Vitality of Norway spruce fine roots in stands infected by *Heterobasidion annosum*

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Abstract

Normally, infection by *Heterobasidion annosum* does not affect the fine roots of Norway spruce. Thus, mycorrhizas may be found with rot-affected conifers. The objective of the given study was to compare the morphological indices and mycorrhization of fine roots for rot-infected and healthy Norway spruce trees. The root samples were collected on 14 plots. In 6 of the plots *H. annsoum* was established. The plots were either on mineral soils or peaty soils.

The major morphological indices of fine roots (such as root length, volume, number of root tips) were found to be substantially higher (α=0,05) for the plots with only healthy Norway spruce trees. Twisted, irregularly thickened mycorrhizas of bunch-like distribution were dominant for the plots with *H. annosum* infected Norway spruce trees.

Introduction

In Latvia, a considerable proportion of Norway spruce [*Picea abies* (L) Karsten] stands suffer from root rot. It has been found that in 60–130 year-old Norway spruce stands of the Dm *Hylocomiosa* and Vr *Oxalidosa* site type the proportion of stems with rot may exceed 80 % (Šica, Huhna, unpublished data). Mycorrhiza (symbiotic association between roots and fungi) is known to enhance the vitality of woody plants, and also enhance their resistance to various diseases (Schönhar 1990). However, a number of researchers believe that rot-suffering conifers may also show healthy, well-developed mycorrhizas. The objective of the present study was to find out how *Heterobasidion annosum* (Fr.) Bres. s. lat. affects the root mycorrhization in Norway spruce and to compare the vitality and morphological indices of fine roots between healthy and *H. annosum* infected Norway spruce stands.

Material and methods

Sample plots

The experimental material was collected in the forest districts of Kandava, Mūsa, Smiltene, Cesvaine, and Madona, and also in the forests of the Forest Research Station (FRS) (Kalsnava and Škēde) as well as in the Trei Forest District of the Riga Forest Agency (Fig. 1).

Altogether 14 stands were now inventoried, of which 6 were characterized by the occurrence of root rot. The sites under study were arbitrarily divided into two groups: Norway spruce stands on mineral soils and spruce stands on peaty soils. The stands on mineral soils represented the following forest site types: As *Myrtillosa mel*. (6 sites); Dm *Hylocomiosa* (4 sites); Kp *Oxalidosa* turf. Mel. (4 sites). The age of the Norway spruce stands studied was 44–96 years.

Fig. 1. Location of sample plots. Healthy stands (sircles), and H. annosum *infected stands (squares).*

Field work

In stands with rot the presence of infection was determined following the availability of macroscopic traits: fungal fruit bodies; rotten stems fallen down; thinning of tree crowns, etc. In clear-cut areas, the presence of rot was determined by inspecting the stumps for patches of rotten wood.

On each sample plot some 10–20 samples of wood containing rot-causing agents were collected by using a sterile Pressler's borer with the sample taken at the height of root collar. The samples were placed in sterile test tubes and taken to the laboratory for storage in refrigerator until further processing. In stands with rot samples of fruit bodies of *H. annosum* were also collected and taken to the laboratory and kept in paper envelopes at the room temperature.

To describe soil horizons and to collect soil samples for chemical analyses a trench revealing the soil profile was dug on each sample site. The chemical analyses were done at the Soil Laboratory of the Latvian Forest Research Institute «Silava». Larger soil samples (20×10×10 cm) were also taken to obtain the material for identifying the dominant mycorrhiza types (Agerer 1987–1991). The root samples were collected next to spruce stems, using a four millimetre high and 100-cm^3-sized metallic cylinder. On each sample plot 25 root samples were taken. The samples around 3–4 stems were taken at random from the topsoil layer within the tree crown projection. For identifying the mycorrhiza species the root samples were fixed in ethyl alcohol.

Laboratory work

At the laboratory the root samples were carefully rinsed. The typological structure of mycorrhiza (mainly the colour) and the vitality (using 5 vitality classes) were studied by using the Leica MZ-7.5 microscope (magnification 6.5–50×). Then the root samples were scanned by calibrated scanner STD-1600+, using the software Win RHIZO 2002 C (Regent instrument^{R}). Scanning was done with the resolution ability 500 dpi [Standard 8 bit; grey tones (256)]. Fourteen classes were introduced for comparing the root diameter: 0–0.1 mm; 0.1–0.2 mm; 0.2–0.3 mm; 0.3–0.4 mm; 0.4–0.5 mm; 0.5–0.6 mm; 0.6–0.8 mm; 0.8–1.0 mm; 1.0–1.2 mm; 1.2–1.6 mm; 1.6–1.8 mm; 1.8–2.2 mm; 2.2–2.6 mm; and >2.6 mm. Win RHIZO 2002 C was employed for the mathematical processing of scanned images. For further processing the data were transferred to the MS Excel, using XL RHIZO V2003a; t-criterion and analysis of variance were used for data treatment.

