Competition of *Scleroconidioma sphagnicola* with fungi decomposing spruce litter needles

O. Koukol, L. Mrnka, A. Kulhánková, and M. Vosátka

Abstract: This study addressed competition of *Scleroconidioma sphagnicola* Tsuneda, Currah & Thormann with saprotrophic ascomycetes and basidiomycetes. We isolated this fungus, previously considered as a preferentially necrotrophic parasite of moss, from spruce needle litter. Competition of *Scleroconidioma sphagnicola* was simulated with strains of the autochthonous litter colonizers *Ceuthospora pinastri* (Fr.) Höhn., *Chalara longipes* (Preus) Cooke, *Setulipes androsaceus* (L.) Antonín (*Marasmius androsaceus* (L.) Fr.), and *Mollisia minutella* (Sacc.) Rehm and the wood-decaying fungus *Hypholoma fasciculare* (Huds.) Quél. Pairings were performed on agar plates with two types of low-nutrient medium made from spruce litter needles. Reisolation on nutritionally rich agar medium following the competition revealed that *Sleroconidioma sphagnicola* was mostly successfully reisolated even though apparently being replaced by the mycelium of other fungi. It formed strongly melanised mycelium and microsclerotia that seem to be responsible for its resistance to fungal competition. All tested strains of needle litter colonizers were outcompeted by *Hypholoma fasciculare*. Enzymatic screening aimed at semiquantitative assay of polyphenol oxidase, peroxidase, and tyrosinace revealed that *Scleroconidioma sphagnicola*, together with other strains, was able to produce polyphenol oxidase and peroxidase on various nutritional media. Activity of tyrosinase was detected only for *Hypholoma fasciculare*. Previous records of *Scleroconidioma sphagnicola* from moss, wood, and our isolations from spruce litter suggest that this species possesses an ability to occupy a wide spectrum of niches.

Key words: competition, spruce needle litter, saprotrophic fungi, *Scleroconidioma sphagnicola*, oxidative enzymes.

Résumé : Cette étude porte sur la compétition du *Scleroconidioma sphagnicola* Tsuneda, Currah & Thormann avec des ascomycètes et basidiomycètes saprophytes. Les auteurs ont isolé ce champignon, autrefois considéré comme un parasite des mousses préférentiellement nécrotrophe, à partir d'aiguilles d'épinette. Ils ont simulé la compétition du *Scleroconidioma sphagnicola* avec des souches de colonisateurs de litières indigènes *Ceuthospora pinastri* (Fr.) Höhn., *Chalara longipes* (Preus) Cooke, *Setulipes androsaceus* (L.) Antonín (*Marasmius androsaceus* (L.) Fr.) et *Mollisia minutella* (Sacc.) Rehm et un champignon de carie du bois, l'*Hypholoma fasciculare* (Huds.) Quél. Les pariades ont été effectuées sur plaques gélosées, avec deux types de milieux faibles en nutriments constitués à partir de litières d'aiguilles d'épinette. Suite à la compétition, on réussi généralement à réobtenir le *Scleroconidioma sphagnicola* sur milieu gélosé riche en nutriments, même s'il avait été apparemment remplacé par le mycélium des autres champignons. Il forme du mycélium fortement mélanisé et des microsclérotes qui semblent responsables de la résistance de ce champignon à la compétition fongique. Toutes les souches de colonisateurs des litières testées sont dépassées par le *Hypholoma fasciculare*. Un tamisage enzymatique visant à tester semi-quantitativement la polyphénol oxydase, la peroxydase et la tyrosinase révèle que le *Scleroconidioma sphagnicola*, tout comme les autres souches, est capable de produire de la polyphénol oxydase et de la peroxydase, sur divers milieux nutritifs. On détecte l'activité de la tyrosinase seulement chez le *Hypholoma fasciculare*. Les mentions antécédentes du *Scleroconidioma sphagnicola* sur mousse et sur bois, ainsi que les isolats obtenus par les auteurs sur litière d'épinette, suggèrent que cette espèce a la capacité d'occuper une large gamme de niches.

