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The role of some saprophytic micromycetes and the fungus Micromucor ramannianus var. ramannianus in forest soils

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Different saprophytic micromycetes were isolated from the humic horizon (H-A 02) of different types of forest soils (barren land of reforested waste dumps, cambisol of spruce, birch, European mountain ash, and blue spruce forests) in several areas (at Most in the Krušné hory (Ore Mts.), Jizerské hory (Izera Mts.)). Besides the spectrum of common species of soil micromycetes (*Penicillium* spp., *Humicola* spp., *Trichoderma* spp., *Paecilomyces* spp., *Scopulariopsis* spp., *Aureobasidium* spp., *Mucor* spp., *Absidia* spp.), the fungus *Micromucor ramannianus* (Möller) Arx var. *ramannianus* (*Mortierella ramanniana* (Möller) Linneman, *Mucor ramannianus* Möller) was regularly isolated from all types of soils, except barren soils of waste dumps. The biological quality of forest soils in connection with other biological characteristics was evaluated in relation to the presence and quantity of this fungus in forest soils. Basic biological processes (basal and potential respiration, ammonification, nitrification) show an increased intensity in forest soils in which the proportion of *Micromucor ramannianus* v. *ramannianus* was not present in the soil of the worst biological quality (lower values of biological soil parameters), e.g. in of waste dumps. It is a topic for discussion whether this fungus can also be an indicator of environmental pollution.

Key words: humic horizon, soil fungi, saprophytic micromycetes, *Micromucor ramannianus* var. *ramannianus*, biological soil functions, respiration, ammonification, nitrification

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Z humusového horizontu (H-A02) různých lesních typů (neplodná půda výsypek, kambisol různých typů – smrk, bříza, jeřáb, stříbrný smrk) v několika místech (výsypky v Mostu, půdy v Krušných a Jizerských horách) byly izolovány různé saprofytické mikromycety. Vedle spektra běžných půdních mikromycetů (*Penicillium* spp., *Humicola* spp., *Trichoderma* spp., *Paecilomyces* spp., *Scopulariopsis* spp., *Aureobasidium* spp., *Mucor* spp., *Absidia* spp.) byla pravidelně izolována ze všech typů lesních půd houba *Micromucor ramannianus* (Möller) Arx var. *ramannianus* (*Mortierella ramanniana* (Möller) Linneman) kromě půd výsypek. Biologická kvalita lesních půd ve spojení s jinými biologickými charakteristikami byla stanovena společně s přítomností a kvantitou této houby v lesních půdách. Základní biologické procesy (bazální a potenciální respirace, amonifikace a nitrifikace) ukazují zvýšenou intenzitu v lesních půdách, ve kterých celkový počet nálezů houby *Micromucor ramannianus* v. *ramannianus* přesahuje 50 % všech přítomných mikromycetů. Na druhé straně houba *Micromucor ramannianus* v. *ramannianus* nebyla přítomna v půdách horší biologické kvality (nižší hodnoty půdních biologických charakteristik) např. v půdách výsypek. Je otázkou diskuse, zda tato houba může být také indikátorem znečištění nebo čistoty životního prostředí.

Introduction

Decomposition of organic matter into humus, gases and inorganic salts is a continuous process in forest soils. Soil micromycetes are extraordinary important participants in this process. Forest soils are habitats of mainly soil-borne saprophytic and some pathogenic fungi. The genus *Penicillium* (Dyr 1940) and its teleomorphs, e.g. *Eupenicillium* (Takada and Udagawa 1983), are the most frequently isolated genera from forest soils. Several new species of *Penicillium* from forest soils have been described. E.g. the new species *Penicillium kananaskense* (Seifert et al. 1994) was isolated from forest soil under *Pinus contorta* v. *latifolia*. New species of the perfect stage of *Eupenicillium* were isolated e.g. from Nepalese forest soils – *E. angustiporcatum* (imperfect stage: *Penicillium angustiporcatum*) and *E. nepalense* (imperfect stage: *Penicillium nepalense*) (Takada and Udagawa 1983). *Talaromyces unicus* and its anamorph were isolated from Taiwanese soil. The anamorph is characterised by mono– or biverticillate penicilli with long-necked lanceolate phialides (Tzean et al. 1992). The genus *Talaromyces* is also dominant in soil in which heat-resistant fungi are present (Jesenská, Piecková and Bernát 1992).

