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Soil saprotrophic micromycetes in Norway spruce forests in the Šumava National Park

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Abstract
Soil saprotrophic micromycetes were studied in different stages of damaged Norway spruce forests in the Šumava National Park. A total of 88 micromycetes including undetermined species and sterile mycelia were isolated from four samplings during 1999–2000. *Micromucor ramannianus* var. *ramannianus*, *Penicillium glabrum*, *P. inflatum*, *P. spinulosum*, *Trichoderma viride*, and *Mortierella* spp. were found the most frequently isolated microfungi species. *Goidanichiella barronii* was firstly recorded from the area of the Czech Republic. No significant differences among studied sites were found on the basis of spectrum of the isolated microfungi species. Quantitative estimations (CFU–counts and mycelial lenght) showed differences among individual samplings and also among studied sites.

Key words: soil saprotrophic micromycetes, Norway spruce forest, bark beetle, gradual extinction of spruce forest

Introduction

Soil saprotrophic micromycetes are very important group of soil organisms taking part in the processes of decomposition of dead organic matter in the soil.

First records about soil zygomycetes in the Bohemian Forest (the Šumava Mts.) are given by Dyr (1941). More detailed information about the occurrence of soil saprotrophic microfungi in the area of the Šumava National Park recorded Kubátová & al. (1998) and records about soil micromycetes from Norway spruce forest on the site Trojmezí reported Nováková & Blažková (2000).

The aim of this study were estimations of the species diversity and quantitative occurrence of the soil saprotrophic microfungi in the Modrava area of the Šumava National Park and the comparison of different stages of the damaged Norway spruce forests (live climax forests, dead forests, clearings) from the view of the occurrence of saprotrophic microfungi.

Material and Methods

Studied sites and sampling. Soil saprotrophic micromycetes were studied on eleven sites in the area of Modrava in the Šumava National Park (the Bohemian Forest) representing series of gradual extinction of the forests as a result of the bark beetle occurrence in the studied area. Studied sites were characterized by different stages of growth, e.g., climax spruce forests, gradual extinction of spruce forests, young and older clearings (see Table 1). Soil samples were collected from mineral horizon (5–10 cm below the soil surface) from five places of

Table 1. – Characteristics of studied sites.

	Site	Locality	Characteristics
Acidophilous series	A0	Studená hora Mt.	poorly acidophilous spruce forest (climax), *Calamagrostio villosae-Fagetum*
	AL1	Špičník Mt. –between Blatný Mt. and Březník Mt	"freshly" dead spruce forest, *Calamagrostio villosae-Fagetum* with *Deschampsia*
	AL2	U Pytláckého rohu, NW of the Blatný Mt.	withered acidophilous forest, *Calamagrostio villosae-Fagetum*, occurrence of bark beetles from 1994, gradual extinction from 1995
	AP1	Studená hora Mt., SE of locality A0	younger acidophilous clearing (by 3 years)
	AP2	near the brook of Roklanský potok	older acidophilous clearing (ca 10 years)
Eutrophic series	E0	Ptačí nádrž	eutrophic spruce forest (climax), *Calamagrostio villosae-Fagetum*
	EL1	Studená hora Mt., near locality A0	"freshly" dead spruce forest, *Calamagrostio villosae-Fagetum*
	EL2	U Pytláckého rohu, NW slope of the Blatný vrch Mt.	eutrophic dead forest, gradual extinction ca from 1996
	EP2	Studená hora Mt., near locality A0	eutrophic older clearing, *Junco-Calamagrostietum villosae*
Without distinction	0	Studená hora Mt., NW slope	climax spruce forest
	P2	Studená hora Mt., NW slope	older clearing (ca 3 years)

each site for preparing of mixed soil samples. Soil sampling carried out in spring and autumn in 1999 and 2000 years.

Isolation of micromycetes and quantitative estimations. Microfungi were isolated using soil dilution plate method (GARRETT 1981) – 1 g of mixed soil was used for preparing of 10^{-4} dilution. Agar with soil extract, beer-wort agar and Sabouraud agar – all with rose Bengal – were used as isolation media (FASSATIOVÁ 1979). Chloramphenicol (200 $mg.l^{-1}$) and streptomycin (100 $mg.l^{-1}$) were used for suppressing of the bacterial growth. Petri dishes were cultivated at 25°C for seven days and then colonies of microfungi were counted and transferred to beer-wort agar. These counts were used for estimation of CFU–counts (counts of colony forming units) per gram of dry soil. Epifluorescent direct microscopic method – dying by calcofluor – (BLOEM 1995) was used for estimation of total lenght of fungal mycelium.

