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Comparison of mycobiota of diverse aged spruce stands on former agricultural soil

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ABSTRACT: The mycological conditions on study plots established in forests growing on former agricultural farm lands were studied. In young spruce stand (8–10 years) reduced and unstable spectrum of macromycetes was found. After approximately 50 years of forest growth the situation became stable and spectrum of macromycetes together with development of mycorrhizal status were similar to a situation found in stands on forest soils. Slightly increased occurrence of saproparasitic species of fungi (e.g. *Heterobasidion annosum* at others) was observed in older growths.

Keywords: ectotrophic stability of forest; species spectrum of macromycetes; mycorrhizae; former farm land; health status of spruce

Afforestation of soils that are not suitable for intensive agriculture is currently in the focus of interest. It is one of the most suitable methods of its economic utilization. The extent of area suitable for forestation is estimated to about tens or hundreds thousands of hectares (Kacálek, Bartoš 2005). These sites are mainly situated in hilly areas or at the foothills of mountains.

Fungi play an important role in decomposition of organic matter in a litter. There are many species of fungi present in different stands according to their localization. In these conditions fungi form specific associations. Major part of these fungi species can form mycorrhizae i.e. symbiosis with roots of trees. Mycorrhizae enable better resorption of minerals than any other fungi. Mycorrhizal symbioses (beneficial associations between plants roots and fungi) are important phenomenon in all debates about a nutrition and growth of trees.

Stability and funcionality of forest ecosystems depend on aggregate impacts of biotic and abiotic factors. Numerous fungi species are considered as sensitive bioindicators of "Ectotrophic Stability of Forest" (ESF) where ectomycorrhizal fungi dominate (Fellner, Pešková 1995; Pešková 2005; Pešková, Soukup 2006, et al.). Changes in this mutual coexistence can be assessed and categorized, and different stadia of enrichment or impoverishment of fungi associations (mycocenoses) can be defined (Fellner, Pešková 1995; Soukup 1996). Occurence, abundance and rate of saprotrophic terrestric and lignicol fungi also reflect the quality of ecosystem.

During the last decades several researches have been published on mycology of the newly afforested agricultural lands in Europe (e.g. Slovakia, Germany, the Netherlands). Most of these were spruce plantations grown on former crop fields or meadows. These studies were mainly focusing on mycorrhizal (Gáper, Lizoň 1995) or terrestrial saprotrophic fungi (Mihál 1998) or both (Arnolds et al. 2004). Stands with trees 50 year old or older, and those 10 year old were noticed to be the most interesting for a comparative analysis of fungi species diversity and abundance of mycorrhizal, saprotrophic terrestrial

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and lignicole fungi. Their determination was based on fructifications. Data was collected by synchronous microscopic study of real mycorrhization of roots found in standard soil probes and with visual quantifications of health status of trees.

MATERIAL AND METHODS

Plot selection

Research was carried out on selected sites in Bystré, located in the foothills of Orlické hory (50°19.7'N; 16°15.1'E; 510–515 m a.s.l.) where we laid out three study plots (2,500 m^2: each divided into 25 subplots): No. I – placed in young plantation (10 years); No. II – medium age (50 years); No. III – old age stand (about 80 years); all plots were relatively compact spruce (*Picea abies* [L.] Karst.) forests on former arable soil. Selected spruce stands were uniform with relatively small intrusion of other tree species.

Evaluation of fungi

Every year during the period June–November we surveyed every 30 days all fructifications of macromycetes. The spectrum was based on detected and determined fructifications. Their abundance and rate (presence/absence on partial subplots 100 m^2) was also assessed. For all species of macromycetes the trophic affiliation was determined (M – mycorrhizal, SL – saprotrophic lignicol and saproparasitic, S – the other saprotrophic mainly tericol and humicol fungi eventually including rare muscicol, fungicol and fimicol fungi).

This same method was applied for a period of 3 years. We suppose that in this period if weather conditions were not extreme approximately 90% of present fungi can be identified from found fructifications. This is sufficient for assessment of the ESF.