Differently broad spectra of micromycetes are usually isolated from forest soils. Grunda and Marvanová (1982) identified the following genera of micromycetes from soil of the forest type group *Ulmi-Fraxinetum carpineum* with *Quercus robur* predominating in the stand: 1) (at a depth of 1–2 cm – horizon F) *Absidia*, *Alternaria*, *Cladosporium*, *Cylindrocarpon*, *Fusarium*, *Humicola*, *Mucor*, *Paecilomyces* abd *Penicillium*, 2) the same genera as in horizon F were isolated at a depth of 2–7 cm (horizon Am), but besides them *Sporothrix* and *Trichoderma* were identified. *Cephalosporium*, *Mortierella*, *Phialophora*, *Stachybotrys* and *Trichocladium* were isolated from a depth of 15–25 cm (horizon Btg). Besides these the following genera were determinated at a depth of 35–45 cm (horizon Btg): *Bispora*, *Phoma* and *Rhizopus*. It is interesting that the fungus *Micromucor ramannianus* v. *ramannianus (Micromucor ramannianus)* was not detected in that soil, although it is present in almost all types of forest soils (Dyr 1940). Grunda (1981) investigated methods of research on soil microflora when he studied the floodplain forest soil in Moravia. The following genera of micromycetes were identified in floodplain forest soil: *Bispora*, *Calcarisporium*, *Chrysosporium*, *Cladosporium*, *Cylindrocarpon*, *Fusarium*, *Humicola*, *Mortierella*, *Mucor*, *Paecilomyces*, *Phialophora*, *Phoma*, *Penicillium*, *Rhizopus*, *Sporothrix*, *Stachybotrys*, *Trichocladium* and *Trichoderma*. Not even in this case the fungus *Micromucor ramannianus* v. *ramannianus* (*Mucor ramannianus* Möller) was detected, even if it is regularly present in forest soils (Dyr 1940).

Saprophytic micromycetes in forest soils were studied by Vláčilíková (1978), who described the presentation of genera of micromycetes in forest soils. Average proportions (in percent) of fungal genera in four types of forest soil were as follows: *Absidia* (7.5), *Circinella* (0.35), *Mucor* (16), *Rhizopus* (0.1), *Zygorhynchus* (0.6), *Cunninghamella* (0.1), *Mortierella* (9.0), *Gymnoascus* (2.7), *Thielavia* (2.3), *Chaetophoma* (0.1), *Truncatella* (0.2), *Aspergillus* (2.0), *Cephalosporium* (4.0), *Gliocladium* (2.0), *Monocillium* (0.5), *Paecilomyces* (0.9), *Penicillium* (51.5), *Spicaria* ((2.1), *Sporotrichum* (0.9), *Trichoderma* (6.4), *Verticillium* (0.4), *Botryotrichum* (0.1), *Cladosporium* (1.9), *Hormiscium* (0.4), *Humicola* (0.9), *Masoniella* (0,4), *Pullularia* (0.1), *Stachybotrys* (1.9), *Cylindrocarpon* (0.6), *Fusarium* (0.3), *Volutella* (0.3), sterile mycelium (0.6), *Myrothecium* (0.3). *Mortierella ramanniana* (Möller) Linneman ranked second among all species of the genus *Mortierella* in forest soils (0, 1.5 %, 13.7 %, 5.1 %). *Penicillium nigricans* (Bainier) Thom was the species with the highest representation in the different soils (%): 2.5, 49, 18.1, 1.1.

Micromucor (*Mortierella*) is the best known genus of forest soil zygomycetes. A relationship of *Endogone* to *Mortierella* (*Mortierellaceae*) has been suggested since in some species, especially *M. nigrescens* and *M. renispora*, the zygospores are surrounded by hyphae, much like the hyphae developed by zygospores in sporocarps of *Endogone* (Benny 1982).

