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Mycorrhiza of *Dryopteris carthusiana* in southern Poland

JULIUSZ UNRUG and KATARZYNA TURNAU

Institute of Botany, Jagiellonian University
Lubicz 46, PL-31-512 Kraków, Poland

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The research on mycorrhiza of *Dryopteris carthusiana* from natural sites and those contaminated by heavy metals (Niepołomice Forest), both on lowlands and mountainous areas in Poland, was carried out. Mycorrhizal colonization of *Arum*-type was higher in ferns growing on tree stumps than in specimens developing directly on the soil. Additionally, an increase in mycorrhiza intensity and arbuscular richness with the rising ground humidity was observed. In comparison to natural sites, mycorrhizas from the areas contaminated by heavy metals were much less developed and the roots were often infected by parasites. Two morphotypes of mycorrhizal fungi have been described. The most common was a fine endophyte (*Glomales*).

Key words: arbuscular mycorrhiza, *Glomales, Zygomycetes, Dryopteris carthusiana.*

INTRODUCTION

Mycorrhiza is one of the most significant mutualistic symbiosis between a plant and a fungus. It occurs in over two-thirds of vascular plants and also in liverworts (Ligrone and Lopes 1989; Smith and Read 1997). Forests of temperate climate are dominated by plant species forming ectomycorrhiza. The presence of other kinds of mycorrhizas developed by plants of herb layer has been mostly overlooked. Among them arbuscular mycorrhiza formed by ferns and members of *Glomales* (*Zygomycetes*) was rarely the subject of ecological studies (Cooper 1976, 1977; Laferriere and Koske 1981; Berch and Kendrick 1982; Jones and Sheffield 1998). The symbiosis may occur in fern thalli, rhizomes and roots, however, it is not ubiquitous. Boullard (1957, 1979) was one of the first to carry out a detailed observation on mycorrhiza in different groups of *Pteridophytes.* Most features of AM colonization in this group are similar to those found in angiosperms. Different patterns of colonization with prevailing coils or with the presence of well visible arbuscules, as in the case of *Pteridium aquilinum, Polystichum aculeatum* or *Osmunda* *regalis*, were described (F o n t a n a 1959; B o u l l a r d 1979). When discussing the relationship between the root system morphology of *Pteridophytes* and the occurrence of AM – B o u l l a r d (1979) suggested that there was a close correlation between fungal colonization and fern evolution. He concluded that the *Ophioglossales* with "primitive" fleshy roots were highly colonized while the *Filicales* with "advanced" fine roots were less colonized or, occasionally, non-mycorrhizal.

Dryopteris carthusiana (Vill.) H. P. Fuchs [= *D. spinulosa* (O. F. Müller) Watt], a member of *Filicidae*, was chosen for observations as a relatively common fern in Poland. Interest has been drawn to this species due to research previously carried out on liverworts (T u r n a u et al. 1999) which showed links between the fern roots and rhizoids of *Pellia endiviifolia*. *D. carthusiana* occurs in lowlands, foothills and in the lower parts of mountains and rarely in the higher parts of mountains (P i ę k o ś-M i r k o w a 1979; Z a j ą c 1998).

The main objective of this study was to determine the mycorrhizal status of *D. carthusiana*. Special attention has been paid to the substratum humidity, nutrient availability and environmental stress (high Zn, Cd, Pb, Al content).

MATERIALS AND METHODS

S a m p l i n g. Samples of *D. carthusiana* roots used for estimation of mycorrhizal colonization were collected at different sites grouped in five regions: Olczyska Valley, Białego Valley, Strążyska Valley (Tatra National Park), Żmiąca Valley (Beskid Wyspowy Mts.) and Brzesko surroundings (Nizina Sandomierska) (Table 1). Ferns were collected from sites situated

T a b l e 1

The list of the sites analysed (areas sampled) and the estimation of mycorrhizal colonization of *D. carthusiana*:
mycorrhizal frequency (F%), mycorrhizal root lenght (M%) and arbuscular richness (A%)

