Changes in the mycorrhizal status of some mountain spruce forests

V. Pešková

Forestry and Game Management Research Institute, Jíloviště-Strnady, Czech Republic

ABSTRACT: In the present study, the defoliation status of spruce stands is related to mycorrhizal conditions and presence of mycorrhizal macromycetes fungi in two localities at the highest sites of the Krkonoše Mts. In the long-term view, the defoliation and the diversity of mycorrhizal fungi have improved but the number of active as well as nonactive mycorrhizae has decreased while their ratio has not changed. Statistically significant is the correlation between the total number of mycorrhizae and pH. The results within last years of observations as well as in comparison to the conditions in the last decade show that mountain spruce stands growing at non-exposed sites are tolerant to a degradation of mycorrhizal conditions resulting from the long-term air pollution impact. The studied parameters of mycorrhizal symbiosis have not shown a clear trend; their results however indicate the stabilized mycorrhizal state. Positive changes can be seen in the total increase of macromycetes diversity as well as in the apparent increase of percentage of macromycetes in the studied localities, which correlates to the moderately improving defoliation.

Keywords: mycorrhizae; root; mycorrhizal fungi; defoliation; *Picea abies*

Development of tree health conditions is influenced by many abiotic and biotic factors that are often correlated to each other. In most cases, a direct influence of individual factors cannot be therefore simply distinguished. It is not often easy to define the causal direction and to define dependent and independent variables.

The health conditions of mountain forests have been intensively studied in the last decades. Their unfavorable development continues even though the major air pollution caused by the power stations have been eliminated. Mountain spruce stands are damaged most seriously. In the present study, their health conditions (e.g. crown defoliation) are evaluated referring to the changes in quality and quantity of mycorrhization of spruce roots. The study follows the author's previous works concerning different forest stands (Soukup et al. 2003; Pešková 2005, 2006).

MATERIAL AND METHODS

Selection of study plots

Two study plots (2,500 m^2 each) were established in the Krkonoše Mts. near the town of Pec pod Sněžkou, in the territory of the Krkonoše Mts. National Park, Horní Maršov Forest District. Both plots represent approximately even-aged spruce stands. The plot Růžová hora is situated on the moraine on the left bank of the Úpa River (50°43'N, 15°44'E, 980 m a.s.l., the vegetation type is *Vaccinio-Piceetum*, the age of the stand 90 years, almost 100% *Picea abies* [L.] Karst.). The plot Sněžka is situated at the locality Pod Kovárnou (50°44'N, 15°44'E, 1,000 m a.s.l., the vegetation type *Calamagrostio villosae-Piceetum*, the age of the stand 80 years, 100% *Picea abies*). In each plot, all trees were numbered.

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Sampling strategy

In 2004–2006, roots and mycorrhizae were sampled during the spring to early summer period (June 5 to July 3), and the autumn period (October 2 to 9). Five samples were taken from each study plot. Each sampling was done roughly at the same place (not identical), approximately in the same distance (about 1 m) from the stem of five selected trees. The cylinder of the soil probe used for this purpose had the inner diameter of 6 cm and the height (depth of space sampled) of 15 cm. The probe had saw rim for cutting roots, and inner plastic tube for stabilizing the sample. After taking soil samples, these were placed in refrigerator until they were processed.

Preparation of root samples

From each probe, all roots were manually prepared with tweezers and preparation needles and separated according to their diameter (Pešková, Soukup 2006). Then they were carefully washed with water in order to remove as much mineral admixtures as possible; the latter complicate determination and quantification with a microscope. The roots with diameter < 1 mm were stored in the fixation solution (glutaraldehyde) to be prepared for determination. The roots with diameter > 1 mm are less usable for the small (6 cm) diameter probe because they are scattered irregularly in the soil and may not be sampled appropriately. These roots were therefore used as additional values for measuring the total weight of root dry matter. After analyses, all prepared roots were dried in the kiln (24 hours at the temperature of 105°C) and weighed with the accuracy of 0.01 g.