We also assumed that the method of ESF assessment (Fellner, Pešková 1995; Soukup 1996) is fully applicable for forests about 50 years old and older. Latent grade of the ESF deterioration is connected with a decrease of ectomycorrhizal macromycetes below 40% while lignicol macromycetes increase to or above 30% from total identified fungi species. Evident inhibition of mycorrhizal fungi fructifications is at same time combined with increase of lignicol fungi and with a stimulation of wood-destroying fungi. Increasing grade of the ESF deterioration is characterized by constantly low percentage of mycorrhizal species (below 40%) while ratio of wood-destroying fungi increase mostly over 40%. Evident decrease of ectomycorrhizal species is followed by an increase of lignicol fungi diversity with their enhanced fructification. Lethal grade is the last and practically irreversible stage: percentage of mycorrhizal species is constantly below 20% from all macromycetes whereas wood-destroying fungi grow over 50%. In our work we use the nomenclature of the Index Fungorum.

Root sampling, extraction and evaluation of mycorrhizal infection

Standard sampling and processing method was used as described earlier (Pešková, Soukup 2006). From selected study plots (Bystré I, II, III) we took standard samples in two periods: in spring (between 17. 5. and 2. 6.) and in autumn (between 25. 9. and 10. 10.). Sampling was carried out in roughly within the same but not identical site, at the same distance from trunks of trees selected in the first year of the study (2005). Five samples were taken from each plot in each period. Soil samples with roots were stored in a refrigerator before further processing in the laboratory.

All roots from soil probes were manually separated using fine tweezers and needles. Afterwards, they were sorted into four groups according to their size (diameter < 1 mm, 1–2 mm, > 2–5 mm and > 5 mm). Remaining mineral matters were gently washed out in water. The finest category i.e. roots to 1 mm were deposited in fixation solution of glutaraldehyd till final evaluation.

Thicker roots show a random and relatively irregular distribution in the soil and they may be absent especially if a small probe is used (e.g. probe 6 cm in diameter) and therefore we used for quantitative evaluation of mycorrhizal infection only roots < 1 mm in diameter. These fine roots form the most adaptive and active portion of root system and thus the figures about all active and non-active mycorrhizae well represent actual status of mycorrhizal activity. Thicker root categories were used in an evaluation of total amount of dry organic matter of roots in samples.

Our standard method envisaged the use as a basic element for evaluation the 5 cm long root sections including all its lateral root branches of lower orders. Numbers of active and non-active mycorrhizae are the main indicators in relation to the total length of such root system. Twenty basic elements were assessed for each sample and average values were calculated.

Numbers of different types of mycorrhizal tips were identified under binocular microscope (mag-

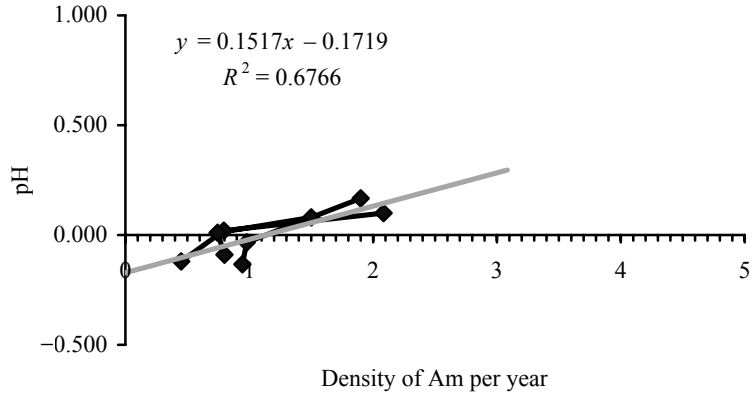

Fig. 1. Relation between the density of active mycorrhizae and changes of pH (pH value transformed as deviations from average values for each plot)

nification 4×) according to their typical features: tips with a hyphal mantel, Hartig net (Peterson et al. 2004), noticeable turgor, without root hair cover, smooth surface and pale coloration are accounted in a group of **Active mycorrhizae** (Am). On the contrary, tips with evident lack of turgor, shringed and wrinkled, without mantle and Hartig net are considered as **Non-active mycorrhizae** (Nm). Some problematic intermediate tips were assessed after inspection of their thin sections under microscope.