Five vitality classes were used to describe root vitality:

I Mycorrhizas well developed and show typical ramification; the root bark is sound.
II Mycorrhizas slightly damaged; mycorrhiza frequency is lower.
III Damaged mycorrhizas found; twisted mycorrhizas having mantle of no uniform thickness predominate.
IV Mycorrhizas heavily damaged; living mycorrhizas rare.
V Fine roots heavily damaged; no living mycorrhizas are found.

Results and discussion

Assessment of root morphological indices

The mean length of roots of healthy spruce trees growing on mineral soils was 238.5±12.8 cm, while for trees in rot-infected stands this length was111.7+7.5 cm. (Table 1). According to the analysis of variance these differences were significant (Table 2).

Table 1. Mean values of the root parameters examined in Norway spruce stands.

Root length, cm	Root volume, cm^3	Number of root tips	Root weight, g
Healthy trees on mineral soils			
238.5±12.8	0.55±0.03	1392±84	0.21±0.11
Trees with rot on mineral soils			
111.7±7.5	0.33±0.02	685±52	0.12±0.009
Healthy trees on peaty soils			
228.0±15.6	0.43±0.03	1331±108	0.16±0.01
Trees with rot on peaty soils			
87.4±20.5	0.12±0.03	536±134	0.05±0.01

Table 2. Analysis of variance: the impact of the *H. annosum* infection on root lenght

Variance	Sum of deviation squares	Degrees of freedom	Mean square	F	P
Factor	1064268.4	1	16458	64.66	< 0.0001
Residual	4377776.4	266			
Total	5442044.8	267			

The impact of the factor is described by η=l9.6 %. Thus, a considerable proportion of the factor under analysis, i. e. the differences in root length for healthy and rot-infected stands, remains unexplained. These differences may be attributed to soil heterogeneity, i. e. the impact of diverse biotic and abiotic factors on root development. The root volume and root weight, too, showed higher values for healthy spruces, and these differences were highly significant (P<0.0001). The number of root tips, which to a great extent characterizes the total number of mycorrhizas, is a significant indicator for the vitality of fine roots. In healthy trees (n=149) the average number of root tips was 1392+84, while 685±52 root tips were scored in diseased trees (n=l19).

When examining root length in the different root diameter classes (Fig. 2), it was found that for the diameter classes in the range 0.10–0.20 mm -0.30–0.40 mm, which represent typical mean diameters for mycorrhizal roots, the differences in root length between healthy and diseased trees were significant (P<0001).

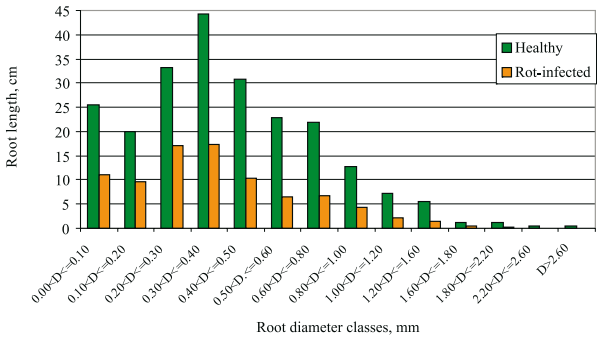

Fig. 2. Distribution of roots into diameter classes (samples from mineral soils).

For the samples originating from peaty soils, too, indices such as the mean root length, root volume, the number of root tips, and the root weight were significantly higher for healthy than for diseased trees. For healthy trees the number of root tips was 1331±108, while in diseased trees 536+134 were scored on average (P=0.001). Also for the other parameters significantly higher values were obtained in healthy trees than in diseased trees (P < 0.0001).

When comparing the distribution of root length within different root diameter classes for peaty soils (Fig. 3), it

was found that, similarly as in mineral soils, the root length up to the diameter class 1.80–2.20 mm was significantly higher for the samples coming from healthy stands than for diseased stands.

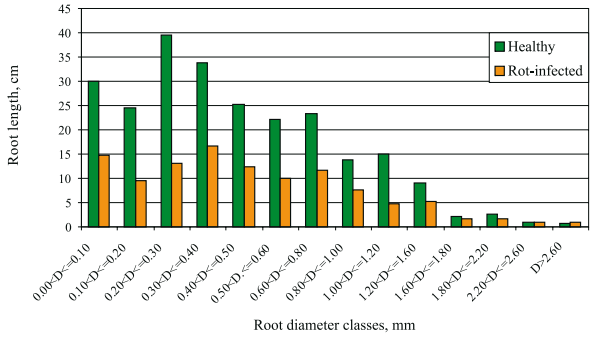

Fig. 3. Distribution of roots into diameter classes (samples from peaty soils).

