Effect of increased nitrogen depositions and drought stress on the development of Scots pine (*Pinus sylvestris* L.) – II. Root system response

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**ABSTRACT:** Effects of drought stress, stress by increased nitrogen depositions and combined effect of the two stress factors on the growth of Scots pine (*Pinus sylvestris* L.) were studied in two experimental series in 1994–1997. The drought stress was induced by reduction of atmospheric precipitation by 60%, the increased nitrogen depositions were simulated by repeated applications of ammonium sulphate at a dose corresponding to 100 kg N/ha per year. All stress factors under study impacted the biomass, vertical distribution, functionality and mycorrhizal infection of fine roots. The root system responded to simulated stresses as early as from the very first year of their effect exhibiting greater damage than the above-ground part of the plant (see PALÁTOVÁ 2001).

**Keywords:** *Pinus sylvestris* L.; nitrogen; drought; biomass of fine roots; vertical distribution of fine roots; functionality of fine roots; mycorrhiza

Forest tree species develop in a permanent interaction with external environment and in the course of their existence they are exposed to natural stress factors such as pathogens and pests, high or low temperatures, excessive water or drought. As suggested by some models worked out in foreign laboratories in connection with the changes in global climate, the occurrence of dry periods in the growing season could increase (CHALUPA 1995), which would mean a considerable risk for the forest tree species at both younger and later stages of development. In recent decades, natural stress factors have been combined with stresses of anthropogenic origin that can directly affect the assimilatory organs of tree species. After deposition into soil many of them can induce changes in the soil environment and affect the plants indirectly through their root system. Apart from sulphur compounds, it is also the case of nitrogen compounds whose emissions have recently increased at an annual rate of 5% according to some estimates (TAMM 1989).

Increased N-depositions favourably affect the growth of the above-ground parts but they can also influence the root systems of tree species that are in direct contact with the soil and reflect all changes occurring in the soil. The most sensitive response is shown by fine roots (diameter < 1 mm) that are most important for supplies of nutrients and water. Although the detailed mechanism of fine root growth regulation is not perfectly known yet, there is a considerable amount of data on morphological and physiological reactions of fine roots induced by changes in soil acidity and nutrient availability. With respect to the fine root development a key role is played by nitrogen which is known for its influence on carbon distribution in the plant, synthesis of defensive substances and sugar supplies (VOGT et al. 1993). A negative relation between soil nitrogen supply and biomass of fine roots was described by many authors (TÖLLE 1967; HEINSDORF 1976; ALEXANDER, FAIRLEY 1983; VOGT et al. 1990, and others). Reduced development of fine roots under the influence of simulated N-depositions was studied by OLSTHOORN et al. (1991) in Douglas fir, by CLEMENSSON-LINDELL and ASP (1994) and KAREN and NYLUND (1997) in spruce. On the other hand, MAJDI and PERSSON (1995) found an increased biomass of fine roots in spruce after addition of ammonium sulphate at a dose corresponding to 100 kg N per hectare/year in the first and third year of application. Similarly, according to RITTER (1990) a high content of nitrogenous matters induced a reduction in the total mass of roots capable of resorption in pine. Increased N doses in experiments made by HEINSDORF (1976, 1991) induced a similarly pronounced reduction of fine root biomass in pine stands. TÖLLE (1969) measured a 45% reduction of fine roots in pine as early as only several months after the...
application of 120 kg N/ha in the form of urea. Changes in soil conditions can also result in the changed representation of fractions of live and dead roots (PERSSON et al. 1995). A response to the changed soil environment can also be a change in the spatial distribution of fine roots in the soil profile, i.e. in the vertical distribution (RASPE 1992).

Increased nitrogen inputs are often taken for being responsible for the disturbance of mycorrhiza that SKEEFFINGTON and WILSON (1988) consider to be one of the possible mechanisms of developing the imbalance of nutrients in the plant. HEINSDORF (1987 in HOFMANN et al. 1990) claimed that increased contents of nitrogen in soil and in pine needles induced an apparently decreased frequency of mycorrhizal roots in the humus layer – from 80% to 50%. More detailed analyses (HEINSDORF 1991) revealed that an increased N-content in pine needles to 2.3% resulted in a decrease in the frequency of short mycorrhizal roots in the humus layer to 55–60%. Similarly, fertilization experiments shown that an application of 3 × 100 kg N/ha suppressed the frequency of mycorrhizas from 87% to 77% and the formation of fruiting bodies by more than 50% (RITTER 1990). Mycorrhizal fungi exhibit dissimilar reactions to the ammonium and nitrate forms of nitrogen. TERMOSHUIZEN et al. (1988) advocated an opinion that the development of mycorrhiza in pine was affected more favourably by the ammonium form of nitrogen than by the nitrate form of nitrogen. According to WALLANDER et al. (1990), N-fertilization affects first of all the vegetative mycelium with the number of mycorrhizas on the roots not showing marked changes.