Identification of microfungi. For determination of microfungi species the mycological keys and monographs were used (e.g., PITT 1979, 1991, ELLIS 1971, 1976, SUTTON 1980, DOMSCH & al. 1980, NELSON & al. 1983, ZYCHA & al. 1969, BISSETT 1983).

Comparison of microfungi associations. Sorensen similarity index – average linkage, SYN–TAX (PODANI 1993) – was used for the comparison of microfungi communities.

RESULTS AND DISCUSSION

A total of 88 micromycetes including undetermined species and sterile mycelia were isolated from four samplings during 1999–2000 (9 taxa belong to Zygomycetes, 1 species belongs to Ascomycetes, 1 species belongs to Coelomycetes and 77 taxa belong to Hyphomycetes), while differences in the species occurrence among successive samplings and also among individual sites were recorded. A sampling in spring 1999 was specifically considerable poor – predominantly *Mortierella* spp. and *Trichoderma* spp. (mostly sterile strains) and poorly *Penicillium* species and other micromycetes were isolated in this sampling. The most frequent

Table 2. – Frequency (%) of micromycetes species isolated in the area of Modrava, Šumava National Park in 1999 and 2000.

micromycetes species	frequency (%)											
	total	AO	AL1	AL2	AP1	AP2	EO	EL1	EL2	EP2	O	P2
Acremonium bactrocephalum W. Gams	**2.3**				25							
Acremonium berkeleyanum (Karsten) W. Gams	**4.5**			25						25		
Acremonium sp.	**2.3**	25										
Arthrinium arundinis (Corda) Dyko et B. Sutton	**2.3**			25								
Aspergillus flavus Link	**4.5**						25	25				
Aspergillus fumigatus Fres.	**2.3**							25				
Aspergillus niger van Tiegh.	**2.3**	25										
Beauveria bassiana (Bals.) Vuill.	**4.5**		25							25		
Beauveria brongniartii (Sacc.) Petch	**15.9**	25	25	25						25	75	
Beauveria sp.	**2.3**	25										
Botryotrichum piluliferum Sacc. et Marchal	**2.3**						25					
Chalara cf. *cylindrica* V.P. Karst.	**4.5**	25	25									
Chrysosporium sp.	**2.3**							25				
Cladosporium cladosporioides (Fresen.) de Vries	**4.5**								25		25	
Cladosporium herbarum (Pers. : Fr.) Link	**15.9**	25		25		25	25	25		25	25	
Cladosporium macrocarpum Preuss	**18.2**	25		25		25	25	25		25	25	25
Cladosporium sphaerospermum Penz.	**4.5**										50	
Cylindrocarpon magnusianum (Sacc.) Wollenw.	**4.5**				25						25	
Emericella nidulans (Eidam) Vuill.	**2.3**	25										
Fusarium sp.	**2.3**			25								
Geomyces pannorum (Link) Siegler et J.W. Carmich.	**11.4**		25		25					25	25	25
Gilmaniella sp.	**4.5**					25		25				
Goidanichiella barronii W. Gams, Steiman et Seigle-Mur.	**2.3**									25		
Micromucor isabellinus (Oudem.) Arx	**9**		50		25					25		
Micromucor ramannianus (Möller) Arx var. *angulisporus* Naumov ex Váňová	**2.3**											25
Micromucor ramannianus (Möller) Arx var. *ramannianus*	**34.1**	50	25	50	25	75				75	50	25
Mortierella parvispora Linnem.	**2.3**											25
Mortierella spp.	**59.1**	100	50	50	75	50	50	75	50	50	50	50
Mucor dimorphosporus Lendn.	**2.3**			25								
Mucor hiemalis Wehmer f. *hiemalis*	**2.3**											25
Mucor hiemalis Wehmer f. *silvaticus* (Hagem) Schipper	**2.3**											25
Oidiodendron griseum Robak	**2.3**							25				
Oidiodendron tenuissimum (Peck) S. Hughes	**11.4**	50						25			25	25
Paecilomyces farinosus (Holmsk. : Fr.) A.H.S. Br. et G. Sm.	**13.6**		25	25	25		25	25			25	
Paecilomyces variotii Bainier	**2.3**								25			