Evaluation of mycorrhizal infection and dry root matter

In this paper, the term *mycorrhiza* is used for an organ that was created after the root had been colonized with a fungus. The main factors analyzed were the absolute numbers of active and nonactive mycorrhizal tips (cf. Pešková, Soukup 2006a) and the dry root matter within the root fraction up to 1 mm. The weight of root dry matter up to 1 mm represents the average value of dry matter obtained from roots separated from five soil samples. These thin roots are the most active part of the root systems; they adapt dynamically to changes of site conditions. The numbers of active and nonactive mycorrhizal tips were calculated from samples selected before drying as average number of tips found in 20 main root sections (< 1 mm in diameter) of 5 cm in length including their side branches. The level of mycorrhizal infection was evaluated using two parameters: density and percentage of mycorrhizal tips. The active (AM) and nonactive (NM) mycorrhizae were counted as an average value of the number of mycorrhizal tips connected with 1 cm of root. The percentage proportion of mycorrhizae was calculated as a ratio of active and nonactive mycorrhizal tips. In both experimental periods, the roots and mycorrhizae were sampled and evaluated according to the same standard method (Pešková, Soukup 2006).

Assessment of defoliation

In the study plots, the health condition of trees was assessed using the standard classification of crown defoliation proposed by Rösel (Rösel, Reuther 1995; Fabiánek et al. 2003). It was also used in some of our other investigations (Fellner et al. 1995; Soukup et al. 2001, 2003).

Assessment of fungi species occurrence

During the investigation period 2004–2006, fruiting bodies of macromycetes were collected in regular intervals, approximately once or twice a month during the main fructification season (from June to November). In all the macromycetes species found in both plots the trophic association was established and the ratio of mycorrhizal species calculated. As for the spectrum of macromycetes species, it is better to evaluate it as a whole after several years. When the weather conditions are more or less normal, at least 90% of the present fungi species can be discovered within three years based on the quantity of collected fruiting bodies sufficient for assessment of the ectotrophic stability of the forest (Fellner, Pešková 1995; Pešková, Soukup 2006a).

Soil and climatic characteristic

The value of pH in soil suspension was used as the major soil characteristic (the standard ČSN ISO 10390 – Soil quality – pH evaluation). The method called *pH–H$_2$O* is based on measuring pH of soil samples in water (five portions of water to one portion of soil); after 5 minutes of agitating and subsequent standing for minimally two hours (not more than 24 hours) pH is measured potenciometrically by means of suitable pH meter with glass combined electrode with available extent of pH 2–9.

The Czech Hydrometeorological Institute provided us with data files with air temperature (°C) and precipitation (mm); they contained average monthly temperature and precipitation from the station of Pec pod Sněžkou (50°55'N, 15°49'E, 816 m a.s.l.).

Evaluation methods

The obtained data can be divided into two groups. The first one comprises average values from two several year periods (1991–1995 and 2004–2006); they indicate a trend but cannot be statistically well evaluated. The second group comprises single measurements that however lack the complexity of the older investigation period.

Matrix of the obtained data was used to calculate correlation coefficients for all variable couples. Basic calculations and graphs were created in the programme Microsoft Excel. For further processing of data, various modules of Statistica 6.0 (StatSoft Inc.) were used. The correlation was tested at the levels of significance of 0.05, 0.01 and 0.001. The used statistical methods belong to the common procedures described in various sources.

RESULTS

Changes in the parameters after ten years

The investigation performed on the two above-described mountain spruce stands in 2004–2006 gave the unique opportunity to compare the results with another investigation conducted in the same localities ten years ago, between the years 1991 and 1995. Even if these older data are nor complete, since some data on the soil quality are lacking, and mycorrhizae were sampled only in the autumn 1994, they indicate some changes that occurred during the last decade. As for the crown defoliation assessment, a significant improvement was recorded after ten years. Average values of primary defoliation can be compared between the plots Sněžka and Růžová hora as follows: in 1994 it was 52% in Sněžka and 56% in Růžová hora, in 2004 the values were 45% and 43%, respectively.