Micromucor ramannianus is a fungus with one of the highest frequencies in forest soils. The genus is classified in the genus *Mucor* by Dyr (1942) as *Mucor ramannianus* A. Möller 1903 (Zeitschr. F. Forst. und Jagdw. 35, p. 330). It differs from other species by the colour of its colonies beeing deep red to soil pink. The cultures reached a height 1.6–2.5 mm only, fungus growth is very dense and velvety. Sporangiophores are usually not ramified. Sporangia are small, 18–35 μm and not ramified, they are deep red in colour and have a slightly melting membrane. The columella is round or disk-shaped, colourless, 10.8–12.7 μm in height and 11–14 μm in width. Sporangiospores are shortly eliptic to round (1.2×2.3, $2.7 \times 3.3 \times 3$ μm). The species was isolated in Germany (Johann 1932), France (Ling-Young 1930), Austria and Yugoslavia (Pispek 1929), Russia (Raillo 1929), Northern America (Povah 1917), Australia (Dale 1914) and Norway (Hagen 1908).

This species is generally present in forest soils, its frequency being the highest of all species of micromycetes and it could probably be isolated at every forest site. The population density of this species is regularly enormous amounting to more 50 % of micromycetes at the site. The ratio of *Mucor ramannianus* to other micromycetes was 25:1 in forest soils of the Jevany district. Sites with the highest population density of the fungus had the highest concentration of sporangiospores in the soil. The fungus population density was low at two forest sites only – in the environs of Dobřichovice (right bank of Berounka river) and in Brdy forest. Different types of forest showed different rates of the fungus (out of the total number of micromycetes). It was 64.2 % in beech forest soils, 39.8 % in spruce forest soils, 37.4 % in pine forest soils, 26 % in oak forest soils, 20 % in alder forest soils, 32.5 % in birch grove soils. This species had modest environmental demands with respect to the extreme soil factors in broad-leaved and coniferous forest stands. Fungus growth in nutrient media in Petri dishes makes up a continuous coating of a typical colour. Fungus growth is very fast. The species is usually at a soil depth of up to 20 cm, in the upper humus layer under it. Kubátová et al. (1998) reporting on the biodiversity of soil microfungi of the Šumava Mts., Czech republic, confirmed that among the most frequent species *Micromucor ramannianus* v. *ramannianus* was. They determined soil micromycetes from 12 localities, including peat-bogs, Norway spruce forest, beech forests, mixed forests in glacial cirques in the period 1993–1996 (121 soil samples). The most frequent species were: *Trichoderma viride* (in 57 % of all samples), *Penicillium spinulosum* (55.4 %), *Micromucor ramannianus* v. *ramannianus* (33.1 %) and *Mucor hiemalis* f. *hiemalis* (24.8 %).

Micromucor ramannianus has not been described as a typical fungus of forest soils in many papers, or its population density is characterised as very low. E.g. Grunda and Vorel (1996) reported a high density of micromycete species with some differences in various types of soils (H-horizons). The following micromycetes were present in the H-horizon of humic acidic Cambisol: *Penicillium* sp., *Aspergillus* sp., *Gliocladium* sp., *Trichoderma* sp., *Cladosporium* sp., *Sporotrichum* sp., *Alternaria* sp., *Humicola fuscoatra* and *Humicola grisea*. Acidic Cambisol contained also these fungi: *Botrytis* sp., *Mortierella nana*, *Chaetomium* sp. The fungi were determined in deep acidic Cambisol in comparison with acidic Cambisol: *Mortierella ramanniana* (*Micromucor ramannianus* at present) and *Verticillium albo-atrum*. In addition, *Absidia glauca* was found in gley acidic Cambisol.

Many of the soil micromycetes showed intensive metabolic activity, e.g. ligninolytic activity in *Penicillium chrysogenum* (Rodriquez et al. 1994). This metabolic activity can last very long. Marfenina (1991) studied the morphological development of microscopic fungi in soil. The life cycle (mycelium and conidium formation) is usually very long.

Many species of *Mortierella* produce a white oily substance in large drops among the aerial hyphae. One group of species, characterised by velvety odourless colonies, is considered by some mycologists to represent a separate genus, *Micromucor* (Benny 1982). The objective of this study was to determine the importance of *Micromucor ramannianus* v. *ramannianus* in soil humic horizons of Czech forests.

