Dynamics of oak mycorrhizas

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ABSTRACT: A decline of macromycetes, especially of ectomycorrhizal species, has been described in the last decade in various parts of Europe. These changes are attributed to direct or indirect effects of air pollution. In Central Europe these changes correlate with visual damage of forest. Many ectomycorrhizal fungi seem to be very suitable bioindicators of the disturbance of forest eutrophic stability. Stages of this disturbance can be linked directly to particular phases of impoverishment of ectomycorrhizal mycocoenoses. These conclusions have been obtained from long-term research on permanent plots (1,000–2,500 m²) in spruce and oak forests in the Czech Republic, but it seems that they are valid generally. For the quantification and study of mycorrhizal activity a special method has been developed and applied. The analyses of mycorrhizas from the same plots in the period 2000–2002 indicate large mycorrhizal dynamics. These data correlate with data obtained from a fruiting bodies survey. Both the percentage of ectomycorrhizal species and the ratio of active mycorrhizas are highly sensitive to outer impacts (air pollution, acidification, fertilization). Their decrease is in correlation with the strong defoliation of trees and can be used for the prediction of further development in comparable stands.

Keywords: ectomycorrhizas; ectomycorrhizal fungi; Quercus – oak; root; forest stability

In the last decades a large decline of the tree health status has been observed in Europe. Different studies did not indicate any simple reason for it. Direct impacts of pathogens and poisoning agents are combined with probably more important dysfunction of the nutritional transfer process. In general, it is a synergic effect of many factors (Thomas et al. 2002) but their particular involvement cannot be clearly detected and their individual role is still discussed. Among others, great attention is focused on the study of root systems and the role of symbiotic fungi.

Mycorrhizal associations

Mycorrhizas are specific forms of symbiosis between plants and fungi. This phenomenon often described as mycorrhizal infection is in fact a complex process. It is known from more than 95% of plant species. Mycorrhizal associations are less often among water plants and they were not detected on many species from ruderal and boggy stands.

Experiments proved that plants with appropriate levels of mycorrhizas revealed increased uptake of nutrients (mainly phosphorus and nitrogen) especially in situations when these elements were present in low concentrations or in an insoluble form. Another important effect is connected with their ability to concentrate in their body mineral nutrients that are slowly released and delivered to the symbiotic plant in a period of insufficiency. Reciprocal transport of organic compounds such as monosaccharides ensures a bilateral advantageous effect.

There are three main types of mycorrhizas from the morphological point of view: ectomycorrhizas, endomycorrhizas and ectendomycorrhizas (Rospal et al. 2003; Peterson et al. 2004).

Ectomycorrhizas

Roots of woody plants from the temperate zone form symbioses with species-specific mushrooms. The majority of ectomycorrhizal (ECM) roots reveals a characteristic anatomic structure. Short ECM

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roots lack root hairs that are characteristic of non-infected roots or roots with endomycorrhizas. They develop predominantly in upper soil layers with high percentage of raw humus.

The surface of roots infected by mycorrhizal fungi is changing being covered by the hyphal mantle. The growth of roots is retarded and the dichotomous branching of root tips is giving them a distinct shape. Hyphae from the mantle often extend to the soil forming here extraradical mycelium and rhizomorphs. In the body of the root, hyphae form Hartig net – a network between root cells. Hartig net is involved in nutrient exchange that was proved by autoradiographic analysis (e.g. Bücking, Heyser 2001). In the period of maximum extent the contact area between host and mushroom hyphae is quite a large interface. The lifespan of ECM roots is dependent on many internal and external factors. The expected maximum lifespan is two years.

Until now, mycorrhizal associations have been identified in about 2,000 plant species. They play an important role in major natural ecosystems, especially in forests as all important woody species (spruce, pine, oak, beech, birch) are characterized by high mycorrhizal activity. It is supposed that about 2,000 species of fungi, mostly Basidiomycetes, can form these associations.

**Endomycorrhizas**

Endomycorrhizal infections do not produce an obvious change. They are not evident with the naked eye. In this case hyphae form the extraradical mycelium and they also grow not only in intercellular areas but also within root cells. Roots are not altered having the normal surface, structure and root hairs. Finely branched intraradical hyphae constitute “arbuscules” responsible for nutrient exchange and enlarged “vesicles” with storage function (Mešťšík 1988).