The number of macromycetes species as well as the percentage proportion of mycorrhizal species of fungi in the plots showed rapid positive changes as follows: in Růžová hora the average number increased after the ten-year period from 42 to 99 species of fungi, the average percentage proportion of mycorrhizal species of fungi increased from 50% to 69%; in Sněžka the increase from 24 to 44 species was observed and from 28% to 52% of mycorrhizal fungi. The relationship between defoliation and proportion of macromycetes is shown in Fig. 1. Figs. 2, 3 and 4 show average numbers of active (AM) and nonactive (NM) mycorrhizae, and proportion of active mycorrhizae (%AM) in individual years. It results from the given data that in both plots relatively high densities of active as well as nonactive mycorrhizae were recorded in 1994; in Růžová hora the nonactive ones were even higher. Ten years later the densities of active mycorrhizae decreased markedly, by 1.05 and 1.32 mycorrhizae to 1 cm of root length in Sněžka and in Růžová hora, respectively. The density of nonactive mycorrhizae decreased by 0.98 mycorrhizae to 1 cm of root length in Sněžka and by 1.55 in Růžová hora. The percentage of active mycorrhizae in Růžová hora in 1994 was only by 2% higher, in Sněžka the percentage of active mycorrhizae was identical.

When comparing the values of both investigation periods the weight of dry matter decreased;