Material and methods

Forest soil samples were taken from the humic horizon (H-horizon) after the F-horizon (fermentation horizon) was disclosed. Soil was sampled into platic bags under sterile conditions, and was processed as soon as it was brought into a laboratory. It was ground through a fine sieve. 5 grams of ground sample was added under sterile conditions into 500 ml of a sterile physiological saline, it was left to clarify for 2 min. 1 ml was pipetted under sterile conditions onto the bottom of sterile Petri dishes and overlaid with cooling down agar (Czapek-Dox agar). After coolong down, Petri dishes were placed in a termostat controlled room at a temperature 24 °C for 10 days. The number of micromycete colonies was determined quantitatively. Qualitative analyses were made and micromycete colonies were determined in microscopic preparations in lactophenol with methylene blue and examined under a microscope. Basic soil characteristics were determined by methods described in e.g. Schilling and Blume's book (1966). The species were typified with the use of different literature (e.g. Domsch and Gams 1993).

Results

The H-horizon (humic) was examined microbiologically in all cases together with F (fermentation) and A (mineral) horizons. Tab. 1 shows the localities of soil sampling. The results of identification of cultivable microscopic fungi (Tab. 2) demonstrate that the fungus *Micromucor ramannianus* v. *ramannianus* is present in spruce, beech, birch and mountain ash soils but not in soils of waste dumps in the Most district, even though they have been reforested. The fungus was also isolated from F and A horizons, but the population density were lower and less regular. The H-horizon was taken as the basic component of forest soils because it contains typical humus (humic acids and fulvoacids). It is evident from Table 2 that the fungus was present in the H horizon of all productive soils. Its rates were higher than 50 % of all cultivable CFU (Colony Forming Units) in soils of higher biological activivity.

Table 1. Localities of soil sampling.

Locality	Mountain range	Stand	Soil type
Jizera river basin	Jizera Mts.	Spruce	Cambisol
Nová Ves v Horách	Ore Mts.	Spruce Blue spruce Birch Mountain ash	Cambisol Cambisol Cambisol Cambisol
Velebudice	Most waste dump	Maple Lime	Artificial ground

Table 2. Micromycetes isolated from H-horizons of cambisol types of forest soils

Locality/Stand	Micromycetes	Proportion / frequency
Jizera river basin/Spruce	*Micromucor ramannianus* var. *ram.*	55%
	Trichoderma viride	20%
	Penicillium expansum	10%
	Paecilomyces farinosus	5%
	Paecilomyces niveus	5%
	Scopulariopsis brevicaulis	2,5%
	sterile mycelium	2,5%
Nová Ves v Horách/Spruce	*Micromucor ramannianus* var. *ram.*	52%
	Trichoderma viride	15%
	Penicillium italicum	15%
	Paecilomyces varioti	7%
	Paecilomyces fulvus	3%
	Scopulariopsis brevicaulis	5%
	sterile mycelium	3%
Nová Ves v Horách/Blue spruce	*Micromucor ramannianus* var. *ram.*	58%
	Scopulariopsis brevicaulis	12%
	Absidia corymbifera	10%
	Paecilomyces fulvus	8%
	Trichoderma viride	5%
	sterile mycelium	7%
Nová Ves v Horách/Birch	*Micromucor ramannianus* var. *ram.*	65%
	Paecilomyces farinosus	8%
	Penicillium expansum	6%
	Scopulariopsis brevicaulis	6%
	Gilmaniella humicola	5%
	Trichoderma viride	2%
	sterile mycelium	8%
Nová Ves v Horách/Mountain ash	*Micromucor ramannianus* var. *ram.*	59%
	Aureobasidium pullulans	10%
	Cladosporium resinae	8%
	Paecilomyces fulvus	6%
	Trichoderma viride	1%
	sterile mycelium	16%
Velebudice/Most waste dump	*Mucor hiemalis*	65%
	Mucor piriformis	16%
	Humicola fuscoatra	15%
	sterile mycelium	4%