Endomycorrhizas are known from about 1,000 plant genera of 200 families but it is supposed that this association is important for much more, perhaps about 300,000 species. On the other hand, the number of involved fungi is very low. These fungi belong to Zygomycetes (Rosypal et al. 2003).

**Ectendomycorrhizas**

Ectendomycorrhizas resemble ectomycorrhizas. They have the mantle and Hartig net but after Hartig net formation, intracellular hyphae develop into epidermal and cortical cells. They are formed between a limited number of ascomycetous fungi and the conifer genera Pinus and Larix.

**Reasons for decline of forests**

According to a majority of authors, the decline of forests in Central Europe is caused by the synergic influence of abiotic and biotic factors. A list of abiotic stress factors comprises: repeated dry periods, limited precipitation in sensitive periods and in general non-optimal distribution of rains, deep winter frosts in some years and mild winters with lack of necessary extent of dormancy, winters without snow cover and fast dramatic changes of weather. Important factors are also long-term changes of site conditions connected with climatic changes (fast water drain and deficit of soil humidity, decrease of water level), anthropogenic changes (emissions followed by acidification, soil wash out processes, deposition of toxic emissions and general changes of soil chemistry) or direct anthropic influence (direct devastation, false husbandry strategy or ecologically incorrect decisions). Other factors are connected with high density of game animals followed by great winter damage by grazing and deer barking with subsequent increase in fungi infections or insect infestations. Trees under attack much more likely reduce the growth of roots and thus mycorrhizal associations are also failing (Fellner, Pešková 1995).

**MATERIAL AND METHODS**

**Dynamics of mycorrhizas on oak study plots**

**Sampling strategy**

Fine roots are concentrated in the depth of 8–30 cm, mainly close to litter and humus horizons. Marks et al. (1967) and Alexander (1985) calculated the optimum volume for borehole samples. The diameter of about 4 cm is enough when only roots smaller than 2 mm are significant. The sample size used in different studies fluctuates from 1.2 to 10 cm of diameter, and sampling depth from 7.5 to 90 cm. Larger samples can contain a more representative composition, but the labour costs of root preparation and analyses grow significantly. We use the cylinder soil probe 6 cm in diameter and 15 cm in height. This seems to be the optimum between sample size and acceptable time for root evaluation. Five samples were taken from each study plot in order to prevent accidental deviations. They are scattered not randomly, but regularly at the distance of 1 m from selected trees. With standard sampling strategy (Pešková 2000) we obtained in total 210 samples.

In the early 90ties, a standard quantification method was developed for comparative studies of mycorrhizas (Pešková 2000). From soil samples...
taken constantly by the soil probe (400 cm$^3$) all root fragments were extracted and, besides others, their number, length and mycorrhizas were surveyed. Evaluation of ectomycorrhizal infection was repeatedly performed on selected study plots together with scoring of the health status of trees (defoliation) and quantification of all fungi fructifications (Macromycetes). Chosen plots represent different ecological environments. Sampling was performed in the period of mycorrhizal growth maximum (spring, autumn).

**Preparation of root samples and their evaluation**

From each probe all roots were separated manually, the total remaining soil was washed out. All roots were classified into four root classes according to root diameters (to 1 mm, 1–2 mm, 2–5 mm and +5 mm), and for detailed analyses only the thinnest roots (to 1 mm of diameter) were stored in the fixation solution (glutaraldehyde). The root dry matter and the total (cumulated) length were measured for roots from all four classes.

**Evaluation of mycorrhizal infection**

The main analyzed factors were absolute and relative numbers of active and non-active mycorrhizal tips. Mycorrhizas were evaluated within the root fraction under 1 mm. We applied the modified method of Jakuc et al. (1986) using 20 root sections of 5 cm in length. The level of mycorrhizal infection was evaluated using two parameters: density and percentage of mycorrhizal tips. The density of active and non-active mycorrhizas was counted as an average value of the number of mycorrhizal tips connected with 1 cm of root. The percentage proportion of mycorrhizas was calculated as a ratio of active and non-active mycorrhizal tips (Vogt et al. 1983; Caisová 1994).