Root systems of tree species can also be influenced by water shortage. LINDBERG and JOHANSSON (1992) consider the length of unsuberized roots that is in negative correlation with water potential to be a sensitive indicator of drought stress. Stress factors can also affect the functionality of fine roots, i.e. their capacity to take up nutrients. According to PERSSON et al. (1995), drought adversely affected the function of fine roots and impaired their capacity of nitrogen uptake. Drought also influences the mycorrhiza. According to KÖZLOWSKI (1971), water deficit restricts the formation of new roots that could be colonized by mycorrhizal fungi while not primarily influencing the fungus itself. Particular fungi exhibit different susceptibilities to water stress (LEHTO 1992). ANONYMOUS (1988) found out an increased development of mycorrhizas in the mineral soil under the influence of drought while the humus layer showed mainly their quiescent stages.

Provided that their development is not disturbed, tree species have a genetically conditioned tendency of maintaining the species-specific shoot to root ratio. The ratio can change under the influence of external environmental factors, i.e. also under the influence of drought or increased N-depositions. Drought would generally increase the root/shoot ratio in a range of plants (GREEN et al. 1994). In contrast, nitrogen is considered to be an element whose higher concentrations show an unfavourable effect on the root system including the mycorrhiza. A dispropor-

**MATERIAL AND METHODS**

**MATERIAL AND GENERAL SCHEME OF TRIAL ESTABLISHMENT**

Effects of increased nitrogen depositions and drought stress were studied on Scots pine (*Pinus sylvestris* L.) in two experimental series: in a Pot Trial that was focused on the study of responses of plants immediately after planting, and in a 12-year pine stand *in situ* (Stand). The drought stress was induced by reducing atmospheric precipitation by 60%. The increased nitrogen depositions were simulated by repeated applications of ammonium sulphate at a dose that corresponded to 100 kg N/ha per year. Variants established in both experimental series were as follows: Control, Drought, Nitrogen, Nitrogen + Drought. A detailed description of the establishment of the two experimental series and of the method of simulating the stress factors – see PALÁTOVÁ (2001).

**METHODOLOGICAL PROCEDURES OF PARTIAL ANALYSES**

**Biomass and vertical distribution of fine roots**

Fine roots are considered to be all roots with the diameter lesser than 1mm; this part of the root system ensures a greater deal of tree nutrition. Soil cores for fine root analyses were taken by a sampler (diameter 5 cm) from a depth of 30 cm. In the Pot Trial at all times 3 soil cores were obtained from a container and a minimum of 8 trees were analyzed in each variant (i.e. min. 24 soil cores). In the closed stand 336 A1 where regular rooting could be anticipated, soil cores were taken at a half distance between the neighbouring rows of trees with a minimum of 20 soil cores for each experimental variant. The soil cores from the Pot Trial were divided into layers 0–10 cm and 10–20 cm, the soil cores from the experimental Stand were divided into humus layer, humus-enriched layer and mineral layer. The reason for adopting different ways of soil core division was homogenized earth used in the Pot
Trial that made the differentiation of soil horizons impossible. Identical portions of soil cores were homogenized in the laboratory separately for the respective variants (fine roots being cut to sections of approx. 1 cm), and 8 samples of 100 ml bulk volume were taken from all resulting soil homogenates for the analyses.

At the separation of fine roots, the to-be-analyzed homogenate samples of respective soil layers were dispersed in Petri dishes with water and fine roots were picked up manually (with the help of painting brush, preparation needle and a pair of tweezers). Cleaned fine roots obtained from the to-be-analyzed samples of respective soil layers were desiccated at 70°C and weighed. The values were used to express total biomass and to assess vertical distribution of fine roots in the soil profile.

**Specific length of fine roots**

The washed fine roots obtained from the to-be-analyzed samples were measured for their length prior to desiccation, the measuring instrument being the Lucia G system of image analysis, version 3.51 ab, installed on a computer with the processor Pentium 90. Root segments obtained from the to-be-analyzed samples were arranged in a dish with water so that they did not create enclosed figures. The system was calibrated and images of individual samples made. The image was segmented by using software and the resulting pseudocoloured binary image was modified by means of a special sub-programme. After entering the mask, a measurement was made of the lengths of binary images of individual fragments with the series of length-expressing figures summed up in the Microsoft Excel spreadsheet. Each sample was measured three times at different parameters of image segmentation and the results of these measurements served to compute the arithmetic mean that was rounded to whole millimeters according to common practice. The use of the image analysis for measuring the length of fine roots was preceded by a check of the correctness of the results by comparing the values found out by measurements with values measured under the stereomagnifying glass and confirmation of repeatability of results (MAZAL 1997, unpublished).