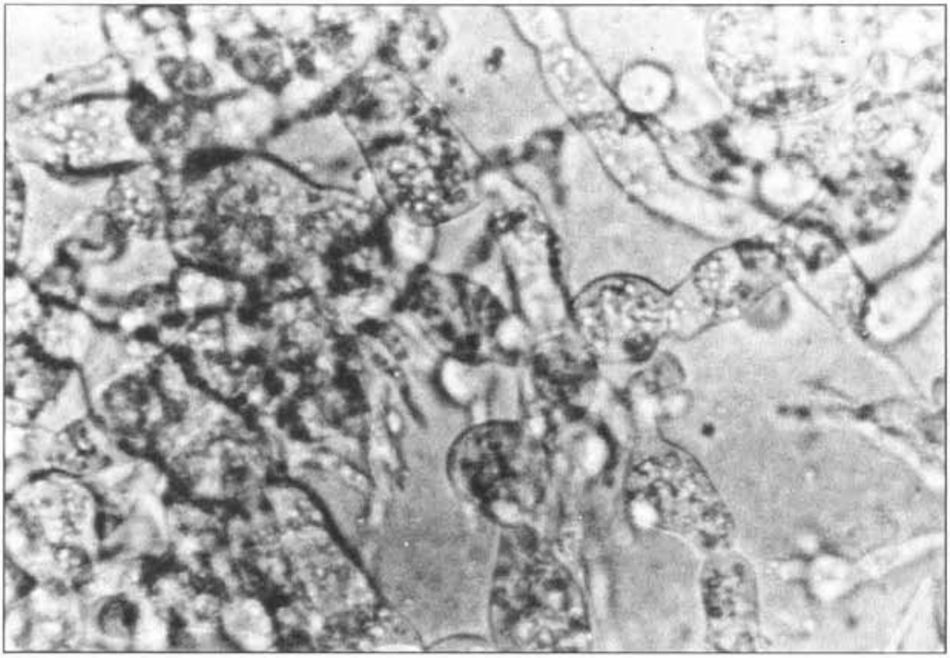

Fig. 1 *Micromucor ramannianus* var. *ramannianus*, mycelium with oil droplets (× 500).

The growth of *Micromucor ramannianus* var. *ramannianus* was satisfactory in artificial media (Czapek-Dox agar) at a temperature of 24 °C. Most colonies produced a red pigment. There exist differences in isolate colour, some isolates are cream-coloured, others are from white to pink, many are brown-red in colour. Sporangia reddish in colour and containing several sporangiospores were exceptionally discovered in the preparats. A major part of the cultures produced a system of sacs containing high amount of oily droplets (Fig. 1).

Table 3 shows the the values of biological soil characteristics from different H-horizons. It is evident from this figure that soils with higher biological activities have higher counts of *Micromucor ramannianus* var. *ramannianus* germs.

Discussion

This paper has many features identical with the papers by Dyr (1942), Grunda and Marvanová (1982), Grunda and Vorel (1996), Kubátová et al. (1998). These authors determined *Micromucor ramannianus* var. *ramannianus* as the basic microfungus of forest soils. The basic paper by Dyr (1942) contains a relatively successful description of its morphology, although the fungus illustration is not

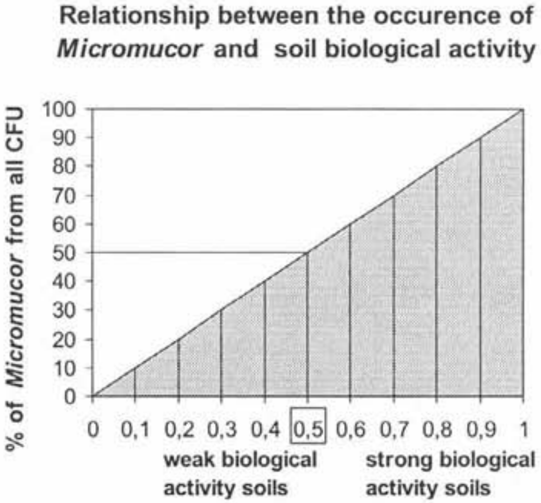

Fig. 2 Incidence of *Micromucor ramannianus* var. *ramannianus* in percent (all microfungi) in relation to soil fertility (scale from 0, 0.1.........0.9, to 1.0).