Comparison of mycorrhiza typological structure and vitality between H. annosum *infected and healthy spruce stands*

Root vitality and the frequency of mycorrhiza types were compared for the samples analysed (Table 3). The mycorrhiza vitality for diseased trees in mineral soils was described by the coefficient 3.2, with this indicator for healthy trees being 2.9 (a lower value of the coefficient points to a higher percent of roots of higher vitality classes). For healthy and diseased stands on mineral soils it was difficult to identify the dominant mycorrhiza types. On Sample Plot 6 with diseased trees light-coloured mycorrhizas (*Piceirhiza* sp.) were found in 50 % of the samples. However, it is probably due to the presence of grey alder and other deciduous trees in the stand.

Table 3. Mycorrhiza frequency (%) and vitality for the root samples analysed (average of 25 samples)

Sample plots	Mycorrhiza type								
	Light-coloured	**Dark**	**Light yellow**	***C.geophilum***	**With external hyphae**	***A.byssoides***	***P.involutus***	***Piceirhiza sp.***	**Vitality**
Healthy trees on mineral soils									
1	12.5	50	8	50	46	46	-	-	2.6
2	76	12	4	-	20	12	-	-	3.1
3	32	-	-	8	48	36	4	4	3.0
4	38	11.5	11.5	58	-	15	8	8	3.0
5	64	16	-	92	64	-	32	32	3.0
Diseased on mineral soils									
6	21	12.5	50	21	42	33	-	-	2.9
7	4	39	39	4	4	4	-	-	3.0
8	54	8	12.5	7.5	-	25	8	21	3.0
9	58	4	-	71	-	5	-	17	3.6
10	20	20	4	-	-	28	-	8	3.6
Healthy trees on peaty soils									
11	16	80	16	40	-	64	-	-	2.3
12	72	-	16	3.0	16	-	12	36	2.9
13	8	-	-	-	-	27	23	19	3.0
Diseased trees on peaty soils									
14	5	11.5	-	-	-	5	21	21	3.3

When comparing soils with a higher proportion of mineral fraction (sample plots 1, 4, 5 compared with sample plots 6, 7, 8) more *Cenococcum geophilum* Fr. was found on the roots of healthy spruce trees than on diseased ones. For healthy trees the mycorrhizal fungus *Paxillus involutus* (Batsch.) Fr. was found in 3 out of 5 sample plots, while for diseased trees on one plot only out of 5 plots. As already mentioned, for diseased trees on peaty soils the material is insufficient for assessing differences between diseased and healthy trees.

Mycorrhiza ramification and morphological traits are also essential for characterising the mycorrhiza vitality. Mycorrhizas showing external hyphae and rhizomorphs were quite often associated with healthy spruce. The mycorrhizal fungi *Amphinema byssoides* (Pers.) J. Erikss., *Piceirhiza* sp., *Cortinarius* sp. and *Piloderma* sp. were also found quite frequently. Clusters of dark (predominantly *Piceirhiza* sp.) and light-brown mycorrhizas were also encountered.

Mycorrhiza ramification and distribution are regarded as typical for the respective species. The mycorrhiza on the roots of diseased spruce showed bunch-like projections and also a lot of damaged mycorrhizas, protruded, twisted and atypically swelled. Meyer (1985) also points out that in *H. annosum* infected spruce trees, the mycorrhizal mantle is poorly developed. There were also lots of heavily damaged roots, which pertain to vitality class 4. On sample site 6 the fine roots were heavily damaged (vitality class 3–4). However, on sample plots 7 and 8, where there is a mixture of grey alder, a good deal of vital mycorrhizal clusters was found. This suggests that the deciduous have a positive effect on the development of mycorrhiza in spruce. The literature, too, suggests that a mixture of deciduous species suppresses the root pathogen in spruce (Piri *et al.* 1990). Yet, it must be pointed out that there are also opposite opinions regarding the role of deciduous in suppressing the spread of *H. annosum* (Werner 1973).

On the sample plots of peaty soils, diseased spruce trees were found in one case only. Also the literature sources indicate that *H. annosum* infection is less common in peaty soils than in mineral soils (Redfern 1997). This is explained by soil acidity. It has been found that on mineral soil plot with healthy spruce trees the soil pH at the depth of 5 cm is 3.6 with the same index on diseased plots being 4.6. At the depth of 20 cm the same indices are 3.9 and 4.8, respectively. No differences in soil acidity have been found for the depth of 40 cm.

In future there is a need to analyse also other factors, which affect the development of mycorrhiza.

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