<table>
<thead>
<tr>
<th>Table 1. Short characteristic of oak study plots</th>
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</thead>
<tbody>
<tr>
<td>Working-plan area</td>
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<tr>
<td>Working-plan area</td>
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<tr>
<td>Organ. unit</td>
</tr>
<tr>
<td>Stand</td>
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<tr>
<td>Forest region</td>
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<tr>
<td>Forest type</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Stand density</td>
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<tr>
<td>Oak proportion (%)</td>
</tr>
<tr>
<td>Height a.s.l. (m)</td>
</tr>
<tr>
<td>Location</td>
</tr>
</tbody>
</table>

**Fig. 1. Relative quantity of active mycorrhizas on oak roots between 2000 and 2002**
RESULTS

Comparison of root development and mycorrhizas in 2000–2002

Comparisons of the main studied parameters, i.e. densities and relative numbers of active (AM) and non-active (NM) mycorrhizas, show large differences from year to year. AM densities and percentages were increasing gradually and the values of NM decreased correspondingly between 2000 and 2002. Average values of dry roots (below 1 mm) were constant (Fig. 5).

The following comparison and statistical tests of AM percentages and densities were performed in the statistic package Kwikstat.

**Table 2. Sampling for root mycorrhizal analyses**

<table>
<thead>
<tr>
<th>Plot</th>
<th>Sample</th>
<th>Tree No</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dřevíč 1</td>
<td>1–5</td>
<td>2, 38, 26, 15, 10</td>
<td>04/05/2000 06/10/2000 25/04/2001 16/10/2001 30/04/2002 15/10/2002</td>
</tr>
<tr>
<td></td>
<td>1a–5a</td>
<td>2, 38, 26, 15, 10</td>
<td>04/05/2000 06/10/2000 25/04/2001 16/10/2001 30/04/2002 15/10/2002</td>
</tr>
<tr>
<td></td>
<td>1b–5b</td>
<td>2, 38, 26, 15, 10</td>
<td>04/05/2000 06/10/2000 25/04/2001 16/10/2001 30/04/2002 15/10/2002</td>
</tr>
<tr>
<td></td>
<td>1c–5c</td>
<td>2, 38, 26, 15, 10</td>
<td>04/05/2000 06/10/2000 25/04/2001 16/10/2001 30/04/2002 15/10/2002</td>
</tr>
<tr>
<td>Postoloprty</td>
<td>1–5</td>
<td>1, 7, 28, 33, 49</td>
<td>16/06/2000 11/10/2000 16/05/2001 16/10/2001 15/05/2002 15/10/2002</td>
</tr>
<tr>
<td>Třebotov</td>
<td>1–5</td>
<td>1, 12, 18, 21, 34</td>
<td>07/06/2000 06/10/2000 11/05/2001 10/10/2001 14/10/2002 14/10/2002</td>
</tr>
</tbody>
</table>

It seems that convenient weather conditions, i.e. higher temperatures and more rains, were positively correlated with all mycorrhizal parameters. There was an increase in AM percentages on all oak study plots except for Postoloprty in 2002 (Figs. 1 and 2) and also an increase in AM densities at all sites (Figs. 3 and 4). Average AM calculated from all study plots were increasing gradually and the values of AM were constant (Fig. 5).
2000: mean = 0.405  s. d. = 0.1828313  n = 12
2001: mean = 0.7091667  s. d. = 0.228173  n = 12
2002: mean = 1.059167  s. d. = 0.4714091  n = 12

Analysis of variance:

<table>
<thead>
<tr>
<th>Source</th>
<th>S.S.</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
<th>Appx P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>5.96</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td>2.57</td>
<td>2</td>
<td>1.29</td>
<td>12.54</td>
<td>0.000</td>
</tr>
<tr>
<td>Error</td>
<td>3.38</td>
<td>33</td>
<td>0.10</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minimum</th>
<th>25th %tile</th>
<th>Median</th>
<th>75th %tile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.18</td>
<td>0.27</td>
<td>0.32</td>
<td>0.61</td>
</tr>
<tr>
<td>1</td>
<td>0.42</td>
<td>0.53</td>
<td>0.66</td>
<td>0.89</td>
</tr>
<tr>
<td>2</td>
<td>0.39</td>
<td>0.58</td>
<td>1.13</td>
<td>1.48</td>
</tr>
</tbody>
</table>

We found statistically significant differences between years in percentage and also in densities of active mycorrhizas.