F [%]	M [%]	A [%]	DESCRIPTION OF THE AREAS SAMPLED
			TATRA MOUNTAINS
96.6	47.8	31.4	Olczyska Valley, very wet soil
98.9	76.7	37.3	Olczyska Valley, soil close to the stream
98.5	44.7	33.3	Olczyska Valley, stump in very shady forest
100	69.7	62.4	Olczyska Valley, wet soil close to the stump
36.7	1.4	0.4	Strążyska Valley, dry soil near the spruce
76.6	5.4	4.4	Strążyska Valley, dry stump
43.3	5.4	0.8	Białego Valley, shallow soil on limestone
56.7	6.5	2.1	Białego Valley, wet soil close to the spruce

F [%]	M [%]	A [%]	DESCRIPTION OF THE AREAS SAMPLED
			ŻMIĄCA VALLEY
61.9	25.3	20.3	wet soil near the stream
61.9	25.3	20.3	wet soil close to the stream
37.0	7.3	0.2	dry soil
80.0	8.3	2.3	stump close to the stream
15.4	1.6	1.3	stump near the stream; PELIA
53.3	18.0	12.6	shallow soil on the tree roots, forest; PELIA
86.7	43.8	42.9	wet stump close to the stream; PELIA
100.0	34.9	27.0	wet soil close to the stream; PELIA
80.0	80.3	27.3	slope near the stream (higher part); PELIA
100.0	34.9	27.0	slope near the stream (middle part); PELIA
85.7	47.5	44.1	slope near the stream (lower part); PELIA
15.4	0.6	0.1	soil close to the stream; PELIA
15.4	1.6	1.3	wet soil near the stream; PELIA
			BRZESKO SURROUNDINGS
40.0	8.3	7.5	Borzęcin Górny, dry soil
50.0	2.1	0.8	Brzeźnica, wet stump
0.0	0.0	0.0	Niedzieliska Górka, sand in pine forest
22.2	0.7	0.2	Rudy Rysie, stump
43.3	6.4	4.1	Rudy Rysie, sand in pine forest
96.7	41.7	32.0	Sufczyn, stump
35.8	0.9	0.1	Niwka, wet stump
46.6	4.1	1.5	Niwka, dry stamp
56.7	21.2	15.0	Niwka, sand in pine forest
			NIEPOŁOMICE FOREST, EXPERIMENTAL PLOTS
22.2	0.9	0.0	Al 5000
83.3	7.6	1.5	Al 5000
20.0	0.2	0.1	Cd 5000
15.0	0.2	0.0	Cd 2000
16.7	0.2	0.0	Cd 2000
93.3	22.9	14.6	Cd 2000
36.5	0.7	0.0	Zn 5000
			NIEPOŁOMICE FOREST, OUTSIDE THE EXPERIMENTAL PLOTS
86.6	42.4	19.1	soil
53.3	3.0	0.2	soil
10.0	0.1	0.0	soil
3.3	0.0	0.0	soil

in forests in different humidity and shade conditions, both on the ground and tree stumps. Another group of sites was situated in the Niepołomice Forest (near Kraków, Southern Poland) on experimental plots treated in 1980 with different doses of cadmium, zinc and copper dusts (100, 500, 1000, 2000, 5000 t ha^{-1}). Some parts of these plots were subsequently fertilized (G r e s z t a et al. 1987; G r e s z t a 1988; T u r n a u 1991).

A total of 83 root samples were collected from 44 sites in 1997 between April and October. Each sample was composed of three fern root subsamples.

S o i l a n a l y s i s. The soil surface samples (0 – 10 cm) were collected with each fern root system. Roots were used for mycorrhizal analysis while the soil was air-dried, crushed and passed through a sieve of 1-mm and 0.5-mm pore size. The pH value (pH-meter N 5170), assimilable elements and organic carbon content (Table 2) were estimated. All the analyses were performed using ammonium lactate buffer. Calcium and potassium were determined photometrically (Flapho-4), whereas magnesium and phosphorus colorimetrically (Spekol). Nitrogen was estimated by Kiejdahl's method while organic carbon by Turin's method (L i t y ń s k i et al. 1976). A detailed description of the soil from the experimental plots in the Niepołomice Forest was given by G r e s z t a et al. (1987).