The values of fine root lengths and weights were used to calculate specific length as a fine root length/fine root weight ratio.

**Mycorrhizal infection**

The method of quantitative glucosamine determination was used to measure mycorrhizal infection with the aim of finding out how the studied stresses affect the development of mycorrhizal fungi. The amount of fungal glucosamine in root samples was determined by acid hydrolysis of chitin of mycorrhizal fungi. The amount of fungal glucosamine in the soil profile was determined by acid hydrolysis of chitin of mycorrhizal fungi. The amount of fungal glucosamine in the soil profile was determined by acid hydrolysis of chitin of mycorrhizal fungi.

**Functionality of fine roots**

Regarding the fact that the longevity of fine roots and their mycorrhizal infection give no complex reply to the question of a possible effect of stresses on the functionality of fine roots and in order to obtain more exact information the functionality was also assessed on the basis of absorption of labelled phosphorus by the fine roots (LANGLOIS, FORTIN 1984). Four trees were subjected to the analysis in each variant of the Pot Trial and the Stand experimental series. Three fine roots long 15 cm at minimum were taken from all trees and immediately placed in portable coolers and transported to the laboratory for processing without delay.

**Volumes of plant parts**

Five plants from all variants in the Pot Trial were measured xylometrically towards the end of the 1997 growing season to detect the volume of the whole root system and that of the above-ground parts and their mutual ratios were calculated.

**Mathematical and statistical assessment**

The results of measurements were processed by common statistical methods. Significance of results was determined by t-test on the 95% level of significance (symbols in Tables: + significant variation; – insignificant variation).

**RESULTS**

Fine roots (i.e. roots with the diameter < 1 mm) represent a dynamic component of the root system that very quickly responds to changed conditions. The pines in both experimental series readily responded to the simulated stress factors from the first year of exposure. The trend of responses was identical to a greater extent in the two experimental series.

Total biomass of fine roots was reduced approximately by a half of the Control in the variant Drought in the Pot Trial after four years of exposure while the response in the experimental series Stand was not so significant (Table 1). Nitrogen depositions had a similar negative influence on the fine roots in both experimental series. The variant Nitrogen in the experimental series Pot Trial exhibited a significant reduction of fine root biomass as early as in the first year with the adverse effect increasing from the second year of exposure. An exception was the last year of research when the biomass of fine roots increased to 80% of the Control. Similarly, the experimental series Stand showed a significant reduction in the biomass of fine roots after the first year of simulated nitrogen depositions. The most severe impact was recorded by the combined stress (var. Nitrogen + Drought) which reduced fine root biomass in the Pot Trial after four years of exposure by 70% and in the experimental series Stand after two years by 31%. The 1997 situation (Pot Trial) is not included in the Table since the insufficient number of plants (due to their sudden dieback) did not make it possible to statistically verify the results.

Apart from the reduced amount of fine roots there were also variations in their distribution across the soil profile.
recorded in the course of the experiment, i.e. variations in vertical distribution (Table 2). The variants of Drought in both experimental series exhibited a gradual shift of fine roots into the upper soil layer.

All stress factors unfavourably affected the specific length of fine roots in the experimental series Pot Trial with the decrease being more pronounced in the variants with induced drought (var. Drought, var. Nitrogen + Drought). The simulated nitrogen depositions also reduced the specific length of fine roots. The response of plants to the stress factors in terms of the specific length of fine roots did not show an unambiguous trend in the experimental series Stand (Table 1).

Mycorrhiza plays an irreplaceable role in the uptake of water and nutrients. It was analyzed by using fine roots from all studied soil layers. The response of mycorrhiza was most pronounced in the Pot Trial in the soil layer 0–10 cm and in the humus layer in the experimental series Stand. Mycorrhizal infection in plants after setting out (i.e. in the Pot Trial) was affected negatively by all stress factors unfavourably affecting the specific length of fine roots. The response of plants to the stress factors in terms of the specific length of fine roots did not show an unambiguous trend in the experimental series Stand (Table 1).