Comparison of macromycete diversity based on their fructifications observed between 2000 and 2002.

The macromycete maximum was found on plot Dřevíč 1 (128 species) and Dřevíč 2 (126 species). The plot of Třebotov was also very rich (107 spe-
partly expected results and also indicated some interesting trends. For example, we confirmed the observation of Jansen (1991) from Douglas fir stands in the Netherlands that the proportion of active mycorrhizal tips was positively, and mostly very closely, correlated with the proportion of mycorrhizal species, and negatively correlated with the proportion of trees with a severe crown decline. However, this observation for beech and oak stands is valid only for trees where crown defoliation exceeds 60% and mostly only in stands with a similar density of mycorrhizas. Similar data were obtained from spruce stands (Fellner, Soukup 1994). The conditions, i.e. similar density of mycorrhizas and severe defoliation seem to be very important factors. Some contradictory results, e.g. Causin et al. (1994), could perhaps be explained by differences between compared stands.

Evaluation of defoliation between 2000 and 2003

The health status of oak (Q. petraea and Q. robur) forests on study plots was evaluated using a standard scoring method. In this period we observed improvement of defoliation (Fig. 6). This amelioration is most probably a result of very convenient weather conditions with high level of precipitation in 2001 and 2002.

DISCUSSION

Our research on mycorrhizas in broad-leaved and coniferous forest on permanent plots produced cies). Other study plots like Březka 1, Březka 2 and Postoloprty reveal low diversity with 84, 54 and 56, respectively. We observed a gradual increase in the species number.
Our data on the dry matter of roots indicates that the finest roots, i.e. those < 1 mm in diameter, are generally the most sensitive to air pollution, fertilization or other external impacts (Fellner 1993). However, the results from our plots are not clear-cut, and only indicate trends. Analyses of data from individual trees may give a clearer picture than data from entire plots.

The fluctuation of the level of mycorrhizal infection reveals a clear correlation between the tree health status and absolute or relative numbers of active mycorrhizas. A conspicuous decline of macromycetes, especially in ectomycorrhizal species, that was described in our study (Fellner, Pešková 1995) and also in the last decade in various parts of the temperate zone of Europe can be attributed to direct or indirect effect of air pollution. As a collateral effect it has an impact on nutrient budgets of oak forests. This can be documented by worse development of mycorrhizas, roots (lower biomass) or finally also by a defoliation of trees.

In view of the fact that in Central Europe these changes preceded the visual damage of forest or even its decline, many ectomycorrhizal fungi seems to be very suitable bioindicators of the disturbance of forest ectotrophic stability. Defined stages of this disturbance can be linked directly to particular phases of impoverishment of ectomycorrhizal mycocoenoses and of enrichment of lignicolous mycocoenoses. These conclusions have been obtained from long-term research on permanent plots 1,000–2,500 m² in spruce and recently also in oak forests in the Czech Republic, but it seems that they are valid generally, at least in temperate forests. The analyses of true mycorrhizas from the same plots confirmed data obtained from fruiting bodies. Both the percentage of ectomycorrhizal species and the ratio of active mycorrhizas are highly sensitive to some outer impacts on forest ecosystems (air pollution, acidification, fertilization). Their decrease is in negative correlation with the strong defoliation of trees and can be used for the prediction of further development in the locality, at least in comparable stands, i.e. in the case of similar density of active mycorrhizas, and water stress.

**CONCLUSIONS**

Despite of the fact that data from individual samples can reveal a relatively large variability, the total values are repeatable and they reliably reflect the parameters tested. Changes of mycorrhizal activity observed on oak plots can be described as follows:

- Between the years 2000 and 2002 we observed an evident increase in active mycorrhizas and a corresponding decrease in inactive ones.
- Gradual increase in active mycorrhizas correlates with an improvement of tree health status measured as grade of their defoliation.
- Between the years 2000 and 2002 in the sum of all macromycete fructifications the percentage of mycorrhizal mushrooms increased correspondingly.
- Dry matter of the root fraction to 1 mm in diameter was roughly constant in the studied period.

The results show that there is a link between the quantity of mycorrhizal infection and the health status of trees. It also seems that many ectomycorrhizal fungi (evaluated in their phase of fructification) can be suitable bioindicators of forest ectotrophic situation. The decline of ectomycorrhizal species can
indicate disturbances and subsequently decline of oak forests.