Different levels of mycorrhization are basically described by two parameters: density of active mycorrhizae (calculated to 1 cm of length) and the density of non-active mycorrhizae including their relative ratio – % (Vogt et al. 1983).

Soil pH and climatic characteristics

The value of pH in soil suspension was used as the major soil characteristic (the standard ČSN ISO 10 390 – Soil quality – pH evaluation). The method called as "pH–H_2O" is based on measuring pH of soil samples to which water is added in volume ratio 1:5, and after 5 minutes of agitating and standing for minimum two hours (and maximum for 24 hours). pH was measured potenciometrically by means of suitable pH meter with glass combined electrod with available extent pH 2–9.

The Czech Hydrometeorological Institute has provided with average data of air temperature (°C) and monthly precipitation (mm) from the closest meteorological station that is in Deštné v Orlických horách. It is situated only 9 km east of the study plots but about 100 m higher in altitude (Bystré 510–515 m a.s.l., Deštné 635 m a.s.l.).

Evaluation of defoliation

Health status of forest trees is characterized by level of defoliation. It is a relative loss of assimilatory apparatus of the crown in comparison with a healthful tree growing on same stand and vegetation conditions. Defoliation of tree is a non-specific symptom of damage that can be caused by many factors which can act individually or in parallel or in a synergic way. Separation of particular factors is difficult (Fabiánek et al. 2004).

A unique figure of defoliation was estimated once a year (August–September) for each plot. It is expressed as a relative number increasing in steps by 5%. Observer biases were minimized by averaging estimates of three observers for each of 25 trees in a plot.

RESULTS

Figures obtained in years 2005–2007 on spruce study plots are summarized in Table 1. We found a total of 75 species of macromycetes (40 mycorrhizal, 21 saprotrophic terrestrial and 14 saprotrophic to saproparasitic lignicol species). In different plots Bystré I, II, III we determined 8, 46 and 41 species, respectively.

Bystré I

In 2005 no fructification of ectomycorrhizal fungi was observed while in 2006 only *Laccaria proxima* was found. In 2007 beside *Laccaria proxima* also *Cortinarius anomalus* and *Hebeloma crustuliniforme* were found. Structure and density: in total 8 species were detected, of which 3 were mycorrhizal species, 5 saprotrophic terrestrial and no one lignicol species. ESF was probably not affected despite species spectrum is low. This plot despite young age revealed standard occurrence of mycorrhizal species.

Bystré II

In total 46 fungi were detected, of which 23 (50%) mycorrhizal species, 11 saprotrophic terrestrial and 12 lignicol species. ESF was not affected. Most