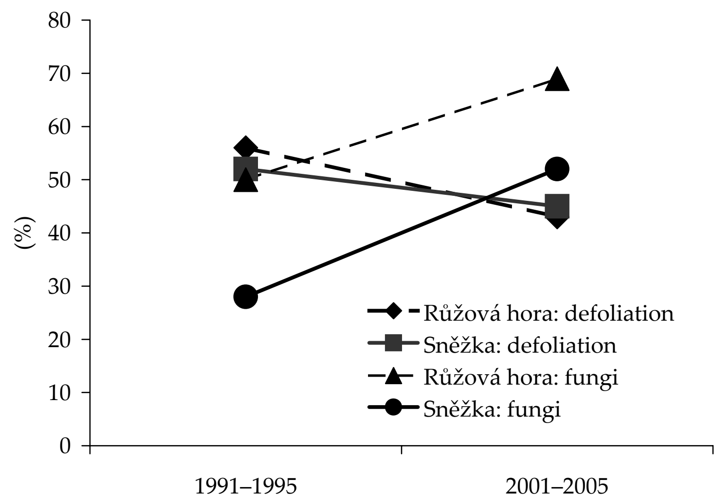

Fig. 1. Changes in defoliation of trees and percentage of mycorrhizal fungi

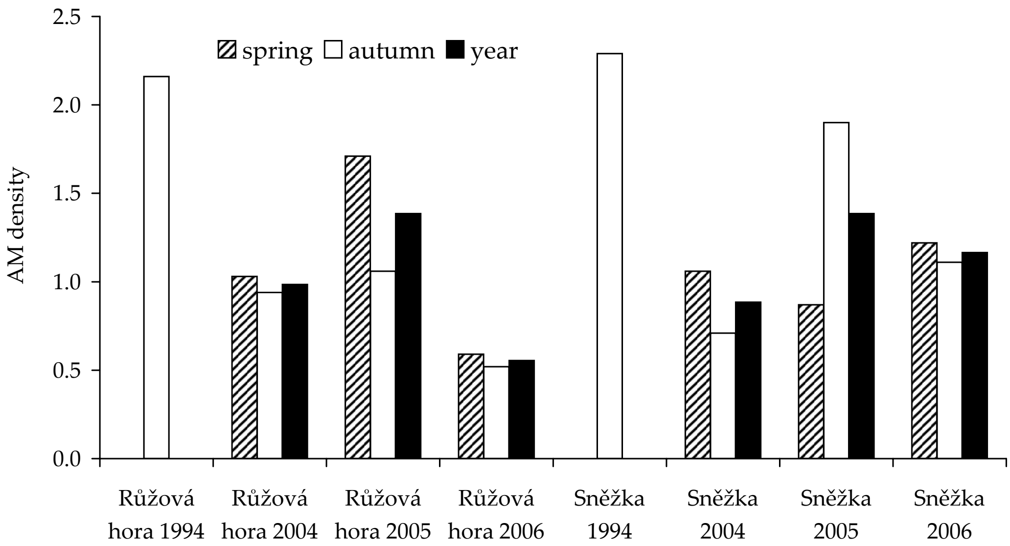

Fig. 2. Index of active mycorrhizae (AM) density in 1994 and 2004–2006

in Růžová hora the average decrease was 0.70 g, in Sněžka 0.81 g (Fig. 5).

Changes in the parameters within the years 2004–2006

Data on mycorrhizal status of roots obtained from spring and autumn sampling comprise the following parameters: density of active and non-active mycorrhizae, their ratio, the total number of mycorrhizae, weight of dry matter of roots up to 1 mm, numbers of mycorrhizae recorded in a year, average defoliation, direct defoliation of the sampled tree and percentage of mycorrhizal species found in all the community of counted macromycetes. Data matrix (6 sampling periods by 26 variables) is completed with abiotic parameters such as altitude, pH, annual precipitation, summer precipitation, average annual and summer temperature (from April to September). In the matrix of these variables the basic statistical values were calculated and the correlations were evaluated. Except for the correlations mentioned in Discussion any other relationships cannot be proved. One of the reason is that the investigation period 2004–2006 is rather short and the influence of weather oscillation from year to year cannot be fully eliminated.

Density of active mycorrhizae (AM, calculated as average values of spring – autumn) varied from 0.55 (Růžová hora 2006) to 1.39 (Růžová hora, Sněžka 2005), with the average of 1.0.