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Table 1. Biomass, specific length and mycorrhizal infection of fine roots in experimental series Pot Trial and Stand

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Year</th>
<th>Pot Trial</th>
<th>Stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass of fine roots (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.0503 ± 0.0065</td>
<td>0.1550 ± 0.0118</td>
<td>0.2279 ± 0.0251</td>
</tr>
<tr>
<td>Drought</td>
<td>0.0525 ± 0.0064</td>
<td>0.0803 ± 0.0115</td>
<td>0.0970 ± 0.0038</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.0264 ± 0.0048</td>
<td>0.0961 ± 0.0028</td>
<td>0.1295 ± 0.0037</td>
</tr>
<tr>
<td>N + Drought</td>
<td>0.0460 ± 0.0055</td>
<td>0.0603 ± 0.0058</td>
<td>0.0749 ± 0.0042</td>
</tr>
</tbody>
</table>

Specific length of fine roots (mm/g)*

| Control             | not measured | 27.9 ± 2.1 | 33.3 ± 1.3 | 28.6 ± 1.1 | 23.5 ± 1.6 | 21.5 ± 1.3 | 21.5 ± 1.3 |
| Drought             | not measured | 22.4 ± 0.2 | 24.2 ± 0.9 | 25.3 ± 2.3 | 26.3 ± 1.9 | 19.9 ± 2.1 | 19.9 ± 2.1 |
| Nitrogen            | not measured | 25.0 ± 3.0 | 24.3 ± 0.9 | 23.1 ± 1.8 | 29.1 ± 3.7 | 21.6 ± 1.8 | 21.6 ± 1.8 |
| N + Drought         | not measured | 24.8 ± 4.0 | 18.7 ± 0.6 | not measured | 24.2 ± 2.6 | 23.6 ± 2.2 | 23.6 ± 2.2 |

Mycorrhizal infection (μg glucosamine . 100 mg/dry weight)*

| Control             | 25.43 ± 0.50 | 23.07 ± 0.49 | 25.58 ± 0.33 | 27.44 ± 0.59 | 20.03 ± 0.50 | 18.36 ± 0.57 |
| Drought             | 10.55 ± 0.61 | 18.04 ± 0.34 | 14.33 ± 0.40 | 14.09 ± 0.56 | 23.67 ± 0.64 | 16.11 ± 0.77 |
| Nitrogen            | 9.21 ± 0.49  | 18.66 ± 0.53 | 17.29 ± 0.53 | 22.37 ± 0.57 | 17.97 ± 0.62 | 17.12 ± 0.57 |
| N + Drought         | 6.98 ± 0.50  | 9.52 ± 0.41  | 6.68 ± 0.76  | not measured | 21.69 ± 0.86 | 16.62 ± 0.39 |

*Results are presented for the layer 0–10 cm in the experimental series Pot Trial, and for the Humus layer in the experimental series Stand.

Table 2. Vertical distribution of fine roots (Experimental series Pot Trial and Stand)

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Year</th>
<th>Layer (cm)</th>
<th>Pot Trial</th>
<th>Stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vertical distribution of fine roots in soil layers under study (%)</td>
<td></td>
<td>0–10</td>
<td>10–20</td>
<td>10–20</td>
</tr>
<tr>
<td>Control</td>
<td>0–10</td>
<td>37.2</td>
<td>30.7</td>
<td>38.6</td>
</tr>
<tr>
<td>Drought</td>
<td>0–10</td>
<td>35.4</td>
<td>45.3</td>
<td>57.7</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0–10</td>
<td>35.6</td>
<td>44.8</td>
<td>65.7</td>
</tr>
<tr>
<td>N + Drought</td>
<td>0–10</td>
<td>42.6</td>
<td>32.5</td>
<td>40.1</td>
</tr>
<tr>
<td>Control</td>
<td>10–20</td>
<td>62.8</td>
<td>69.3</td>
<td>61.4</td>
</tr>
<tr>
<td>Drought</td>
<td>10–20</td>
<td>64.6</td>
<td>54.7</td>
<td>42.3</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>10–20</td>
<td>64.4</td>
<td>55.2</td>
<td>34.3</td>
</tr>
<tr>
<td>N + Drought</td>
<td>10–20</td>
<td>57.4</td>
<td>67.5</td>
<td>59.9</td>
</tr>
<tr>
<td>Control</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Drought</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N + Drought</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Table 3. Functionality of fine roots in experimental series Pot Trial and Stand

<table>
<thead>
<tr>
<th>Experimental series</th>
<th>Pot Trial</th>
<th>Stand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.1837 ± 0.265</td>
<td>0.0592 ± 0.0021</td>
</tr>
<tr>
<td>Drought</td>
<td>0.0873 ± 0.0064+</td>
<td>0.0258 ± 0.0033+</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.1191 ± 0.0063+</td>
<td>0.3811 ± 0.0039+</td>
</tr>
<tr>
<td>N + Drought</td>
<td>0.0747 ± 0.0023+</td>
<td>0.0271 ± 0.0028+</td>
</tr>
</tbody>
</table>

Absorption of $^{32}$P (mg KH$_2$PO$_4$·1g/DM)

Table 4. Volumes of plant parts in the experimental series Pot Trial at the end of the experiment (1997)