Table 1. List of macromycetes found on plots in years 2005–2007

Taxon	Trophicity	Bystré I	Bystré II	Bystré III
Amanita fulva	M			1/1
Amanita muscaria	M		21/6	
Amanita pantherina	M		4/1	1/1
Amanita porphyria	M			4/2
Amanita rubescens	M		25/5	17/7
Amanita spissa	M		2/2	2/2
Amanita vaginata	M		2/2	
Cortinarius (Seric.) *anomalus*	M	8/3		
Cortinarius (Telam.) cf. *castaneus*	M		1/1	
Cortinarius (Telam.) sp.	M		2/1	
Dermocybe cinnamomea	M		7/1	
Dermocybe crocea	M		17/4	
Gomphidius maculatus Lx	M	5/2		
Hebeloma crustuliniforme	M	8/4		
Hygrophorus pustulatus	M		36/5	
Laccaria amethystina	M		2/1	
Laccaria laccata s.l. (Be)	M	(4/2)	2/1	4/1
Laccaria proxima	M	3/1		
Lactarius mitissimus	M		12/2	
Lactarius necator	M			1/1
Lactarius rufus	M		63/8	13/3
Lactarius tabidus	M		80/3	30/3
Leccinum scabrum Be	M	4/2		
Paxillus involutus	M		7/3	2/2
Russula aeruginea	M		19/6	
Russula azurea	M			1/1
Russula badia	M			2/1
Russula cyanoxantha	M			4/1
Russula emetica	M			2/1
Russula fragilis	M			1/1
Russula integra	M		1/1	
Russula ochroleuca	M		14/4	12/6
Russula puellaris	M			1/1
Suillus grevillei Lx	M	12/4	22/4	1/1
Thelephora palmata	M		4/1	
Thelephora terrestris	M		50/1	13/2
Tricholoma psammopus Lx	M	6/2		
Xerocomus badius	M		7/3	32/12
Xerocomus chrysenteron	M		9/3	3/1

Table 1 to be continued

Taxon	Trophicity	Bystré I	Bystré II	Bystré III
Xerocomus subtomentosus	M			2/2
Clavulina cristata	S		6/1	4/1
Clitocybe incilis	S	5/1		
Clitocybe metachroa	S			4/1
Collybia asema	S		16/4	12/3
Collybia butyracea	S		7/3	2/2
Collybia dryophila	S	4/1		
Collybia maculata	S			1/1
Coprinus cf. *ephemerus* Lx	S	2/1		
Hygrophoropsis aurantiaca	S		4/2	
Lepista nebularis	S			3/1
Lycoperdon foetidum	S		3/2	2/1
Marasmius graminum	S	1/1		
Mycena citrinomaginata	S	1/1		
Mycena epipterygia	S		14/2	12/1
Mycena filopes	S		8/2	
Mycena pura	S		7/4	
Mycena sp. (Lx)	S	(1/1)	1/1	
Phallus impudicus	S		9/4	7/3
Rickenella fibula	S			10/1
Rickenella swartzii	S	4/2		
Setulipes androsaceus	S			107/6
Antrodia serialis	SL		30/1	
Bjerkandera adusta	SL		10/1	20/1
Calocera viscosa	SL		8/5	5/3
Dacrymyces stillatus	SL		170/2	50/1
Heterobasidion annosum	SL		1/1	
Hypholoma capnoides	SL		112/8	
Nectria cinnabaria	SL		60/1	
Panellus stipticus	SL			20/1
Pluteus cervinus	SL			1/1
Stereum sanguinolentum	SL		20/1	24/2
Trametes hirsuta	SL		5/1	
Tricholomopsis rutilans	SL		3/1	2/1
Tyromyces caesius	SL		11/4	2/1
Tyromyces stipticus	SL		1/1	

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

Lx or Be behind taxon's name – fungus bound to larch or birch trees, respectively. Trophicity: M – mycorrhizal, SL – lignicole saprotrophic or saproparasitic, S – the other saprotrophic

Table 2. Average values of mycorrhizal densities and percentages of active mycorrhizae (2005–2007)

Plots	Density of active mycorrhizae			Density of non-active mycorrhizae			% of active mycorrhizae		
	2005	2006	2007	2005	2006	2007	2005	2006	2007
Bystré I	0.95	0.98	1.90	0.28	0.12	0.37	78	89	85
Bystré II	0.45	0.80	2.09	1.36	0.45	1.03	31	64	69
Bystré III	0.80	0.75	1.50	1.38	0.37	0.86	38	67	64

common mycorrhizal species found were: *Lactarius rufus*, *Amanita muscaria*, *Russula aeruginea* and *Hygrophorus pustulatus*. These were accompanied by saprotrophic *Hypholoma capnoides*. Also rare species like *Dermocybe cinnamomea*, *Dermocybe crocea* and *Cortinarius* (*Telamonia*) sp. were found. This plot was characterized by *Dermocybe* and *Cortinarius* (*Telamonia*) sp. and also the species *Russula aeruginea*.

