1				
Rhodocollybia butyracea f. *asema*	S							*	1/1	**	12/3		
Rhodocollybia butyracea f. *butyracea*	S	*	1/1			*	3/1			*	2/2		
Rhodocollybia maculata	S									*	1/1		
Rickenella fibula	S									*	10/1		
Rickenella swartzii	S											*	4/2
Russula aff. *azurea*	M									*	1/1		
Russula badia	M									*	2/1		
Russula cyanoxantha	M									*	4/1		
Russula emetica	M	**	20/8	***	22/9					*	2/1		
Russula fellea	M					*	5/1						
Russula fragilis	M									*	1/1		
Russula mustelina	M	*	1/1										
Russula ochroleuca	M	***	133/17	***	174/15	**	6/3			**	12/6		
Russula paludosa	M			*	2/1								
Russula puellaris	M									*	1/1		
Russula rhodopoda	M			*	2/1								
Stereum sanguinolentum	Sl					*	20/1			**	24/2		
Strobilurus esculentus	S	*	1/1	**	3/2								
Stropharia sp.	S					*	1/1						
Thelephora caryophyllea	M			***	15/1								
Thelephora terrestris	M									*	13/2		
Trichaptum abietinum	Sl					***	220/4						
Tricholomopsis decora	Sl	***	8/4										
Tricholomopsis rutilans	Sl									*	2/1		
Xerocomus badius	M	***	12/6	***	24/15	**	3/1			***	32/12		
Xerocomus ferrugineus	M	**	1/1	*	1/1								

Table 4 to be continued

Taxon	Tr	KR90	KR80	OH20	OH15	B80	B10
Xerocomus chrysenteron	M			*** 2/1		*** 3/1	
Xerocomus subtomentosus	M					* 2/2	
Xeromphalina campanella	Sl	** 255/6					
Xylaria hypoxylon	Sl			** 100/1			
Total species		59	62	53	29	41	11
Total mycorrhizal species		38	33	20	5	21	4
% mycorrhizal species		64.4	53.2	37.7	17.2	51.2	36.4

*number of years with positive evidence

Tr = Trophic categories: M = mycorrhizal, Sl = lignicolous saprotrophic or saproparasitic, S = other saprotrophic, Pm = parasitic muscicolous

tions (air pollution, weather-related stress). Those soils have low pH and are relatively well supplied with nutrients in the upper ca. 20 cm of soil horizons. At deeper horizons which are deficient in base cations, especially calcium, magnesium and potassium, relatively unbalanced N/K ratios occur in the highly damaged stands (Šrámek et al. 2009). The results of 10-year measurements of atmospheric sulphur deposition in the Krkonoše Mts. show that even despite a major decline in sulphur deposition in the last decades, the nutritional nitrogen level is still significantly critical. Therefore the combined critical load of sulphur and potassium in total is still exceeded, especially because of nitrogen depositions (Hošek et al. 2007).

Evaluation of fungi incidence

As the intensity of fructification is dependent on climatic conditions in any given year, a practicable method seems to be to monitor the plots for 3 years at least and to summarize all the species detected for the entire period for incidence evaluation, while

Table 5. An overview of macromycete species found on different plots

Comparison of similar plots	KR90	KR80	OH20	OH15	B80	B10
Total number of species	89		68		50	
Species in both plots	32		14		2	
Different species	27	30	39	15	39	9
Total number of mycorrhizal species	43		23		24	
Mycorrhizal species in both plots	20		2		1	
Different mycorrhizal species	16	7	18	3	20	3

Summary for mountain study plots in KR and OH	KR	OH
Total number of species	89	68
Species in both plots	26	
Different species	63	42
Total number of mycorrhizal species	43	23
Mycorrhizal species in both plots	12	
Different mycorrhizal species	31	11

KR = Krkonoše Mts.; OH = Orlické Mts.

for quantifying a given species the highest values from the most fructuous year are used (Table 4).

Comparing the cumulative findings for the plots in the Krkonoše and Orlické Mts. shows large shares of species in common for the Krkonoše and Orlické Mts. (Table 5). For all trophic groups of fungi, 26 species are in common, i.e. 31% and 37%, respectively, and for mycorrhizal species it is 12 species, i.e. 21% and 48%, respectively. The detail shows that this is largely due to the older of the two young Orlické Mts. stands, which is much closer in this respect to the stable advanced-age Krkonoše Mts. stands. The issue of changing the species composition is discussed separately.