Table 2. – continued

micromycetes species	frequency (%)											
	total	AO	AL1	AL2	AP1	AP2	EO	EL1	EL2	EP2	O	P2
Penicillium aurantiogriseum Dierckx	**4.5**		25				25					
Penicillium brevicompactum Dierckx	**2.3**										25	
Penicillium cf. *canescens* Sopp	**4.5**		25								25	
Penicillium chrysogenum Thom	**4.5**								25	25		
Penicillium citrinum Thom	**18.2**			25	25		25	25	25	50		25
Penicillium cf. *citrinum* Thom	**2.3**						25					
Penicillium commune Thom	**4.5**			25		25						
Penicillium corylophilum Dierckx	**2.3**			25								
Penicillium decumbens Thom	**9**	25					25	25	25			
Penicillium funiculosum Thom	**2.3**								25			
Penicillium glabrum (Wehmer) Westling	**52.3**	50	100		100	50	50	50		50	50	25
Penicillium cf. *griseofulvum* Dierckx	**2.3**		25									
Penicillium inflatum Stolk et Malla	**52.3**	100	100	25	100	25	25	50	25	75	50	25
Penicillium janczewskii K.M. Zalessky	**29.5**	50	50	25	25			25	50	75	25	
Penicillium janthinellum Biourge	**9**	25			25					25	25	
Penicillium lanosum Westling	**2.3**									25		
Penicillium lividum Westling	**11.4**		50	25	25	25						
Penicillium miczynskii K.M. Zalessky	**18.2**		25		25		25	25	50		25	25
Penicillium minioluteum Dierckx	**6.8**			25				25			25	
Penicillium pinophilum Hedgc.	**11.4**	25			25		25	25		25		
Penicillium cf. *primulinum* Pitt	**2.3**	25										
Penicillium purpurescens (Sopp) Biourge	**11.4**	25	25		50							25
Penicillium rugulosum Thom	**4.5**	25						25				
Penicillium simplicissimum (Oudem.) Thom	**6.8**		25		25					25		
Penicillium spinulosum Thom	**50**	25	75	25	75	75	100	50	50	25		50
Penicillium variabile Sopp	**4.5**	25	25									
Penicillium verrucosum Dierckx	**2.3**	25										
Penicillium sp.	**11.4**		50							50		25
Phoma sp.	**2.3**	25										
Rhizopus arrhizus Fisch.	**2.3**								25			
sterile dark mycelium**	**29.5**	25	50	75	25	50	25	25	25	25		
sterile pink mycelium	**6.8**					25		25	25			
sterile white mycelium**	**31.8**	25	75	25	25		50	75	25	25	25	
sterile yellow mycelium	**2.3**			25								
Thysanophora penicillioides (Roum.) W.B. Kendr.	**2.3**		25									
Tolypocladium cylindrosporum W. Gams	**6.8**		25		25		25					
Tolypocladium geodes W. Gams	**4.5**				25					25		
Tolypocladium niveum (Rostrup) Bissett	**11.4**		25					50			50	
Tolypocladium sp.	**2.3**		25									
Trichoderma atroviride Karsten	**13.6**		25				50			50	25	

Table 2. – continued

micromycetes species	frequency (%)											
	total	AO	AL1	AL2	AP1	AP2	EO	EL1	EL2	EP2	O	P2
Trichoderma harzianum Rifai	**9**		25								25	50
Trichoderma koningii Oudem.	**9**					25	25			25		25
Trichoderma longibrachiatum Rifai	**4.5**									25		25
Trichoderma polysporum (Link : Fr.) Rifai	**11.4**	25				25		25	25		25	
Trichoderma viride Pers. : Fr.	**45.5**		25	75	50	75	25	50	50	25	75	50
Trichoderma spp.	**77.3**	75	100	75	100	75	75	75	75	75	75	50
Verticillium chlamydosporium var. *catenulatum* (Kamyschko ex G. L. Barron et Onions) W. Gams	**9**			25	50				25			
Verticillium dahliae Kleb.	**2.3**	25										
Verticillium fungicola (Preuss) Hassebr.	**6.8**	25				25	25					
Verticillium lamellicola (F. E. V. Sm.) W. Gams	**2.3**									25		
Verticillium lecanii (Zimm.) Viégas	**2.3**								25			
Verticillium sp.	**4.5**	25				25						
undetermined species of Moniliales	**2.3**	25										
totaly isolated taxa	**88**	**32**	**31**	**24**	**25**	**18**	**23**	**27**	**20**	**30**	**26**	**21**

genera were found to be *Penicillium* (28 species), *Trichoderma* and *Verticillium* (6 species). Summary of all fungi isolated is listed in the Table 2.