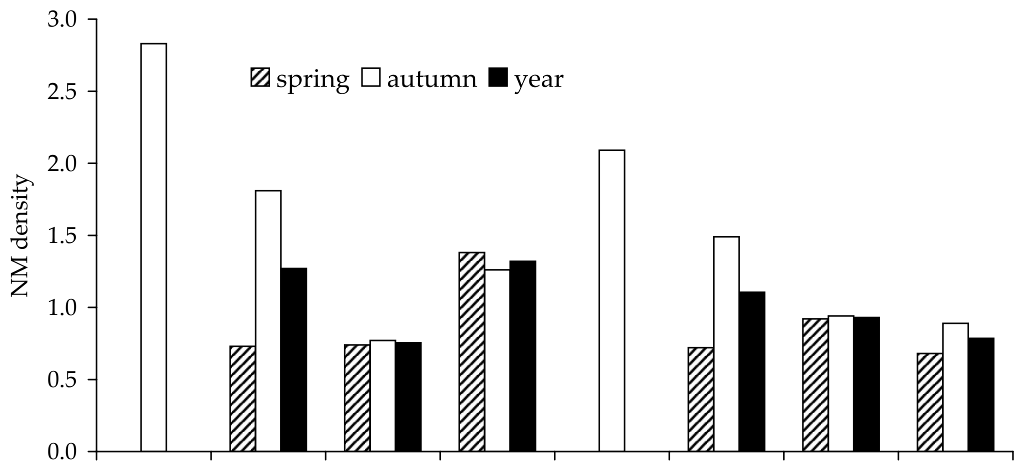

Fig. 3. Index of nonactive mycorrhizae (NM) density in 1994 and 2004–2006

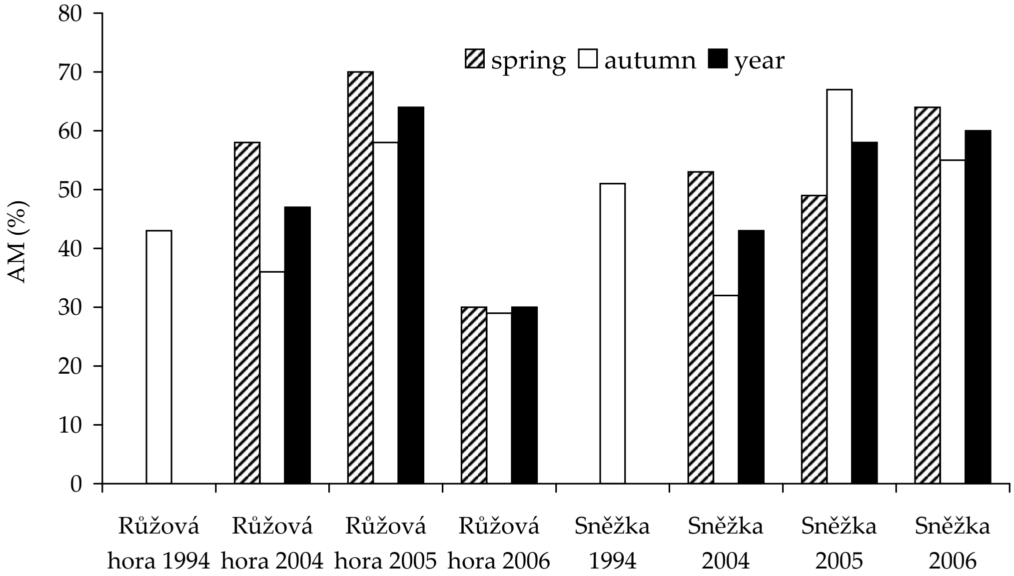

Fig. 4. Percentage of active mycorrhizae on spruce roots in 1994 and 2004–2006

Density of nonactive mycorrhizae (NM) varied from 0.76 (Růžová hora 2005) to 1.32 (Růžová hora 2006), with the average of 1.0. Their ratio (%AM) remained very similar reaching about 50%.

DISCUSSION

The average primary defoliation improved after ten years by 13% in Růžová hora and by 7% in Sněžka, which corresponds to stabilization or even improvement of tree health published in recent articles. The positive results presented here are in agreement with the results obtained in other stands in the Krkonoše Mts. (Fellner, Landa 2003), even in localities that were under heavier air pollution impact in the past. According to Fabiánek et al. (2003), who assessed the forest health conditions in the monitoring plot Pec pod Sněžkou from 1986 to 2003, the average defoliation increased until 1997, then decreased by 3% in 1998, and stagnated until 2003. Vacek et al. (2007) published the results received from permanent trial plots in the Krkonoše Mts. showing that the first signs of spruce stands destruction arose after 1982, and in the first half of the 1980s the health conditions of stands gradually worsened. In the period after 1988 health improvement and decline of the stands alternated in individual years. In the years 1996–1999, several stands within the study plots were destroyed but others were stabilized or moderately improved (1–3% per year).

The improvement of defoliation and the simultaneous increasing proportion of mycorrhizal species fully agree with the hypothesis about their relation. Data correlation within a year (however, weakened