The situation in the younger Orlické Mts. stands is illustrated by a low number of mycorrhizal species in the younger one, having only two mycorrhizal species in common, and it is especially surprising that their variety does not increase by the addition of new species, but rather by a change in the species composition.

When comparing the typical mycorrhizal species of mountain spruce stands in the Krkonoše Mts., such as *Dermocybe crocea*, *D. sommerfeltii*, *Hygrophorus olivaceoalbus*, *Entoloma venosum* or *Inocybe napipes*, we found that all of them appeared on the Orlické Mts. plot in the 20-year-old stand while in the 15-year-old stand only one of them, *Inocybe napipes*, occurred. This also evidences some differentiation when comparing the younger of the Orlické Mts. stands and the wholly successful introduction of a larger variety of mycorrhizal species that began between the 18th and 20th years of the OH 20 stand.

Bystré 10 also showed a relatively low number of mycorrhizal species, comparable to OH 15 (Table 6), as well as poor growth of fungi in general. We presumed a constraining influence of planting onto agricultural soil, but the progression on the plot was nevertheless favourable. A comparison with Bystré 80 suggests a significant change in the species composition with a minimum of species in common for all the trophic representations in general and especially for the mycorrhizal ones, but this was the case over the long term. Actually, numerous species of the genera *Amanita, Russula* and *Lactarius* are present, which are more typical of secondary coniferous and mixed forests of middle and lower elevations.

In the older Krkonoše Mts. stands (KR 80, KR 90), the ratios stabilized at higher values for mycorrhizal species especially due to higher species diversity of the *Cortinariaceae* – *Cortinarius* and *Dermocybe*, which are not usually so high in young stands (Table 5).

In the overall FES evaluation, for which the incidence and diversity of fungi are useful indicators, we consider not only the determined quantifications for the given year, but also summary comparisons of the values for each plot and its respective age, as the number of mycorrhizal fungal species present in a stand grows more rapidly in the first decades, and in the subsequent decades it gradually approaches a plateau.

The specific FES evaluation based on comparing the incidence ratios of mycorrhizal and other macromycete species, which can be reliably applied to stands over 50 years of age, becomes less reliable (e.g. due to greater inaccuracy in determining the incidence of negligible species) in a young stand, and it seems useful also to determine the specific succession process for the various mycorrhizal fungal species within the stand both quantitatively and qualitatively.

A number of authors have referred to the relationship between the disruption of mycorrhizal conditions or regression of mycorrhizal fungi and the influences of atmospheric pollution (Schlechte 1986; Termorshuizen, Schaffers 1987; Arnolds 1989; Fellner 1989, 1993) or to the relationship with visually assessable damage to forest trees (Jakucs et al. 1986; Fellner, Soukup 1991; Jansen 1991). The regression of a formerly large range of ectomycorrhizal fungi proceeds along with the general

Table 6. Comparison of all observed macromycetes species, mycorrhizal species and frequency of mycorrhizal species from all study plots – Goodness of fit test for all study plot combinations

Site	All sp.	Mycorrhizal sp.	Frequency
OH 15/OH 20	**	**	***
OH 15/B 10	***	–	–
OH 15/B 80	–	**	***
OH 15/KR	***	–	***
OH 20/B 10	***	***	***
OH 20/B 80	–	–	–
OH 20/KR	–	*	***
B 10/B 80	***	*	***
B10/KR	***	–	***
B 80/KR	–	–	***

Statistically significant deviations from equal representation for * for $P < 0.05$; ** for $P < 0^*.01$; *** for$P < 0.001$; – not significant

