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Filamentous Fungi Associated with the Fine Roots of *Erica herbacea* L. from the Area influenced by the Žerjav Lead Smelter (Slovenia)

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S u m m a r y

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Filamentous fungi have been isolated from nonmycorrhizal, apparently healthy fine roots of *Erica herbacea* L. from four sites at different distances from the Žerjav lead smelter during different seasons of the year. The collection sites vary in terms of the contents of heavy metals (Pb, Cd, Zn) in the soil. The overall infection rate of the roots is directly dependent on the sampling location. Sampling sites differ regarding fungal infection rates and fungal taxa recorded. Approximately 107 different types of filamentous fungi were recorded; 58 % of the root samples (i.e. 561 isolations from 960 root segments) were found to be infected. The dominant species found were: dark septate endophytes (DSE) (18 % of all isolations), *Colletotrichum gloeosporioides* (Penz.) Sacc. (8 %), *Penicillium* sp. (11 types) (6 %), *Scytalidium lignicula* Pesante (5.5 %), and *Pestalotiopsis funerea* Desm. (5 %).

Introduction

Ericoid mycorrhiza is present in the majority of plants belonging to the *Ericaceae* family. Dark septate endophytes (DSE) resembling *Mycelium radicis atrovirens* (MELIN 1921, AHLICH & SIEBER 1996, HOLDENRIEDER 1989) are the dominate fungal group of ericaceous hosts in addition to mycorrhyzal fungi. DSE are composed of heterogeneous genera, species and strains of Hyphomycetes (WANG & WILCOX 1985),

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although their role in its hosts is unknown. Soil pollution with heavy metals has a considerable influence on the microorganisms in the soil. Such pollution may have a significant effect on the expansion growth and variation of filamentous fungi in plant tissues and other fungi in the soil. It is also known that ericoid mycorrhizal endophytes can overcome heavy-metal toxicity in ericaceous host plants by sequestrating metals in fungal mycelia reducing metal transfer to the shoot (BRADLEY & al. 1982). This process of avoidance is of great ecological importance for the successful growth of *Ericales* on polluted sites (SMITH & READ 1997). The aim of the present study was to examine and identify the filamentous fungi associated with the fine roots of *Erica herbacea* L. and to find out if soil pollution with Pb, Cd and Zn in the area influenced by the Žerjav lead smelter has effected root associated fungi.

Materials and Methods

Erica herbacea plants were collected from four heathland sites in May 1998 and September 1998. The sampling sites were at different distances from the source of pollution: 3 sampling sites were in the Žerjav area (700-750 m a. s. l.) and one was at an unpolluted location in Mežica (485 m a. s. l.). All sites are situated in the Alpine high – mountain area (East Karavanke Mountains) of Slovenia, with rendzina on limestone and dolomite as the predominant soil types.

Small terminal portions of the root system were excised in distilled water and washed under running tap water for 24 hours. 0.3 cm long root segments were surface-sterilised by two methods (M1 and M2) using the following sequence of immersions: 1 min 50 % ethanol, 5 min sodium hypochlorite (M1: 1.5 % of Cl^-, M2: 2.6 % of Cl^-), and 1 min 50 % ethanol (JURC & JURC 1995). The segments were blotted dry and plated onto 2 % (w/v) malt extract agar (MEA - Malt Extract, Biolife S.r.l., 20 g l^{-1}, Agar Bios Special LL, Biolife S.r.l., 20 g l^{-1}) in 90 mm d Petri dishes. Petri plates were then incubated at 23^0C and examined weekly for ten weeks. Mycelial outgrowths from the segments were subcultured and identified. A total of 960 root segments were processed.

Soil analysis and analysis of heavy metal presence in the soil were carried out using AAS (atomic absorption spectroscopy; Perkin-Elmer Analyst 100) for Zn and ETAAS (Perkin-Elmer SIMAA 6000) for Cd and Pb. The analyses were carried out by ERICo, Velenje.

Results and Discussion

The fine roots of *E. herbacea* have a rich mycoflora, but there are only few fungi which predominate. In total, 107 different types of filamentous fungi were isolated, 7 species were determined, and 7 taxa were determined to the level of the genus (Table 1). 58 % of the root samples (561 isolations from 960 segments) were found to be infected. The dominant species, i.e. those which appear in more than 4 % of all isolations, were: dark septate endophytes (DSE) (18 % of all isolations), *Colletotrichum gloeosoporioides* (Penz.) Sacc. (8 %), *Penicillium* sp. (11 types) (6 %), *Scytalidium lignicula* Pesante (5.5 %), and *Pestalotiopsis funerea* Desm. (5 %). One species (*Chaetonium globosum* Kunze ex Steud.) formed teleomorphs in culture.