very satisfactory. Sporangia were discovered rarely during our studies. Dyr (1942): "Of all micromycete species, the frequency of this species is highest in forest soils, and it is likely to be isolated from every forest site." We observed during our studies that the fungus was not present in soil of reforested waste dumps and that its population density varied in different types of soils. *Micromucor ramannianus* var. *ramannianus* is dominant in "physiological" ("helthy") forest soils while soil germ counts of this fungus decrease in soils exposed to degradation processes. A worldwide distribution is typical of this fungus (Johann 1932, Ling-Young 1930, Pispek 1929, Reillo 1929, Povah 1917, Dale 1914, Hagen 1908). Dyr (1942) reported different frequencies in forest soils (beech 64.2 %, spruce 39.8 %, pine 37.4 %, oak 26 %, alder 20 %, birch 32.5 %), but he did not take into into account environmental and soil pollution that was substantially lower sixty years ago than (recently or) at present. Air pollutants (mainly sulphur dioxide) had negative impacts on the environment and forest soils while bacterial counts, especially of the genus *Thiobacillus,* increased (Lettl 1984) and total counts of micromycetes in forest soils decreased. The population densities of *Micromucor ramannianus* v. *ramannianus* are also likely to fluctuate snd decrease, although it has not been verified experimentally in the last sixty years. Since this fungus is not present in less nutritive soils, it is to deduce that it needfs forest soil humus for its life. Some researchers did not discover this fungus in forest (Grunda 1981). Due to its high frequency in forest soils, it is possible to anticipate the fungus' high

Table 3. Types of forest soil according to biological characteristics.

	High fertile soils	Moderately fertile soils	Weakly fertile soils	Non-fertile soils
pH (H_2O)	6–7	5–6	4–5	4–3
pH (KCl)	5–6	4–5	3–4	3–2
% humus	20	15–19	10–14	5–9
% C	20–15	15–10	10–5	5–0
% N	2–1,5	1–1,5	1–0,5	0,5–0
C: N	20	19–15	10–15	5–10
		20–25	25–50	30–40
Basal respiration	> 1000	> 500	> 250	< 250
Potential respiration	4×	3×	2×	< 1×
Ammonification	> 50	> 25	> 10	< 10
Nitrification	100–80	80–50	25–50	25–0
Number of aer. bact.	> 5 mil.	> 0,5 mil.	> 10 000	> 1000
Number of am. bact.	> 1 mil.	> 0,5 mil.	> 10 000	> 1000
% *Micromucor* from CFU	80–100	50–80	< 50	

Values: Basal respiration – in mg CO_2 released after 24 h at 21°C
Potential respiration – how many times is higher than basal respiration
Ammonification – in mg ammonia nitrogen after 14 days at 21°C
Nitrification – in mg nitrate nitrogen after 14 days at 21°C

metabolic activity like e.g. in *Penicillium chrysogenum* (Rodriquez et al. 1994). The genus *Penicillium* was isolated from many types of forest soils and there dominates (Tzean et al. 1992). Even new species of *Penicillium* and their perfect stages were isolated (Takade and Udagawa 1983) A very broad spectrum was isolated by Vláčilíková (1978), although *Mortierella ramanniana* ranked second among all isolated species in her study (*Penicillium* was a dominant genus), the rate of the fungus appeared to be low. Many authors did not indicate the horizons where the fungus was detected. The spectra of soil micromycetes were determined at different depths (1–2 cm, 2–7 cm, 7–15 cm, 15–25 cm) (Grunda and Marvanová 1982). The genus *Mortierella* was isolated from a depth of 15–25 cm, although it was not explicitly described as *Micromucor ramannianus*. The spectrum of micromycetes detected in our study was not so broad as reported in above paper. Mutual interactions of many of the reported genera are possible in vitro as well as in soil. It would also be interesting to study their antagonistic effects (Veselý 1997). Soils can undergo development during the year, and micromycetes along them, as was investigated by Marfenina (1991). They can have fungistatic effects on soil micromycetes. Kubátová, Váňová and Prášil (1998) determined the soil micromycetes from Šumava Mts. (Bohemian Forest). Amongst 139 fungal species *Micromucor ramannianus* v. *ramannianus* was on the third place from most frequent species (33.1 %) and this species occurred on all localities.

This paper was exclusively focused on the species spectrum of soil micromycetes that were associated with the presence of a fungus typical of forest humic horizons – *Micromucor ramannianus* v. *ramannianus*. The fungus appears to be a basic micromycete of forest soils underlying biological functions of soil (respiration, ammonification, nitrification).

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