T a b l e 2

Element content and pH values of the soil collected from the areas sampled

pH (in H_2O)	pH (in KCl)	C	N	C/N	K_2O mg/100g	P_2O_5 mg/100g	MgO mg/100g	CaO mg/100g	SITE
4.2	3.3	2.4	1.9	15.0	15.6	2.8	22.5	61.6	Olczyska Valley, very wet soil
3.5	2.8	13.4	0.2	11.1	58.8	6.4	6.0	75.5	Olczyska Valley, soil close to the stream
3.2	2.6	13.6	0.6	23.9	39.0	4.6	1.0	36.4	Olczyska Valley, stump in very shady forest
3.6	3.0	30.4	1.2	11.5	26.0	13.2	7.0	254.8	Strążyska Valley, dry soil near the spruce
7.3	7.0	9.0	1.6	18.7	16.4	4.0	600.0	940.8	Białego Valley, shallow soil on the limestone
6.7	6.6	8.0	0.7	12.1	19.4	9.0	175.0	722.4	Białego Valley, wet soil close to the spruce
3.8	3.3	3.2	0.2	15.8	10.0	1.0	14.0	39.2	Żmiąca Valley
5.5	5.5	5.3	0.3	19.9	2.0	24.0	4.7	25.2	Niepołomice Forest Al 5000
5.7	5.6	3.8	0.3	14.3	3.0	24.0	6.0	30.8	Niepołomice Forest Zn 5000
4.2	3.8	1.9	0.1	18.2	3.0	6.2	3.3	11.2	Niepołomice Forest Cd 5000
3.8	3.6	3.6	0.2	15.2	2.0	9.6	3.7	11.2	Niepołomice Forest Cd 2000
3.9	3.3	4.1	0.2	19.2	5.0	3.0	3.7	21.0	Niepołomice Forest, outide the experimental plots

Estimation of mycorrhizal colonization. Root samples of *D. carthusiana* were stained according to the modified method of Philips and Heyman (1970). The whole procedure was carried out at room temperature. Roots carefully washed with tap water were cleared in 7% KOH for 24 hours, washed with water and bleached in H_2O_2 containing NH_3 (10:1) for 5 minutes. The roots, washed again, were treated with 5% lactic acid solution (in distilled water) for 12–24 hours and stained with 0.01% aniline blue solution in lactic acid approximately for 24 hours. The root samples were deposited in pure lactic acid. They were finally cut into 1cm long pieces and placed in lactoglicerol or lactic acid on a slide, smashed with cover glass and observed under a light microscope. Mycorrhizal frequency (F%), mycorrhizal root length (M%) and arbuscular richness (A%) were estimated according to Trouvelot et al. (1986).

Statistical analysis was carried out using StatSoft Inc. programme (1997), Statistica for Windows: nonparametric test ANOVA Kruskall-Wallis ($\alpha \leqslant 0.05$) was used.

RESULTS

Mycorrhizal colonization. The observations revealed that *D. carthusiana* was in most cases mycorrhizal (87% of the analysed root samples). The mean value of mycorrhizal colonization (M) was 19,2% and arbuscular richness (A) 14.6%. High arbuscular richness was described for the Tatra Mountains and Żmiąca sites while much lower values of this parameter were assessed for ferns collected from the Niepołomice Forest (both experimental plots and the control) (Fig. 1). The increase in mycorrhizal colonization and arbuscular richness was correlated with the rising soil moisture (Fig. 2). Higher mycorrhizal colonization was found within the root system of ferns growing on tree stumps, especially those soaked with water, than directly on soil. The mycorrhizal colonization and arbuscular richness were much higher in root samples which were not colonized by fungal or animal parasites.