The species composition found in this work on *E. herbacea* L. roots is similar to that recorded by OBERHOLZER-TSCHÜTSCHER 1982 for the same host. In their case, species like *Colletotrichum gloeosporioides* and *Chaetomium globosum* were isolated from the stems of *E. herbacea*, fungi of the *Phialophora Hoffmannii*-group were found in the roots and stems, and *Verticillium* sp. was present in the roots of the same host. *Cryptosporiopsis* sp. was found to be very common in the roots of *E. herbacea* and was assumed to be associated with the degradation of mycorrhiza in Norway spruce (HOLDENRIEDER 1989). *Cryptosporiopsis* sp. also causes cortical and vascular infections in seedlings in vitro (HAUG & al. 1988). *Epicoccum purpurascens* Ehrenb., which was also present in the stems of *E. herbacea*, has been described as a cosmopolitan and pathogenic species (DOMSCH & al. 1993). *C. globosum* was also present in the dead plant material and is involved in the »soft rot« of the wood (DOMSCH & al. 1993). Species of the genus *Truncatella*, are often saprophytic in dead plant material and sometimes even parasitic in weakened hosts (NAG RAJ 1993). Abundant root colonisation by DSE is known for plants growing on an environmentally stressed glacier forefront on soil low in N and organic matter. It was shown that DSE may function as beneficial root symbionts improving mineral nutrition of the host (JUMPPONEN & al. 1998).

Table 1. Species composition of fungi associated with fine ericaceous roots at different locations (May 1998, September 1998).

Sampling location	0 Mežica		1 Žerjav		2 Žerjav		3 Žerjav	
Type of sterilisation	M1	M2	M1	M2	M1	M2	M1	M2
Number of segments	120	120	120	120	120	120	120	120
Fungal species or type	Infections							
Chaetonium globosum Kunze ex Steud.								1
Colletotrichum gloeosporioides (Pers.) Sacc.		9	3	14	13			
Colletotrichum trichellum (Fr. ex Fr.) Duke		1				2		
Cryptosporiopsis sp.						1		
Epicoccum purpurascens Ehrenb.		1						
Hypoxylon sp.			4	3				
Mucor sp. (3 types)			2		2	1	3	3
Penicillium sp. (11 types)	13	17	2	7	40	23	17	10
Pestalotiopsis funerea	3	2	16	8				
Truncatella sp. (2 types)				7				
Phialophora Hoffmannii-group	1							
Phoma sp. (2 types)			4	3	5		1	
Scytalidium lignicula Pesante					19	7		
Trichocladium canadense Hughes			3	1				
Verticillum sp.							1	
dark septate mycellium (DSE) (3 types)	20	19	16		1	20	17	10
dark sterile mycellium (8 types)	8	4	2	6	1	2	7	1
hyaline sterile mycellium (6 types)	3	2	5	4	4	1	2	
other unidentified types 63	9	24	20	20	19	20	11	10
Total	57	79	77	73	104	77	59	35

The soil in the sampling locations has been classified as highly polluted by metals, according to current regulations (OFFICIAL GAZETTE RS, No. 68/96). The warning Cd value is exceeded in locations 0 and 3, while the critical Cd value is exceeded in locations 2 and 3. The critical value for Pb is exceeded in locations 1, 2 and 3; the critical value for Zn is exceeded in locations 2 and 3 (Table 2).

The site most polluted by heavy metals is site 2, followed by 1, 3 and 0, respectively. However, the greatest number of infections of root samples were observed in location 2, followed by 1, 0 and 3, respectively. Thus, the frequency of colonisation was the greatest in *Erica* roots from the most polluted location. The majority of the identified fungal species are saprophytes. We hypothesise that the prevalence of saprophytic root associated fungi is connected to the weakness and decay of host plants affected by soil pollution.

Table 2. The amount of heavy metals in the soil.

Soil sample (depth in cm)	$C_{Cd}/\mu gg^{-1}$	%RDS	$C_{Pb}/\mu gg^{-1}$	%RDS	$C_{Zn}/\mu gg^{-1}$	%RDS
0 Mežica (0-5cm)	1.4	0.5	171	0.5	61.8	0.5
1 Žerjav (0-5cm)	35.8	1.1	5422	0.5	582	0.5
1 Žerjav (5-15cm)	15.8	0.7	1480	0.5	233	0.5
2 Žerjav (0-5cm)	87.7	0.6	31320	2.2	1330	0.6
2 Žerjav (5-15cm)	46.0	1.3	4415	1.1	727	0.5
3 Žerjav (0-5cm)	1.7	3.9	2568	0.5	151	0.5
3 Žerjav (5-15cm)	6.9	0.6	667	0.5	177	0.5
3 Žerjav (15-30cm)	3.4	0.5	95.7	0.9	133	0.5

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