Bystré III

In total we identified 41 fungi of which 21 (51%) were mycorrhizal species, 12 saprotrophic terrestrial and 8 lignicol species. ESF was not affected. Most common mycorrhizal species were *Xerocomus badius*, *Amanita rubescens*, *Russula ochroleuca* and *Lactarius tabidus*. Only one saprotrophic fungi *Setulipes androsaceus* was detected. Rare species found here were: *Russula azurea*, *Russula badia*, *Russula emetica*, *Amanita fulva*, *Amanita porphyria*. This plot, typical of submountain and mountain natural acidic spruce associations, was characterized by remarkable occurrence of *Xerocomus badius* and *Russula ochroleuca*.

Study plots in the area of Bystré showed rich and healthy communities with a favorable situation for future development of the forest (high ratio of mycorrhizal species found on all plots: I, II – 50%, III – 51%). On all of them only *Laccaria laccata* and *Suillus grevilei* fructified.

In the study plot Bystré II the fructifications of *Heterobasidion annosum* were identified. This species is considered as important damaging agent of conifers planted on former arable soils. This fungus was also identified nearby other plots.

Evaluation of mycorrhizae

Average year density values of Am and Nm are compared in Table 2.

Study plot Bystré I revealed the highest density of Am in fall 2007 (2.41 cm) and the lowest in fall 2006 (0.81 cm). Density of Nm was lowest in fall 2006 (0.11 cm) and highest in fall 2007 (0.57 cm). The proportion of Am was highest in spring 2006 and 2007 (89%) and lowest in fall 2005 (77%).

Study plot Bystré II showed highest density values of Am also in fall 2007 (2.19 cm) and lowest in spring 2005 (0.30 cm). Lowest value of Nm was detected in spring 2006 (0.44 cm) and highest in spring 2005 (2.05 cm). Relative quantity of Am was highest in spring 2007 (76%) and lowest in spring 2005 (13%).

On Bystré III, the highest density of Am was found also in fall 2007 (1.71 cm) and lowest in fall 2006 (0.42 cm). Lowest value of Nm was found also in spring (0.31 cm) and highest in fall 2005 (1.68 cm).

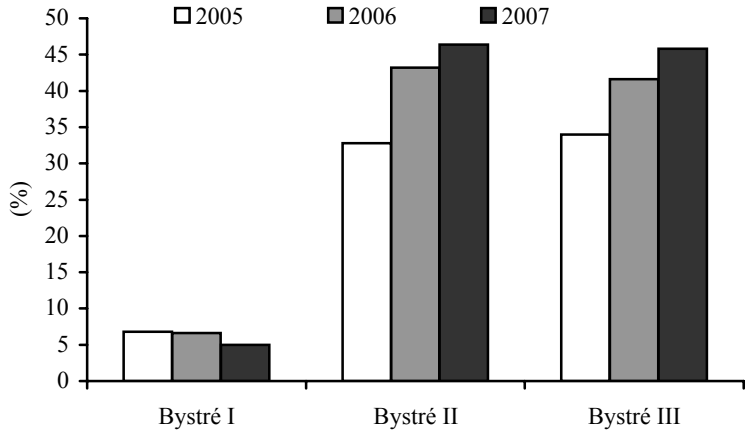

Fig. 2. A comparison of spruce defoliation during period 2005–2007

Table 3. Basic meteodata from the observatory Deštné v Orlických horách (2005–2007)