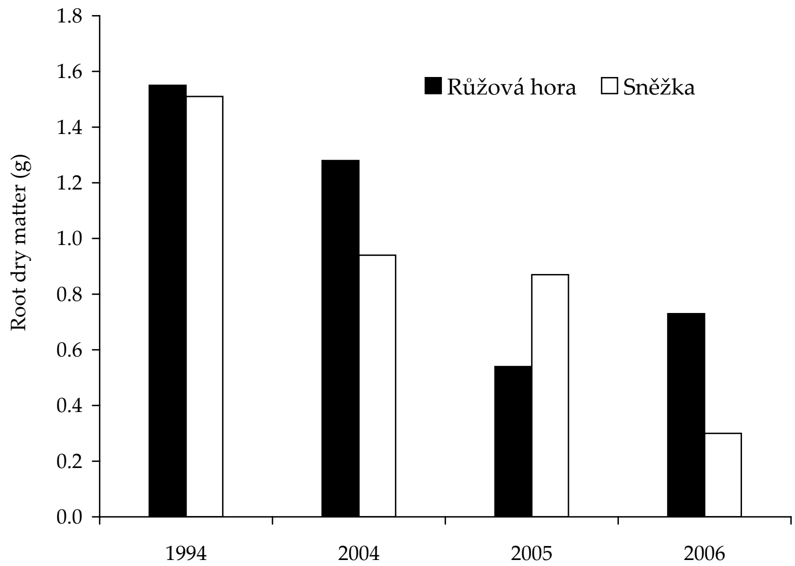

Fig. 5. Average values of root dry matter (root section to 1 mm in diameter) in years 1994–2006

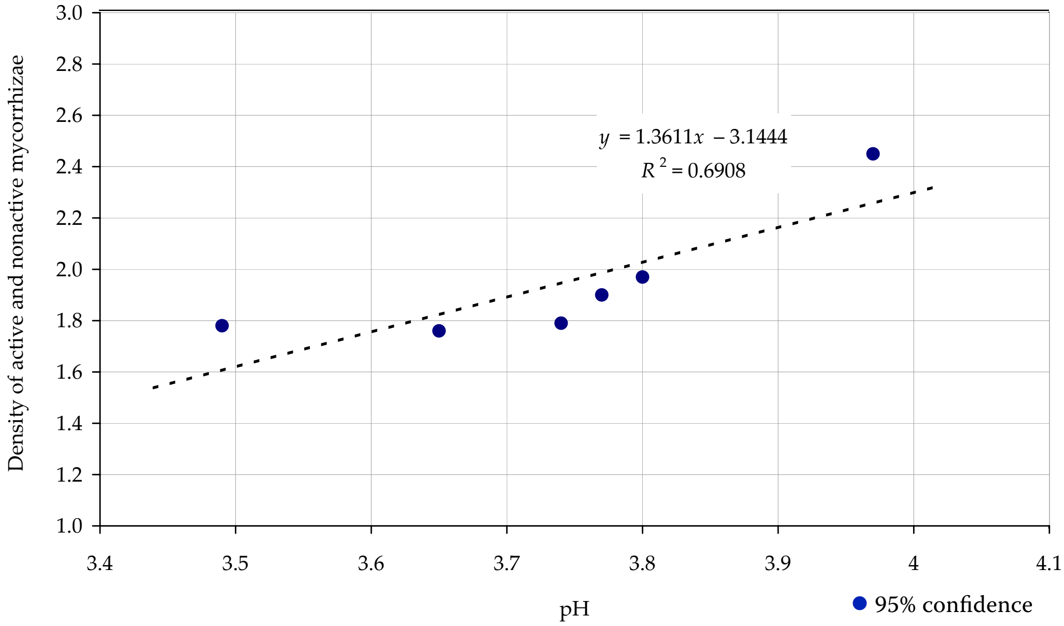

Fig. 6. Possible correlation between density of all (active and nonactive) mycorrhizae, and pH

in consequence of variable occurrence of macromycetes that are best evaluated within longer period!) only indicates this relationship even though the test of correlation coefficient (R^2 = 0.427) was inconclusive (P = 0.079).

Correlation of mycorrhizae in mountain spruce stands and summer precipitation was not proved, contrary to very high correlation of oak mycorrhizae with summer precipitation in lower altitudes (Pešková 2006). Generally, poor precipitations in lower altitudes belong among the partly limiting factors whereas regular and rich precipitations in mountains (Sněžka: 1,250–1,450 mm per year) are oversupplying and sufficient soil humidity probably does not reduce the fungal activity (mainly reduced by droughts). No influence of summer temperature (average monthly temperature from April to September) to any of mycorrhizal parameters was observed.