<table>
<thead>
<tr>
<th>Volume of above-ground part (ml)</th>
<th>%</th>
<th>Volume of root system (ml)</th>
<th>%</th>
<th>Shoot:root ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>283 ± 44</td>
<td>100.0</td>
<td>130.5 ± 20.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Drought</td>
<td>137 ± 17+</td>
<td>48.4</td>
<td>34.8 ± 1.7+</td>
<td>26.7</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>342 ± 81+</td>
<td>120.8</td>
<td>91.0 ± 9.6+</td>
<td>69.7</td>
</tr>
<tr>
<td>N + Drought</td>
<td>67 ± 13+</td>
<td>23.7</td>
<td>24.3 ± 2.8+</td>
<td>18.6</td>
</tr>
</tbody>
</table>

DISCUSSION

Plants respond to natural or simulated stress factors by changes in their above-ground parts and root systems. While there is a range of easily detectable parameters to assess the response of the above-ground parts, the range of methods to measure the response of root systems is considerably lesser due to the enormous labour and time consumption of rhizological analyses. The most frequently used methods include measurements of fine root biomass, changes in the vertical distribution of fine roots, changes in the number of root tips and mycorrhizas, changes in the number of live and dead root tips, changes in the chemical composition of fine roots, changes in the uptake of nutrients measured by labelled elements, etc. (Clemensson-Linell 1994). Some of these methods were used in this study.

Fine roots in both experimental series responded to the induced stresses most intensively and most rapidly of all other plant parameters under study. Although the exposure to drought in the Pot Trial induced a slight increase in total biomass of fine roots (that was insignificant, though) in the first year of exposure, the number of fine roots continually decreased in the following years. Similarly, in the experimental series Stand, drought induced a reduction of the fine root biomass that was however less pronounced. The reduced production of fine roots under the influence of drought was found in spruce by Blanck et al. (1995) and the fact that the root system of spruce responds to drought was also confirmed by research results published by Beier et al. (1995), who detected a decreased production of fine roots even under the influence of a temporary short-term drought. Nitrogen is known to affect carbon allocation in the tree species. It results in a negative

Table 4. Volumes of plant parts in the experimental series Pot Trial at the end of the experiment (1997)
correlation between the nitrogen supply and the biomass of fine roots (VOGT et al. 1990), which was corroborated in our experiments. The pine trees in both experimental series responded to the nitrogen depositions by decreasing the total biomass of fine roots although the reduction was somewhat lower than that observed in response to drought. There is a number of authors who informed about the reduced development of fine roots under the influence of increased nitrogen depositions. The dependence was found both in spruce and in Scots pine – both in the experiments with simulated nitrogen depositions and after fertilization. TÖLLE (1969) recorded a reduction of fine roots by approx. 45% in a medium-aged pine stand as early as just several months after the application of 120 kg N/ha in the form of urea. The correlation computations repeatedly suggested a negative relation between the N-content in needles (HEINSDORF 1991) or the N-concentration in the upper soil layer (TÖLLE 1967; HOFMANN et al. 1990) and the amount of fine roots. KÖSS and MURACH (1996) made a comparison of pine stands under different stresses, studying the amount of fine roots into a depth of 40–50 cm. They found a higher number of roots with diameters < 2 mm in a locality less loaded with N-depositions. The roots in the low-loaded locality also reached greater maximum depths. The soil with lower N-depositions exhibited a much denser rooting with the number of live fine roots being by 50% higher. The variations occurred mainly in the humus horizon and in the upper mineral soil. The results of some authors (KRAUSS 1965; HEINSDORF 1976) document that the number of fine roots increased after the fertilization of pine plantations while it decreased in mature stands. Based on these findings TÖLLE and TÖLLE (1995) assume that the development of fine roots does not depend on the nutrition only but it is also affected by other factors such as stand age, stand density and soil vegetation. The results of our experimental series do not corroborate different effects on pines of different age. The strongest unfavourable influence on the fine root biomass was that of combined stress in both experimental series. In the Stand, the negative effect of the combined stress showed fast and fine roots were reduced by 31% as early as after the second year of exposure. The plants in the Pot Trial exhibited an even more expressive response with the number of fine roots in the last year of research, i.e. after four years of exposure to the combined stress, being reduced to 30% of the Control. (The value is not included in the Tables since the insufficient amount of plants surviving in this variant did not make it possible to exactly assess the statistical significance of results.) This was probably the main reason for a sudden dieback of 75% plants in the variant that exhibited poor bud bursting in the spring and began to dry off.

In addition to the reduced fine root biomass we also recorded a change in the distribution of fine roots across the soil profile, particularly under the influence of drought that induced a gradual displacement of fine roots into the upper soil layer. The simulated N-depositions did not induce any significant change in the vertical distribution, which is in good agreement with findings of MAIDI and PERSSSON (1995) in spruce.