2005			2006			2007		
Month	T (°C)	precipitation (mm)	month	T (°C)	precipitation (mm)	month	T (°C)	precipitation (mm)
1	–2.3	197.5	1	–6.7	43.3	1	1.2	169.8
2	–5.0	95.7	2	–5.5	96.6	2	0.9	88.6
3	–1.5	54.8	3	–2.3	89.1	3	3.5	55.2
4	7.2	37.6	4	5.7	96.0	4	8.2	7.5
5	10.7	184.3	5	10.7	153.2	5	12.8	64.6
6	13.9	67.8	6	14.9	64.8	6	16.3	91.4
7	16.1	167.6	7	19.8	17.8	7	15.8	158.9
8	14.2	93.0	8	13.3	343.8	8	15.9	62.4
9	13.1	69.0	9	13.6	41.9	9	9.2	139.3
10	8.0	12.5	10	8.7	71.4	10	5.4	34.5
11	0.5	55.4	11	4.1	165.8	11	–0.2	139.3
12	–2.8	163.5	12	1.1	65.2	12	–2.7	66.0
Average	**6.0**		average	**6.5**		average	**7.2**	
Sum		**1,198.7**	sum		**1,248.9**	sum		**1,077.5**

Relative number of Am was highest in spring 2006 (74%) and lowest in fall 2005 (30%).

During the study period we detected a mild improvement of pH. This abiotic effect probably positively influenced the numbers of active mycorrhizae as they are generally very sensitive on even small changes of pH. Studied plots had principally different basic pH levels. For better insight we compared in graph 1 the pH deviations from average values of each plot. Correlations of other studied parameters (summer and winter temperatures, summer and winter precipitation, defoliation, dry biomass of roots and others) did not show uniform results. This may be caused by different age of growths or extreme weather fluctuations (spring 2006 with abnormal precipitation, whole year 2007 with supernormal temperatures and subnormal precipitation – these figures are compared in Table 3).

Evaluation of deforestation

In all plots and years except one (Bystré I) we recorded an increase of defoliation (Fig. 2). But in general, the health status of trees improved even in Bystré I, where it stabilized, and this growth, according to our data, seemed viable (average values of defoliation even decreased from 7% in 2005 to 5% in 2007). Average values of defoliation decreased slightly between 2006 and 2007 perhaps as a result of increased fall of needles in 2007 due to low precipitation between September 2006 and May 2007.

DISCUSSION

Spruce growths on forested agricultural lands reveal differences in studied parameters caused by differences in age, pH of the soil, elevation of the sites or even minor variations of stands homogeneity. These are probably main factors affecting presence and activity of different fungi species.

Bystré I plot with trees about 10 years old reveals a succession of fungi in early stadium while mycocenosae on Bystré II (50 years) and Bystré III (80 years) are rich and stable. However, some species disappeared here but in total they can still be enriched by some other new species. Older growths fully represent conditions for assessment of ESF whereas extremely young growths show fast development of fungi structure and especially mycorrhizal species are usually relatively lower. Initial composition of mycoflora existing on former fields and meadows are quite different in absence of any mycorrhizal species. After forestation this group of fungi infiltrate slowly in natural conditions unless it is artificially introduced.

Variation of soil pH values between 3.9 and 4.9 (i.e. acidic or lightly acidic) is relatively small to influence on a species structure. A younger stand seems less

acidic (Bystré III – 3.9, Bystré II – 4.2, Bystré I – 4.9). However, initial geological conditions (underbads are characterized by metabasits and phylits) can influence this situation.

Mycological conditions on ten years old growth in Bystré I

Species like *Laccaria*, *Hebeloma*, *Cortinarius*, *Inocybe* and also e.g. *Lactarius detterimus* are known as ectomycorrhizal fungi of early succession. Gáper and Lizoň (1995) found in total 9 species in young forests younger than 10 years with higher abundance: *Cortinarius* sp., *Hebeloma crustuliniforme*, *Laccaria laccata*, *Lactarius detterimus*, *Chalciporus piperatus*, *Amanita muscaria*, *Inocybe lacera*, *Hebeloma perpallidum*, *Hebeloma sinapizans*. In another study, Arnolds et al. (2004) identified four species: *Hebeloma mesophaeum*, *Laccaria laccata* s.l., *Laccaria proxima*, *Cortinarius flexipes* ss. Kühn.