Mots clés : compétition, litière d'aiguilles d'épinette, champignon saprophyte, *Scleroconidioma sphagnicola*, enzymes oxydantes.

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Introduction

Dematiaceous anamorphic ascomycetes form a substantial part of the mycoflora of coniferous litter needles. Among them, the strongly melanized fungi allied with the Dothideomycetes represent a widespread group of fungi present on plant litter (Söderström 1975) and woody debris (Vasiliauskas et al. 2005) and that have become adapted to various environments, including those with stress conditions (Sterflinger et al. 1999). Apart from their resistance to harsh environmental conditions, there is less information about their response to biotic factors, namely competition with other fungi. Various approaches have been used to provide lists of ascomycetes colonizing coniferous litter, quantify their relative occurrence, and state their succession (i.e., on pines: Kendrick and Burges 1962; Brandsberg 1969; Toku-

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masu 1978; Ponge 1991; Tokumasu et al. 1994; Tokumasu and Aoiki 2002; on spruce: Söderström 1975). Interactions and competition of these fungi have not been studied in much detail. Only a few studies have considered evidence of fungal competition in litter needles (Tokumasu 1998; Wardle et al. 1993; Gourbière et al. 2001).

During fungal isolations from *Picea abies* (L.) Karst. litter needles, we isolated six strains of dematiaceous hyphomycetes with thick-walled hyphae and hyaline endoconidia whose morphological description did not enable an obvious identification within known saprotrophic fungi colonizing coniferous litter. The polymerase chain reaction (PCR) analysis of the rDNA internal transcribed spacer (ITS) region revealed that the six isolates belonged to the recently described species *Scleroconidioma sphagnicola* Tsuneda, Currah & Thormann (Tsuneda et al. 2000). Originally, this fungus was described as a parasite causing necrotic lesions of the peat-forming moss *Sphagnum fuscum* (Schimp.) Klinggr. Recent investigation also identified this fungus as an airborne colonizer of fresh spruce wood (Vasiliauskas et al. 2005). As there were no *Sphagnum* species growing in our locality and only sparse clumps of *Dicranum scoparium* Hedw. with no evidence of infection, we assumed that the fungus was capable of saprotrophic life in spruce needle litter. These data suggested that *Scleroconidioma sphagnicola* possessed two life strategies and broader ecological plasticity than originally expected.

To address the lack of data on competition with ascomycetes and litter colonizers, we used a series of competition experiments on low-nutrient agar media made with spruce needles. The experiments used strains of basidiomycetes and ascomycetes isolated mostly from spruce needle litter. The strain of *Scleroconidioma sphagnicola* isolated from its new revealed niche was included to estimate how efficient it might be in fungal competition. The main questions that we investigated were how efficient are selected ascomycetes during competition with basidiomycetes and is *Scleroconidioma sphagnicola* able to compete with other saprotrophic spruce litter colonizers in vitro. The success during competition was confirmed after a reisolation test. Furthermore, as fungal competition is closely related to enzymatic activities, namely the production of oxidative enzymes (Boddy 2000), we estimated the production of three oxidative enzymes by our strains as well.

Materials and methods

Fungal strains

Fungal strains used for experiments were selected from strains isolated from spruce litter needles collected in October 2002 and September 2003. The sampling site was located in a well-preserved 80–100 year old spruce monoculture forest (*P. abies*) near "Novohuťský močál" swamp in the Bohemian Forest (Šumava National Park, Czech Republic). The uppermost litter layer O_l (2–3 cm thick) was removed and needles from O_f soil horizons were collected into sterile polyethylene bags. In the laboratory, individual needles were surface sterilized for 30 s with hydrogen peroxide (30%) and washed three times in sterile water. The first washing had the addition of 0.1 mL of Tween 20 to remove fungal spores from the needle surface. Washed needles were incubated in Petri dishes containing malt extract agar (1.5% MEA) (Sigma-Aldrich Co., St. Louis, Missouri) at room temperature. Growing mycelium was transferred to half-strength potato dextrose agar (50% PDA) (Sigma-Aldrich Co.) and pure cultures were preserved in the Culture Collection of Basidiomycetes (CCBAS) of the Institute of Microbiology (Academy of Sciences of the Czech Republic) and the Culture Collections of Fungi of the Faculty of Sciences (Charles University, Prague, Czech Republic).