Mycorrhizal colonization and arbuscular richness within the root samples collected from the experimental plots in the Niepołomice Forest were significantly lower than in the case of ferns growing in the mountains or non-polluted areas. Significantly lower mycorrhizal colonization and arbuscular richness were observed in the case of the fertilized plot treated with Cd 5000 t ha^{-1}, non-fertilized plots treated with Cd 2000, Al 5000 and Zn 5000 t ha^{-1}. No statistically significant differences between the experimental plots and the control area outside the plots in the Niepołomice Forest were observed.

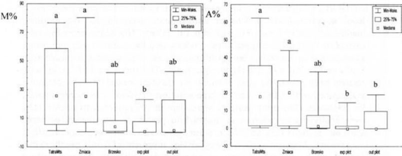


Fig. 1. Mycorrhizal colonization (M) and arbuscular richness (A) of *D. carthusiana* roots in the areas sampled (different letters in the upper columns indicate statistically significant differences at $p < 0.05$)

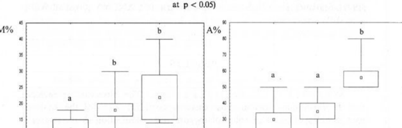
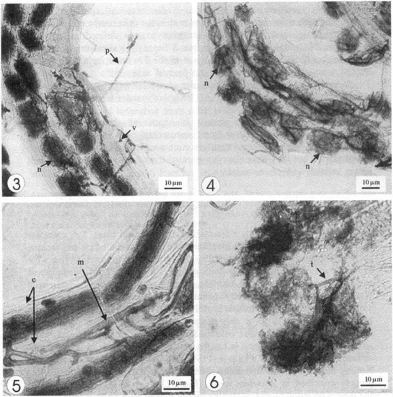


Fig. 2. Mycorrhizal colonization (M) and arbuscular richness (A) of *D. carthusiana* under different moisture conditions (different letters in the upper columns indicate statistically significant differences at $p < 0.05$)

Morphotypes of arbuscular fungi. The mycorrhizal colonization observed within the roots of *D. carthusiana* was characterized by the dominance of intercellular growth. Individual coils within root cortical cells were arranged in rows and connected by the mycelium extended along well developed intercellular air spaces. The plant cells were full-filled with the coils. The arbuscules were found abundantly as delicate lateral branches formed mostly within the coils. The colonization of the root was usually started by the penetration through the hair roots (Fig. 3). In some cases intercellular hyphae were growing parallel to the surface of the root forming several branches subsequently penetrating the nearest cortical cells in which the coils were formed.



Figs 3 – 6. Mycorrhiza of *D. carthusiana*: Figs 3 – 4. – colonization by fine endophyte with net-like structures (n) surrounding internal delicate arbuscules, typical vesicles (v) and hyphae penetrating hair roots (p); Figs 5 – 6. – broad endophyte colonization within the fern cortex cells (c) with intercellular thick mycelium (m) and arbuscules with branched trunk hyphae (t)

Two morphotypes of arbuscular mycorrhizal fungi (*Glomales*) were distinguished. The first one, much more common (found in almost 70% of the analysed roots), was identified as a fine endophyte. It was characterized by strongly stained and very narrow hyphae (less than 1 μm in diameter) with net-like growth pattern and fan-shaped structures. The mycelium spread intercellular within the cortex of *D. carthusiana*, sometimes formed coil-like structures and very delicate, comparatively abundant arbuscules inside the fern cells (Fig. 4). Oval vesicles of 4–6 μm in diameter and spores of about 60–70 μm, were quite frequently formed within the root cortex cells and also within the hair roots. The second morphotype was defined as a broad endophyte. No spore formation was found. Strongly stained mycelium was much thicker (2,5–5 μm) than in the case of the fine endophyte (Fig. 5). The hyphae were growing intercellular and formed many coils and arbuscules. Trunk hyphae and branches of arbuscules were relatively thicker than in the case of the first morphotype (Fig. 6). Irregular vesicles were rarely formed between root cells.