As the fine roots play a crucial role in the uptake of nutrients and water by tree species having at the same time a number of other beneficial effects. The presence and condition of mycorrhizal symbiosis is usually assessed on the basis of the frequency of short mycorrhizal roots (HEINSDORF 1991), ultrastructure of mycorrhizas (BRUNNER, SCHEIDEGGER 1994; STROHMAYER 1992) or production of fruiting bodies (RITTER 1990; BRAND-RUD 1995). Mycorrhiza in our study was assessed by the method of chemical survey, i.e. determination of glucosamine (building element of chitin) as recommended by WALLANDER et al. (1990). In our experiments the mycorrhiza was unfavourably influenced by all simulated stresses. The consistingly low mycorrhizal infection found after the first year in all experimental variants of the Pot Trial could also be due to the fact that the plants were subjected to stresses just after rooting and apparently they did not produce enough carbohydrates available to symbiotic organisms. The mycorrhizal infection considerably increased in the following year but it did not reach the Control values in any of the stresses under study. Drought had an unfavourable effect on the mycorrhiza and reduced the mycorrhizal infection in the upper soil layer to about 50% of the Control with the reduction being less pronounced in deeper soil layers. In the experimental series Stand, drought was initially stimulating for the mycorrhiza both in the humus and in the nutrient-enriched layer. It does not correspond with the conclusions of ANONYMOUS (1988) according to whom the increasing drought stimulates the production of new mycorrhizas in the mineral soil while mycorrhizas in the humus layer that is first to be affected by drought pass into quiescence. In contrast to the conclusions of the above-mentioned author about the development of new mycorrhizas in the mineral soil being stimulated by drought, we did not record any increase although it apparently follows from our results that the mycorrhiza finds more favourable conditions for its existence in deeper soil layers. The mycorrhiza reduction
– although not so pronounced – was detected in drought-impacted spruce also by MAUER and PALÁTOVÁ (1996). There are frequent references in technical literature that superfluous nitrogen has an adverse effect on the mycorrhiza (HEINSDORF 1991; ARNEBRANT 1994; BRUNNER, SCHEIDEGGER 1994; HOLOPAINEN et al. 1996, and others). For example HEINSDORF (1987 in HOFMANN et al. 1990) found out an apparent decrease in the frequency of mycorrhizas in the humus layer from 80% to 55% after the soil nitrogen content increased. It was corroborated by our experiments in both experimental series. Although the pine trees on the stationary experimental plot Stand responded to the increased soil nitrogen content by increasing mycorrhizal infection in the nutrient-enriched and mineral layers in the first year, in the following year the mycorrhiza was affected in a negative way in the whole profile under study. Very low values of mycorrhizal infection were recorded in the variant with the combined stress in the Pot Trial. After the initial stimulation the same variant in the experimental series Stand showed no significant difference in the values of mycorrhizal infection from those in the Control. We assume that it is to be attributed to the fact that the measure of stress was apparently lower than that in the Pot Trial and that in the case of the “Stand” we can speak about a better stabilized ecosystem. MEYER (1962) informed that the mycorrhiza in soils with high microbial activity was protected against superfluous nitrogen better than in soils with low microbial activity. Biological activity of soil was measured in the experimental series Stand by a respirometric test (the results are not presented). We followed up a presumption that CO₂ amount and dynamics are in close correlation with the intensity of mineralization processes in the soil and with the general activity of soil micro-organisms. It was found out during our measurements carried out on the stationary research plot Stand that all stresses had a negative effect on soil respiration. In line with the conclusions arrived at by MEYER (1962), the suppressed microbial activity could be one of the reasons for the reduction of mycorrhiza in spite of the fact that the needle N-contents did not reach the critical level yet.

The impact of stress factors can also affect fine root functionality, i.e. the capacity of fine roots to take up nutrients. In our experiments we found a considerably decreased absorption of labelled phosphorus by roots from the variants with induced drought in both experimental series. Similar results were described by PENNEL et al. (1990 in CLEMENSSON-LINDELL, ASP 1994) in loblolly pine (Pinus taeda L.) and by CLEMENSSON-LINDELL and ASP (1994) in spruce. The effect of nitrogen depositions on the uptake of labelled phosphorus varied during the experiment. After the initial reduction, a suddenly increased uptake of labelled phosphorus by the fine roots was detected in the variants with simulated nitrogen depositions in the Pot Trial in the third year and in the experimental series Stand in the second year. The increased values in the Pot Trial decreased in the following year but the decrease was not large enough to reach the level detected in the previous years. As the experiment was brought to end, it is difficult to assume nowadays whether it was a trend or a deviation induced for example by different dates of sample collection because as demonstrated e.g. by LANGLOIS and FORTIN (1984) in Abies balsamea, the uptake has certain dynamics in the course of the growing season. Although the sampling was made in the autumn, it was unfeasible to observe precisely the identical month of analyses due to the supplier’s commitments elsewhere.