Six strains of the fungus *Scleroconidioma sphagnicola* were isolated. Other fungi representing the autochthonous mycoflora of the locality included the saprotrophic basidiomycete *Setulipes androsaceus* (L.) Antonín (*Marasmius androsaceus* (L.) Fr.) (CCBAS 859/I) and three saprotrophic ascomycetes: *Chalara longipes* (Preus) Cooke (CCF 3367), *Ceuthospora pinastri* (Fr.) Höhn. (CCF 3551), and *Mollisia minutella* (Sacc.) Rehm (CCF 3550). The white rot basidiomycete *Hypholoma fasciculare* (Huds.) Quél. (CCBAS 858/VI) was included as a representative of strong competitors among wood-decaying fungi whose growth and morphological characteristics in culture have been intensively studied (Griffith et al. 1994*a*, 1994*b*). *Hypholoma fasciculare* was isolated from fruit bodies collected in a spruce forest about 50 km from the previously described locality in October 2003.

Identification of *Chalara longipes* and *Ceuthospora pinastri* was based on the morphology of conidia, conidiophores, and pycnidia (Nag Raj and Kendrick 1975; Nag Raj 1993). *Setulipes androsaceus* and *Hypholoma fasciculare* were identified in the field from their fruit bodies (Moser 1983). The identification of *Scleroconidioma sphagnicola* and *Mollisia minutella* was based on the PCR analysis of the ITS region of rDNA. Genomic DNA was first extracted from pure cultures using the Dneasy plant mini kit (Qiagen GmbH, Hilden, Germany) and the ITS1 region amplified by PCR using ITS1F and ITS2 primer pairs. PCRs were performed with an iCycler Thermal Cycler (BioRad Laboratories, Hercules, California). PCR products were purified using a commercial kit (QIAquick PCR Purification Kit, Qiagen GmbH) according to the protocol and sequenced by MWG-Biotech AG (Ebersberg, Germany). Later, DNA isolation was repeated using the sorbitol method (Štorchová et al. 2000) and amplified by PCR using ITS1F and LR21 primer pairs to obtain a longer sequence (including ITS1, the 5.8S rDNA subunit, and the ITS2 region). PCR products were purified with a High Pure PCR product purification kit (Roche Diagnostics Co., Basel, Switzerland) and sequenced by GATC Biotech AG (Konstanz, Germany). Obtained sequences were compared with sequences available in the GenBank database of the National Center for Biotechnology Information (NCBI) using Blast software.

Agar media

Two agar media were prepared for pairing tests to simulate different nutritional levels. Sieved dried needles (2 mm sieve) were soaked in distilled water for 24 h (50 g of needles per 1.5 L). The suspension was then filtered through filter paper (Filtrak Nr. 288) to obtain a pure extract of leached nutrients but without any solid particles (SPEA, spruce extract agar). The filtered needles were returned to

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fresh distilled water to obtain a suspension with 5% content (*w*/*v*) of spruce needles (SPNA, spruce needles agar). Agar (15 g/L) was added to both the fractions and these media were autoclaved for 20 min. Additional nutrients or inorganic elements were not added. The elemental analysis of the needles used for the SPNA revealed that the C:N ratio was 22:1 with the P content less than 0.2 mg/L. Most of the P was extracted in the distilled water giving a concentration of 5.92 mg/L in the SPEA medium. The total phenol concentration expressed as the content of gallic acid in the SPEA and SPNA agar reached 0.93 and 1.62 g/L, respectively (Singleton and Rossi 1965).