The fine endophyte was present in almost all the samples. It was a dominant morphotype in ferns from experimental plots in the Niepołomice Forest. In 20% of the analysed sites fern root samples were colonized by both morphotypes, but they were always separated from each other by a few plant cortex cells devoid of colonization.

DISCUSSION

Dryopteris carthusiana is a facultatively mycorrhizal plant. The colonization by arbuscular mycorrhizal fungi seems to vary depending on soil factors, such as moisture, pH and availability of nutrients. Fine endophytes were especially abundant under wet conditions and at low pH. The features of these endophytes are in accordance with the description of *Glomus tenue* (Greenal) Hall (Hall 1977), originally named *Rhizophagus tenuis* (Greenal 1963). The position of the fungus is still unclear. It is possible that more than one species is hidden under this name. The endophyte is one of the most common forms of arbuscular colonization, especially in uplands and acidic soils (Walker 1987) and is known as enhancing growth of plants under certain circumstances (Wilson 1984; Abbot and Robson 1991). Among the ferns the double colonization by coarse and fine endophytes was already described in *Athyrium* roots (Boullard 1979). The presence of the fungus on wood blocks or stumps totally soaked with water is note worthy. In such places the fern was often accompanied by a liverwort *Pellia endiviifolia*. As indicated earlier both plants can be interconnected by the fungus (Turnau et al. 1999). *D. carthusiana* might be an important donor of the symbiont on the steep banks of streams where roots may also serve as a mechanical support for developing thalli. Both plants are characterized by *Arum*-type of mycorrhizal colonization (Gallaud 1905) with the intercellular growth of the mycelium and fine arbuscules developed as lateral branches of the coils. In the case of *D. carthusiana* the arbuscular richness was much higher than in the cases described so far. Nowadays *Arum*-type colonization is considered to be a "typical VA mycorrhiza" (Smith and Read 1997).

The present study indicated the decrease in mycorrhizal colonization and arbuscular richness in polluted places. Experimental plots in the Niepołomice Forest had already been investigated concerning mycorrhizal fungi. Higher mycorrhizal colonization on plots than outside was found in the case of *Oxalis acetosella* (Turnau et al. 1996). Among the ferns *Pteridum aquilinum* was investigated and the presence of heavy metals in both symbionts was indicated (Turnau et al. 1993). With regard to mycorrhiza development in *P. aquilinum*, no differences in comparison with natural sites were found. The mechanisms regulating the development of symbiosis under heavy metal stress are completely unknown (Leyval et al. 1997). Although, the treatment with high doses of industrial dusts took place nearly 20 years ago, the plant populations are still scarce suggesting that there is still high toxicity of the dust within the soil, which is certainly much higher than in the forest outside plots; however the whole area belongs to a polluted region. The Niepołomice Forest, including experimental plots, might be an interesting source of AM fungal strains which could be used later for inoculation of strongly polluted places.

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Mikoryza *Dryopteris carthusiana* w Południowej Polsce

Streszczenie

Badania kolonizacji mikoryzowej *Dryopteris carthusiana* prowadzone były na siedliskach naturalnych i skażonych pyłami o wysokiej zawartości metali ciężkich w Puszczy Niepołomickiej, zarówno na terenach nizinnych jak i górskich. Kolonizacja mikoryzowa typu *Arum* była wyższa u paproci rosnących na pniakach niż u tych roślin, które rozwijały się bezpośrednio na podłożu glebowym. Dodatkowo zaobserwowano wzrost intensywności kolonizacji mikoryzowej i poziomu arbuskulacji we wzrastającym gradiencie wilgotności podłoża. W porównaniu do siedlisk naturalnych kolonizacja mikoryzowa na terenach skażonych metalami ciężkimi rozwijała się w znacznie mniejszym stopniu a korzenie paproci często zainfekowane były przez pasożyty. Opisano dwa morfotypy grzybów mikoryzowych. Najpowszechniejszym był przedstawiciel rzędu *Glomales*.