Comparing studied sites, numbers of isolated microfungi species are in the range of 18 (acidophilous older clearing) to 32 (acidophilous climax spruce forest). The highest number of micromycetes species in acidophilous series was isolated from sites A0 (climax – 32 species) and AL1 (freshly dead spruce forest – 31 species), the lowest micromycetes species number was found in site AP2 (older clearing – 18 species). Low soil humidity in this site was probably the cause of estimated low species number. Other situation was recorded in eutrophic series – the highest number of isolated species was recorded in site EP2 (older clearing – 30 species) and the lowest number of isolated species was found in site EL2 (older dead forest – 20 species). Site EP2 is completely covered by grass growth with thick layer of grass litter. Higher species number in this site in comparison with aforested sites was probably caused by richness of autochthonous soil mycoflora of spruce forest soil with microfungi species typical for grassland stands. Species number isolated from sites without distinction (O, P2) were estimated between boundary values of corresponding forest stages in acidophilous and eutrophic series.

Comparison of species richness among sites and total frequency of microfungi in 44 soil samples are given in Table 2. *Micromucor ramannianus* (Möller) Arx var. *ramannianus* (34.1% of all samples), *Penicillium glabrum* (Wehmer) Westling (52.3%), *P. inflatum* Stolk et Malla (52.3%), *P. spinulosum* Thom (50%), *Trichoderma viride* Pers.: Fr. (45.5%), and *Mortierella* spp. (59.9%) were the most frequently isolated species in studied area during 1999–2000 years. The majority of microfungi occurred in low frequency, even many species were found only once. With the exception of sites P2 and EL2, on all sites were found one to three very frequent species, a few of other frequent fungi and many of rarely isolated microfungi species. The same results are also recorded by KUBÁTOVÁ & al. (1998). *Penicillium inflatum, P. spinulosum, Trichoderma viride* and *Mortierella* spp. were also frequently isolated from spruce forest site on Trojmezí (NOVÁKOVÁ & BLAŽKOVÁ 2000), while *P. glabrum* and *Micromucor ramannianus* var. *ramannianus* were not found in this site.

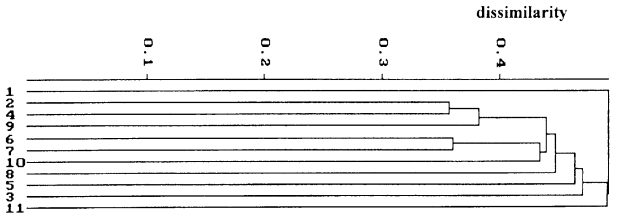

Fig. 1. – Cluster analysis of studied sites (Sorensen similarity index, SYN-TAX). 1 – A0, 2 – AL1, 3 – AL2, 4 – AP1, 5 – AP2, 6 – E0, 7 – EL1, 8 – EL2, 9 – EP2, 10 – 0, 11 – P2.

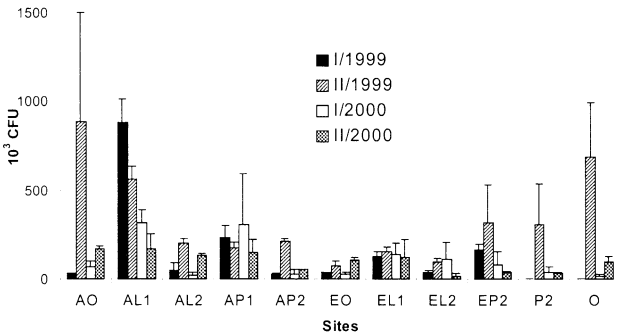

Fig. 2. – Values of the CFU-counts per gram of dry soil in 1999–2000.