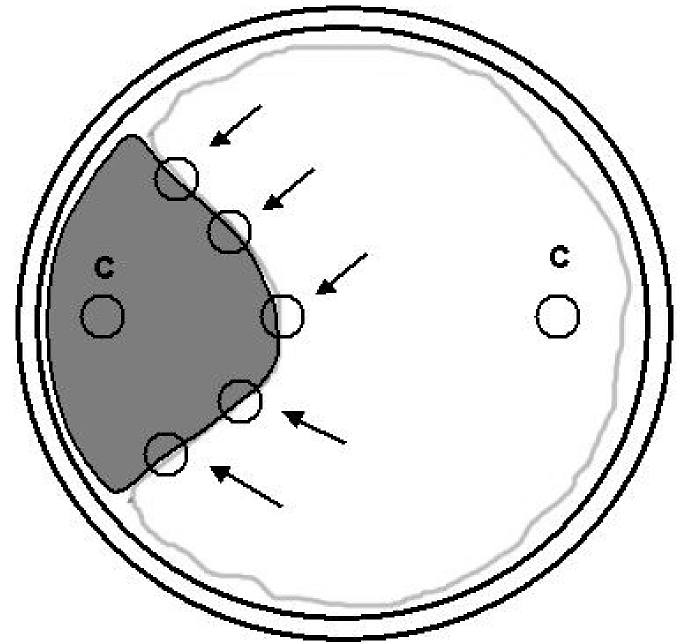
Experiments on agar media

All *Scleroconidioma sphagnicola* strains possessed identical growth characteristics; thus, only strain CCF 3545 was used for further competition experiments. Pairings between isolates of individual species were performed in 9 cm diameter plastic Petri dishes. For each fungal isolate, mycelial disc inocula 5 mm in diameter were cut from the margin of actively growing colonies on 50% PDA. Discs were placed opposite one another and 3 cm apart providing equal distance between colonies and from the colony to the margin of the Petri dish. Control pairings were performed between mycelia of identical strains; discs were taken from the same colony. Five replicates were set up for each combination including the control. Growth rate of strains was expressed as radius of the control colony measured 9 d after inoculation. Description of interactions and growth patterns (pycnidial production, colour, and growth changes) were recorded at 1 week intervals beginning 6 d after inoculation. Three months later, the observed results of competition were quantified after fungal reisolation (adapted from Holmer et al. 1997). Five discs of 5 mm diameter were cut from the contact zone between mycelia and two discs (one per isolate) from areas with presumed growth of only one individual fungus (Fig. 1). The reisolation discs were placed in Petri dishes containing 1.5% MEA. The outgrowing mycelium was identified by comparing it with the original strain and the number of discs reisolated per individual species was counted.

Enzyme production

The production of oxidative enzymes was estimated using “spot tests” described by Gramss et al. (1998). Solutions of chromogenic phenolics were applied to assess production of the following enzymes: polyphenol oxidase (EC 1.14.18.1) reacts with 2.5% guaiacol dissolved in ethanol to form red to dark reddish coloration, tyrosinase (EC 1.10.3.1) reacts with *p*-cresol at 0.1 mol/L dissolved in ethanol to form a yellow to red colour, and peroxidase (EC 1.11.1.7) reacts with separate solutions of 0.2% H_2O_2 and 0.5% pyrogallol in water (*w*/*w*) to form a yellow to brown colour. This method enables only semiquantitative assessment of production and the coloration was only characterized as weak, medium, or strong after 1 and 24 h. The tests were performed in duplicate. Our previous usage of this method showed that enzyme production within 24 h was dependent on the agar media (O. Koukol, data not included). Therefore, we performed the tests with colonies growing on 1.5% MEA, 50% PDA, and SPEA.

Fig. 1. Design of the reisolation test from a Petri dish with two competing mycelia (shaded and open areas). The reisolation discs were taken from fives spots in the contact zone (circles marked with arrows) and from the colony margins as control (circles marked with C).