Fructifications found on Bystré I (mainly in last year of study when this growth was 10 years old) were similar to species identified by Gáper and Lizoň (1995) in a growth 8 year old where the most abundant were *Cortinarius* sp. and *Hebeloma crustuliniforme,* while a year before and a year after these species were less numerous. *Laccaria laccata* was most abundant in the second year of growth age. Similarly, according to Arnolds et al. (2004) *Hebeloma mesophaeum*, *Laccaria laccata*, *Laccaria proxima* a *Cortinarius flexipes* ss. Kühn were the most abundant species in 10th year of the growth. We found remarkable high degree of similarity in species composition, timing and density. It may indicate stable processes of succession and also a standard development of spruce mycorrhization in Bystré I.

From other study of 16 year old plantation Arnolds et al. (2004) reported 22 species. This place was rich in nutrients (mainly nitrogen). Increased number of fungi correlates with advanced succession.

Mycological conditions in older growths

Changes in the mycorrhizal and saprotrophic terrestrial trophic groups are the most informative. We can extend our results appending published data (Gáper, Lizoň 1995; Arnolds et al. 2004). While in early stadia of succession we have more published results available, data from growths over 50 year old were till now scarce. Figures show clear increase of fungi number that correlates with stand aging process. Growths younger than 10 year old usually host about 10 species, growths younger than 30 year old about 20 species and growths 50–80 year old about 30 fungi species of this trophic group. However, at the same time the variation increases according to respective conditions of the stands. Increase between age category 30 and 50–80 years is followed by smaller changes or stable situation over this age. It seems that the growth of fungi spectra is limited and mycocenosis is saturated.

CONCLUSIONS

During three-year study of mycological situation in spruce plots on former agricultural non-forest grounds in foothills of the Orlické hory a presence of 75 fungi species was identified simultaneously with a health status situation (described in terms of defoliation) and mycorrhizal activity assessed. Records from the middle and old age stages of growths are so far missing in the literature where mostly only successions in young stands were studied. Results show that the number of mycorrhizal fungi increases with the age of growths reaching 20 or 30 species or even more. Less frequent mycorrhizal species found in young stands disappeared. Older stands seem to be gradually better adapted. Aged growths on non-forest lands are becoming identical in quality and appearance to growths on forest soil. A lower number of fungi found can be probably caused by generally less suitable meteorological conditions in years of the study.

Three year period of the study is minimal for assessment of fungi occurrence as fructifications are strongly dependent on weather conditions. Determination of mycorrhizal activity is less sensitive to actual weather conditions because their development and function has long-standing effect that is not considerably influenced by inter and within year fluctuations. Mycorrhizal conditions seem appropriate not only in older but also in young stands. Slight positive effect in manifestation of mycorrhizal activity was observed in correlation with narrow decrease of acidity of soil on all stands. In 2007, highest intensity of fructification occurred on all plots. However, this can be partly caused by relatively dry seasons 2005 and 2006 with a reduced level of fructification beside an effect of pH changes.

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Srovnání rozvoje mykobioty na různě starých smrkových stanovištích na původně zemědělských půdách

ABSTRAKT: Na plochách v lesních porostech založených na bývalých zemědělských půdách v severovýchodních Čechách (podhůří Orlických hor) byly studovány jejich mykologické poměry. Ve smrkových porostech ve věku do 10 let bylo druhové spektrum makromycetů poměrně úzké a nestálé, od 50 let věku se situace stabilizovala a spektrum makromycetů i kvalita mykorhiz již byly obdobné jako u porostů rostoucích na lesních půdách. V padesátiletých a starších porostech byl registrován mírně zvýšený výskyt saproparazitických druhů hub (*Heterobasidion annosum* aj.)

Klíčová slova: ektotrofní stabilita lesa; druhové spektrum makromycetů; mykorhizy; bývalá zemědělská půda; zdravotní stav smrku

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