All isolated fungi are saprotrophic soilborne species (Domsch & al. 1980). Noteworthy, some of these microfungi are also entomogenous (*Beauveria bassiana, B. brongniartii, Beauveria* sp., *Paecilomyces farinosus, Tolypocladium cylindrosporum, T. geodes, T. niveum*, and *Verticillium lecanii*), phytopathogenous (*Cylindrocarpon magnusianum, Phoma* sp., *Verticillium* sp., *Fusarium* sp., and *Penicillium brevicompactum*), and fungicolous (*Acremonium berkeleyanum, P. brevicompactum, P. glabrum, P. spinulosum*, and *Trichoderma viride*) (Domsch & al. 1980, Kubátová & al. 1998). *Thysanophora penicillioides* and *Chalara* cf. *cylindrica* are typical for soils under coniferous forests and for wood, needles etc. of coniferous tree (Ellis 1971).

One micromycete species, *Goidanichiella barronii* W. Gams, Steimann et Seigle-Mur., was not yet published from the area of the Czech Republic (Řepová 1989a, b; 1990a, b).

The Sorensen similarity index was used to compare microfungal communities of all studied sites – cluster analysis is recorded in Fig. 1. On the basis of spectra of the isolated microfungi species, no significant differences among the studied sites were found, because the frequently isolated species were more or less similar in all studied sites and differences in the occurrence of rarely isolated microfungi was not so conspicuous.

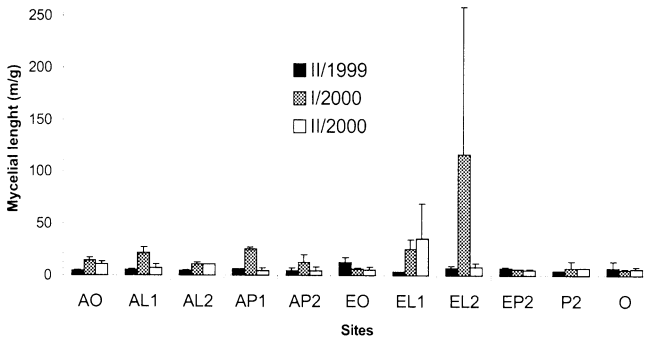

Fig. 3. – Mycelial lenght (m) per gram of dry soil in 1999–2000.

The highest CFU–counts (Fig. 2) were recorded in acidophilous climax forest (A0) in autumn 1999. The CFU–counts were found higher predominantly in autumn than in spring (sites A0, AL2, AP2, E0), but opposite is true by sites AL1 and AP1. Mycelial lenght in 1 g of dry soil (Fig. 3) was found the highest in site EL2 (eutrophic older dead forest) in spring 2000 – 116 m. The highest values of mycelial lenght were recorded in the most part of studied sites in the spring sampling in 2000 in the range of 5 to 12 m (A0, AL1, AL2, AP1, AP2, EL2, P2), while in sites 0 and E0 were the highest values found in autumn 1999. In the autumn sampling, the highest mycelial lenght (35 m) was estimated in site EL1 (eutrophic younger dead forest), while in the eutrophic older dead forest was mycelial lenght in the same time only 8 m and at the same time CFU–counts were also very low in this site.

The present study may be considered as the first contribution to the biodiversity and quantitative occurrence of the saprotrophic microfungi in soils under Norway spruce forests in the Šumava National Park with particular regards to gradual extinction of the forests as a result of the bark beetle occurrence in the studied area.

Conclusions

A total of 88 micromycetes including undetermined species and sterile mycelia were isolated from four sampling during 1999–2000 (9 taxa belong to Zygomycetes, 1 species belongs to Ascomycetes, 1 species belongs to Coelomycetes and 77 taxa belong to Hyphomycetes).

Micromucor ramannianus var. *ramannianus*, *Penicillium glabrum*, *P. inflatum*, *P. spinulosum*, *Trichoderma viride*, and *Mortierella* spp. were the most frequently isolated species in studied area during 1999–2000 years.

Goidanichiella barronii W. Gams, Steimann et Seigle-Mur. was firstly recorded from the area of the Czech Republic.

Using the Sorensen similarity index no significant differences among studied sites were found on the basis of spectrum of the isolated microfungi species.

Quantitative estimations (CFU–counts and mycelial lenght) showed differences among individual samplings and also among studied sites.

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