Under undisturbed development tree species have a genetically conditioned tendency to maintain the species-specific shoot to root ratio. This ratio can change under the influence of external environmental factors, i.e. drought or increased nitrogen depositions. In agreement with a number of literary data, in our Pot Trial nitrogen supported the development of the above-ground parts (their volume) while showing an unfavourable effect on the root system, which was reflected in a significant increase in the shoot/root ratio. Nevertheless, the rule that the more unfavourable the moisture conditions in the soil, the better the root system development (LYR et al. 1967; ZIMMERMANN, BROWN 1974) did not prove to be true. The root system volume was dramatically reduced in both variants with simulated drought.

Comparing the results obtained for pine with the results of similar experiments made in spruce (MAUER, PALÁTOVÁ 1996), we can claim that the responses of the two species to the studied stresses were similar although pine surprisingly appeared to be more sensitive in some aspects than spruce. Regarding the fact that the comparison of results obtained in situ in the stands could be affected by different climatic conditions (precipitation amounts in particular) in the respective years, by different ecotopes and even by the age of analyzed stands, we consider appropriate to make a comparison with the values recorded in pot trials established in a similar way where the plants of approximately the same age developed under comparable conditions. In spite of the fact that drought suppressed the height increment in both species, the reduction was greater in spruce but the combined stress was tolerated better by spruce than by pine. A similar difference could be seen in the response of the two species to increased nitrogen depositions. Spruce showed a positive response but no increased increment was recorded in pine. The needle length response to the drought stress was greater in spruce and pine responded to the combined stress more sensitively again. While 58% of spruce trees died under the influence of drought, the number of pines dying within a comparable time horizon amounted to 8% only. The combined stress was responsible for the dieback of only 46% spruce trees but the percentage of dead pines was 79%. A crucial difference was that spruce trees declined gradually when subjected to the combined stress, but pine exhibited a sudden dieback of 75% plants after 3 years of exposure to the stress. The reduction of fine root biomass was comparable in the two experimental series – with the exception of the combined stress that induced a more severe reduction of fine root biomass in pines. Mycorrhizal infection was suppressed considerably
more in pine than in spruce under the influence of all stress factors, the reduction being higher than a half under the combined stress. It is possible to suggest that while the negative effect of drought was eliminated by nitrogen in some cases in spruce, nitrogen enhanced the stress in pine. There arises a question why spruce – which is considered to be a water-demanding species – proved highly drought resistant while pine, which is considered a drought-resistant species, surprised by its high susceptibility to the combined stress in particular.

Similarly like birch or aspen, pine is taken for a pioneer species naturally colonizing nutrient-poor sites. Sites of better qualities are taken up by more competitive broad-leaved species – beech and oak (Ritter 1990). Pine is accommodated to the low supply of soil nutrients that are gained through mycorrhizal symbiosis. However, the mycorrhizal symbiosis is susceptible to increased nitrogen depositions that at the same time lead to the change in carbon allocation and manifest in the suppressed formation of fine roots. The different response of the two tree species can be caused by a higher reduction of mycorrhiza in pine. According to Hofmann et al. (1990), disturbance of mycorrhiza can also be reflected in an unfavourable influence on the health condition of pine stands because the protective function against pathogenic micro-organisms within the fine roots cannot show and the stands are infested by the honey fungus (Armillaria mellea [Vahl ex Fr.]). From this point of view, the reduction of mycorrhiza can represent another destabilizing factor for the pine stands. It also followed from our experiments that – similarly like spruce – pine would not respond to drought by increased development of fine roots. It is also evident that the very existence of the species can be severely threatened in localities with low water supplies and affected by high nitrogen depositions. Our experiments demonstrated that pine responded to drought by displacing the fine roots into the upper soil layers. Should we take into consideration also the fact that the growth of grasses (Anders 1996; Koss, Murach 1996) whose roots accumulate in the upper soil layer is extensive under the influence of nitrogen depositions, then the competition within this space and the high consumption of water by the dense grass cover can result in further impairment of water supply to pine trees and most probably even in their dieback.

CONCLUSIONS

Effects of drought stress, stress by increased nitrogen depositions and combined effect of the two stress factors on the growth of Scots pine (Pinus sylvestris L.) were studied in two experimental series in 1994–1997 with special emphasis on root system responses. The experimental series represented different age stages of the experimental material: plants immediately after setting out (Pot Trial) and a 12 years old stand (Stand). The drought stress was induced by reduction of atmospheric precipitation by 60%, the increased nitrogen depositions were simulated by repeated applications of ammonium sulphate at a dose corresponding to 100 kg N/ha per year.