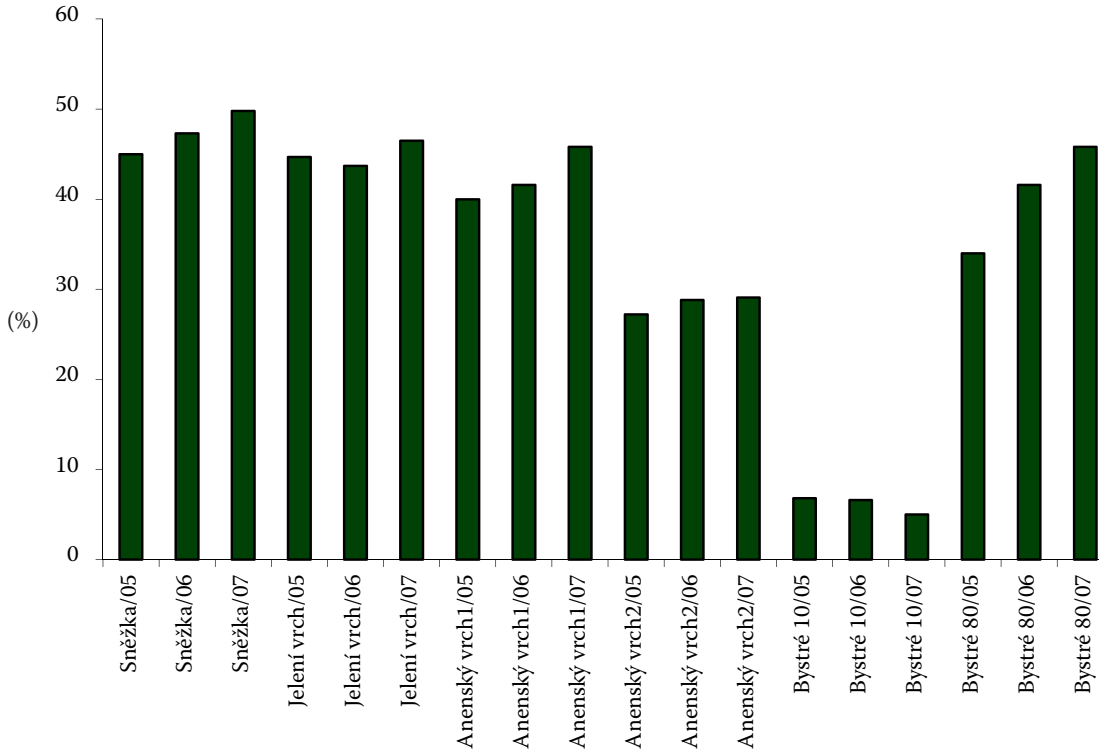

Fig. 1. Average tree defoliation on all study plots in 2005–2007 (relative values, for details see Material and Methods)

weakening of the stand on mountain and foothill plots (Lepšová et al. 1987). Our findings indicate that the situation in the Orlické Mts. may be unfavourable in this sense. However, the age differences among the various stands compared also play a role.

The results of scientific observations of FES development and the forest-fungi-mycorrhizae interactions in relation to environmental influences have been monitored in mountain spruce stands in the Krkonoše Mts. for a long time (e.g. Fellner, Landa 2001). Research conducted by these authors in the 1980's and 1990's shows a strong increase in the abundance and frequency of the mycorrhizal and other macromycete species in the late 1990s, which is associated with a significant decrease in SO_2 emissions.

Because the reviews of fungi and mycorrhizae in the older Orlické Mts. stands are not available yet, it seemed appropriate to choose mountain spruce stands in the Krkonoše Mts. for the purposes of comparison, where similar methods have been used.

Evaluation of defoliation

During the three years of study, tree defoliation was generally increasing on the given plots (Fig. 1). The situation at Bystré 10 plot was much more favourable, as the spruce health stabilized and the average defoliation even decreased. The average values for the respective localities were as follows: Bystré 10 – 6%, Orlické Mts. – 35%, and Krkonoše Mts. – 46%. The highest values were detected on the Sněžka plot – 47%, followed by Jelení vrch – 45%, Anenský vrch 1 – 43%, Bystré 80 – 40%, and Anenský vrch 2 – 28%. The high needle fall in 2007 can be attributed especially to a significant precipitation deficit in September 2006 to May 2007.

CONCLUSION

Comparing the quantities of mycorrhizae, numbers of macromycete species determined, and their frequency of incidence on four mountain spruce plots and two foothill plots proved crucial differences in the evaluated parameters of forest ectotrophic stability depending not only on the age of the stands but also on the geographical location. A fundamental difference in mycorrhizal fungal species between the young stands compared and their radical changes in the second decade of age were demonstrated. The conditions detected in the Orlické Mts. locality suggest a certain disruption of the FES, and especially for very young stands.

The research illustrates pronounced differences in the composition of macromycete species in mountain spruce stands compared to that on formerly agricultural soils in the foothills. While in younger mountain spruce stands of the Orlické Mts. the expansion of macromycete species composition began to develop in a fashion similar to that in the Krkonoše Mts., the comparison of older stands (80 years old) with those on formerly agricultural soils in the Orlické Mts. foothills and of Krkonoše Mts. stands shows that the macromycete species composition developed quite differently, but, according to the overall evaluation, with a similar degree of success.

The mycorrhizal status of younger (10 years) and older (80 years) stands on formerly agricultural soils was comparable with 80- and 90-year-old forests on standard forest soils, but the young spruce stands in the Orlické Mts. replanted on forest soils do not surprisingly show any favourable mycorrhizal characteristics.

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