Statistical analysis

The success of strain A in a particular pairing with strain B was quantified as the sum of discs reisolated by the strain from one Petri dish. These sums were transformed as follows:

$$s = \ln\left[(A+1)(B+1)^{-1}\right]$$

where *s* is the score quantifying success of a particular strain in the pairing, ln is the natural logarithm, and *A* and *B* are the means of reisolated discs by strain A and strain B (n = 5). The A + 1 and B + 1 values were used to prevent dividing by zero (when one of the fungi was not reisolated). The scores were used instead of the sum of captured discs, as the scores also included information about the number of reisolated discs of the opposing strain. Growth rates of fungal strains were compared and analysed using one-way ANOVA (SPSS statistics program).

Results

Molecular identification

The PCR analysis performed with the ITS1F and ITS2 primer pair yielded sequences of ±220 bp length. Sequencing of the obtained products revealed that all strongly melanized fungi isolated from Norway spruce needles were identical to sequences of *Scleroconidioma sphagnicola* (NCBI accession Nos. AY220610 and AY805592). Further PCR analysis performed with the ITS1F-LR21 primer (obtained approximate size 1050 bp) confirmed these findings. The sequence of our strain CCF 3545 was deposited under accession No. DQ182416 in the NCBI GenBank. The identification of strain CCF 3550 resulted in a 99% homology

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Table 1. Growth rate of fungal strains on SPEA and SPNA agar media measured as radius of control colonies 9 d after inoculation.

	Mean colony radius (cm)	
Fungal strain	SPEA	SPNA
Ceuthospora pinastri	15±0.89*	20.4±0.8
Chalara longipes	7±0.0	6.6±1.02
Hypholoma fasciculare	22±0.0*	23±0.0
Setulipes androsaceus	5.2±0.75*	16±1.41
Mollisia minutella	7.8±0.75*	11±0
Scleroconidioma sphagnicola	14±0.89*	21.8±1.83

Note: The values are means ± SD (n = 5); *, significantly lower growth on SPEA than on SPNA at $p < 0.05$ using one-way ANOVA.

with the *Mollisia minutella* sequence (NCBI accession No. AJ430223).

Competition studies

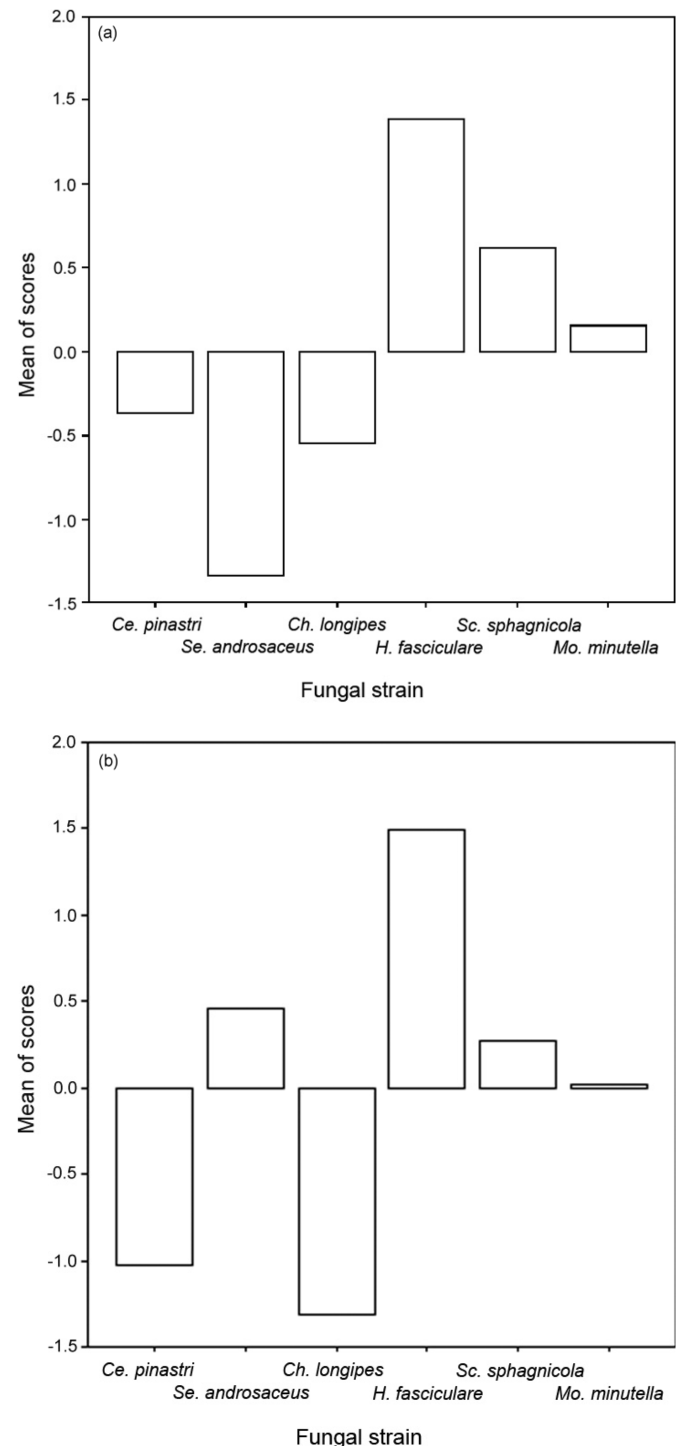
All fungal strains were able to grow on the two low-nutrient agar media, but their growth rates differed substantially. The fastest growing mycelium, that of *Hypholoma fasciculare*, grew approximately three times faster than mycelium of *Chalara longipes* (Table 1). With the exception of *Chalara longipes*, all strains grew faster on SPNA medium than on SPEA medium and the difference was significant ($p < 0.05$).