The evaluations of various root and mycorrhizal parameters indicate that the density of mycorrhizal tips is affected by the long-term quality of local soil conditions while the proportion of active mycorrhizal tips is a more sensitive indicator of present factors such as drought stress, air pollution, use of fertilizers, etc. Despite of these general correlations it is not yet completely known what factors directly influence the mycorrhizas and how they affect their development.

References


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Dynamika dubových mykorhiz

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ABSTRAKT: V práci jsou uvedeny výsledky výzkumu ze šesti trvalých zkusných dubových ploch v ČR (Březka 1 + 2, Dřevíč 1 + 2, Postoloprty, Třebotov). Na vybraných plochách byly provedeny odběry kořenů a mykorhiz, hodnocen zdravotní stav dubů, sbírany plodnice makromycet. Z výsledků rozborů kořenů a mykorhiz je od r. 2000 do r. 2002 patrný zřetelný nárůst aktivních mykorhiz
V posledních desetiletích došlo v Evropě ke zhoršení zdravotního stavu lesních porostů. Příčiny nelze jednoznačně určit. Vedle přímého působení patogenních organismů a znečištění prostředí má hlavní úlohu i narušení nutričního mechanismu. Velká pozornost je v současnosti věnována výzkumu kořenových systémů a funkci symbiotických hub.

Ve druhé polovině osmdesátých let publikovali v Maďarsku výsledky mnohaletých výzkumů o eko logických souvislostech odumírání dubů v Maďarsku. Při interpretaci svých závěrů zvažovali vlivy nej různějších činitelů, které mohou vést k poškození lesních porostů, i nejrůznější projevy negativních změn, které lze v poškozovaných lesních ekosysté mech zjistit. Scénář základních příčinných vztahů v tomto složitém procesu degradace lesních stano vivií vychází z klíčové úlohy vzdušného znečištění, vedoucí k zániku mykorhizních hub, což ve svých důsledcích navozuje procesy odumírání kořínků a inhibici mykorhiz.

Mykorhizní houby mohou sloužit jako bioindikátory narušení ektotropní stability lesa. Dosavadní výsledky výzkumu ukazují na diagnostic ký význam stanovení procentuálního podílu mykorhizních druhů makromycet vzhledem k nemy korhizním druhům.

Studium biomasy jemných kořenů je důležitým parametrem pro poznání dynamiky lesních ekosystémů. Dynamika rozvoje jemných kořenových systémů a ek tomykorhiz je rízena jednak vnitřními faktory dřeviny, podmínkami půdního prostředí (dostupností vody, aciditou, dostupností minerálních látek, obsahem org anické hmoty v půdě atd.) a povětrnostními vlivy.

Během výzkumu mykorhiz na dubových plochách bylo kořenovou sondou v letech 2000–2002 odebíráno pět vzorků, celkem bylo získáno 210 vzorků kořenů a mykorhiz. Odběry kořenů a mykorhiz byly realizovány v období růstového maxima mykorhiz (na jaře a na podzim), a to vždy na stejném místě.

Úroveň mykorhizních poměrů byla hodnocena s využitím dvou parametrů: hustota mykorhizních špiček a jejich procentuální podíl. Hustota aktivních a neaktivních mykorhiz je počítána jako průměrná hodnota zjištěného počtu mykorhiz vztažená na 1 cm délky kořene. Procentuální podíl mykorhiz je kalkulován jako poměr aktivních a neaktivních mykorhiz.


Výzkum přinesl tyto výsledky:
– Celkově je od r. 2000 do r. 2002 patrný zřetelný nárůst aktivních mykorhiz a naopak pokles neaktivních mykorhiz.
– Postupný nárůst aktivních mykorhiz koresponduje s pozvolným zlepšováním zdravotního stavu (hodnoceným především stupněm defoliace).
– Srovnání údajů procentuálního zastoupení mykorhizních hub na sledovaných dubových plochách v r. 2000–2002 ukazuje zvyšující se podíl mykorhizních hub v celkovém druhovém spektru makromycet.
– Výsledky analýzy sušiny kořenů v r. 2000–2002 vykazují celkem vyrovnané hodnoty, bereme-li v úvahu nejcitlivější frakci do 1 mm.

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