In the years 2004–2006 the values of pH in Růžová hora varied from 3.65 do 3.97, those in Sněžka were similar 3.49–3.77. A high negative influence of the increase in acidity (even small differences in pH were significant) on the number of mycorrhizae proven in oak stands (Pešková 2006) was statistically proven in spruce mycorrhizae only in the total number of mycorrhizae (active and nonactive) in the sampling in spring 2004–2006. It was the only parameter probably positively correlated with pH (R^2 = 0.691, P = 0.040) (Fig. 6).

Considering the obtained data, it is hard to define why the number of mycorrhizae in the plots dropped after ten years; it concerned the rapid decrease of density of active as well as nonactive mycorrhizae. However, their percentage proportion remained unchanged after ten years. This rather surprising decrease in the number of active and nonactive mycorrhizae (Figs. 2 and 3) can be connected with the changes in acidity as it was reported by Vacek et al. (2007). Chemical analyses of the soil from the spruce stands in the Krkonoše Mts. in years 1980, 1993, 1998 and 2003 gave unsteady values. In this period, pH markedly increased (from 3.59 in 1980 to 4.77 in 1998) and in the 2004–2006 investigation period it decreased to 3.78. Although the pH values measured in the plot are not available for the oldest mycorrhizal analyses (sampling in 1994) it can be supposed that a similar decrease in acidity could have had a positive influence.

The total average values of active mycorrhizae density in spruce stands are about 1.0/cm, which is moderately higher than those found in oaks (Pešková 2006; 0.74/cm), however, the average of nonactive mycorrhizae is markedly lower in spruces (1.0) than in oaks (1.65). This corresponds to the higher percentage of active mycorrhizae (50%) found in spruces compared to oaks (30%).

CONCLUSIONS

The results of the 2004–2006 investigation period show that some mountain spruce stands growing at non-exposed sites are more tolerant to irreversible degradation of mycorrhizal conditions resulting from the long-term air pollution impact; this is confirmed by the comparison of the results with data obtained in the 1990s. The studied parameters of mycorrhizal status do not show a clear trend but indicate the stabilized mycorrhizal state. Positive changes in the total increase of macromycetes diversity as well as the apparent increase of percentage of macromycetes can be seen in the studied localities, which correlates to the moderately improving defoliation.

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Změny mykorhizních poměrů v některých horských smrčinách

ABSTRAKT: Práce přináší výsledky sledování zdravotního stavu smrkových porostů na dvou studijních plochách v nejvyšších polohách Krkonoš ve vztahu ke změnám kvality a kvantity mykorhizace jejich kořenů a prezenci mykorhizních hub. V dlouhodobém pohledu došlo ke zlepšení úrovně defoliace a zastoupení druhů mykorhizních hub, avšak k poklesu počtu aktivních i neaktivních mykorhiz při zachování jejich vzájemného poměru. Statisticky významnou korelací se ukázal vztah celkového počtu mykorhiz a pH. Změny v posledních letech – stejně jako srovnání s poměry před deseti lety – ukazují, že horské smrkové porosty situované na poněkud chráněných místech jsou i přes stále ne zcela uspokojivý zdravotní stav odolné vůči nevratné degradaci mykorhizních poměrů v důsledku dlouhodobé imisní zátěže. Studované parametry mykorhizní symbiózy neprokázaly jednoznačný trend, jejich hodnoty však reprezentují stabilizovaný mykorhizní stav. Pozitivní je celkový nárůst druhové diverzity makromycetů i výrazný trend v nárůstu procentuálního podílu makromycetů na studovaných plochách, který odpovídá i mírně se zlepšující defoliaci.

Klíčová slova: mykorhiza; kořen; mykorhizní houby; defoliace; *Picea abies*

Corresponding author:

Ing. Vítězslava Pešková, Ph.D., Výzkumný ústav lesního hospodářství a myslivosti, v.v.i., Jíloviště-Strnady, 156 04 Praha 5-Zbraslav, Česká republika
tel.: + 420 257 892 299, fax: + 420 257 920 648, e-mail: peskova@vulhm.cz