The results of 4-year research in the Pot Trial and 2-year research in the experimental series Stand can be summarized into the following conclusions:

- Drought induced reduction of fine root biomass and at the same time displacement of fine roots into the upper soil layers, considerably decreasing functionality and mycorrhizal infection of fine roots.
- Simulated nitrogen depositions induced fine root biomass reduction and decreased mycorrhizal infection of fine roots. Functionality of fine roots was initially affected unfavourably but the effect was positive in the Pot Trial from the third year and in the experimental series Stand from the second year of exposure.
- Combined stress induced a conspicuous (and most severe) reduction of biomass, functionality and mycorrhizal infection of fine roots.
- The root system (fine roots in particular) responded to simulated stresses from the first year of exposure although the above-ground parts did not show any significant changes and were considerably more impacted than the above-ground parts of plants.
- Drought showed to be a stronger stress factor than single N-depositions in both experimental series in the period under study.
- The most severe effect was that of the combined stress factors.
- The negative effects of the stresses under study do not necessarily show trends increasing with the increasing time of exposure. Partial analyses revealed that the adverse effect was followed by certain regeneration that can be of short duration, however. Pine responds sensitively to all studied stress factors but mainly to the combined stress. Should its negative reaction be repeatedly recorded and proven, it will not be possible to recommend its planting in localities threatened with drought and increased nitrogen depositions.

References


BEIER C., GUINERSEN K., RASMUSSEN L., 1995. Experimental manipulation of water and nutrient input to a Norway spruce plantation at Klosterhede, Denmark. II. Effect of tree...
Vliv zvýšených depozic dusíku a stresu suchem na vývoj borovice lesní (*Pinus sylvestris* L.) – II. Reakce kořenového systému

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**ABSTRAKT:** V letech 1994 až 1997 byl sledován ve dvou experimentálních řadách vliv simulovaného stresu suchem, stresu zvýšených depozic dusíku a souběžného působení obou stresových faktorů na růst borovice lesní (*Pinus sylvestris* L.). Stres suchem byl vyvolán redukcí atmosférických srážek o 60 %, zvýšené depozice dusíku byly simulovány opakovaným dodáváním siranu amonného v dávce odpovídající 100 kg N/ha za rok. Všechny sledované stresové faktory ovlivnily biomassu jemných kořenů, vertikální distribuci, funkčnost a mykorhizní infekci jemných kořenů. Kořenový systém reagoval na simulované stresy již od prvního roku jejich působení a byl negativněji ovlivněn než nadzemní část (PALÁTOVÁ 2001).

**Klíčová slova:** *Pinus sylvestris* L.; dusík; sucho; biomasa jemných kořenů; vertikální distribuce jemných kořenů; funkčnost jemných kořenů; mykorhiza


Ve snaze o pokud možno komplexní posouzení reakce borovice na uvedené stresy byly hodnoceny parametry nadzemní části i kořenového systému. Práce navazuje na publikaci PALÁTOVÁ (2001), informující o reakcích nadzemní části, a shrnuje výsledky hodnocení jemných kořenů (kořenů o průměru menším než 1 mm, které zajišťují v převážné míře příjem živin a vody). Jemné
kořeny byly získávány z půdních výkrojů, odobíraných pomocí speciální sondy o průměru 5 cm do hloubky 20 cm, resp. 30 cm (Porost). V experimentální řadě Ná-dobový pokus byly výkroje děleny na vrstvy 0–10 a 10–20 cm, v experimentální řadě Porost na vrstvy humus, obohacená humusem a minerální. Z homogenátů příslušných vrstev byly získávány jemné kořeny a hodnocena jejich biomasa a vertikální distribuce, specifická délka (délka kořenů v mm/g sušiny), mykorhizní infekce (stanovením množství glukosaminu po kyselé hydrolýze chitinu), funkčnost (na základě přijmu značeného fosforu), objem nadzemní části a kořenů (xylometricky) a jejich vzájemný poměr.

Výsledky získané po čtyřech letech působení simulovaných stresových faktorů v Nádobovém pokusu a dvou letech působení v Porostu lze shrnout do následujících závěrů:

– Sucho vyvolalo snížení biomasy, mykorhizní infekce a funkčnosti jemných kořenů.
– Simulované deponice dusíku snížily biomassu a zvýšily funkčnost jemných kořenů.
– Souběžný stres výrazně snížil biomassu, mykorhizní infekci a funkčnost jemných kořenů.
– Kořenový systém byl vždy ovlivněn více a rychleji než nadzemní část.
– Rostliny po výsadbe reagovaly na stresy výrazněji.

Vzhledem k citlivé reakci borovice na sledované stresové faktory a zejména na souběžný stres nedoporučuje se její výsadba na lokality ohrožené suchem a zvýšenými deponicemi dusíku.

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