In competition tests, the five replicates gave almost identical growth patterns. The scores from reisolation revealed the following results of competition. For litter-colonizing fungi, *Scleroconidioma sphagnicola* was the most successful strain on the agar media from SPEA (Fig. 2*a*). It was not affected by the absence of needles. The presence of spruce needles in the SPNA medium strongly enhanced the competitive abilities of *Setulipes androsaceus* (Fig. 2*b*). This strain dominated among the strains of litter colonizers. Dense "mycelial ridges" produced in abundance on SPNA medium (Fig. 3) were probably a reaction to the needles embedded in the agar and not a direct response to the opposite mycelium. All selected strains of needle litter colonizers were weaker competitors than the representative of wood-decaying basidiomycetes, *Hypholoma fasciculare*, which was able to outcompete the rest of the strains regardless of the medium.

The outcome of interactions observed after contact between mycelia was in first sight in agreement with deadlock (including noncontact inhibition) (Fig. 5), partial replacement (Fig. 6), and complete replacement (Fig. 4) as defined by Boddy (2000). Although complete replacement is supposed to result in elimination of the weaker competitor, reisolation tests revealed that the mycelium of *Scleroconidioma sphagnicola*, replaced by *Hypholoma fasciculare* and *Mollisia minutella* mycelia, showed some ability to grow from reisolation discs (Table 2). *Hypholoma fasciculare*, *Setulipes androsaceus*, and *Mollisia minutella*, which frequently replaced other strains, did not change their mycelial morphology. However, during competition of *Hypholoma fasciculare* with *Ceuthospora pinastri* on SPNA medium, the former produced branched mycelial cords after contact with the mycelium of the latter (Fig. 4).

Most of the pairings with *Scleroconidioma sphagnicola* resulted in noncontact (Fig. 5). Growing mycelia almost met forming a distinct zone not exceeding several millimetres. The morphology of colony along the zone exhibited the growth strategy "phalanx" in which frequently branched but shortened hyphae were produced on the colony margin facing the opposing mycelium. This strategy is typical for anamorphic ascomycetes (Carlile 1995). Reisolation after noncontact deadlock resulted mostly in growth of both strains, even from the same disc. The same result of reisola-

Fig. 2. Mean reisolation of model fungal species from all competition pairings on SPEA (*a*) and SPNA (*b*) agar. Bars refer to higher success (bars above zero) or lower average success in competition (bars under zero).



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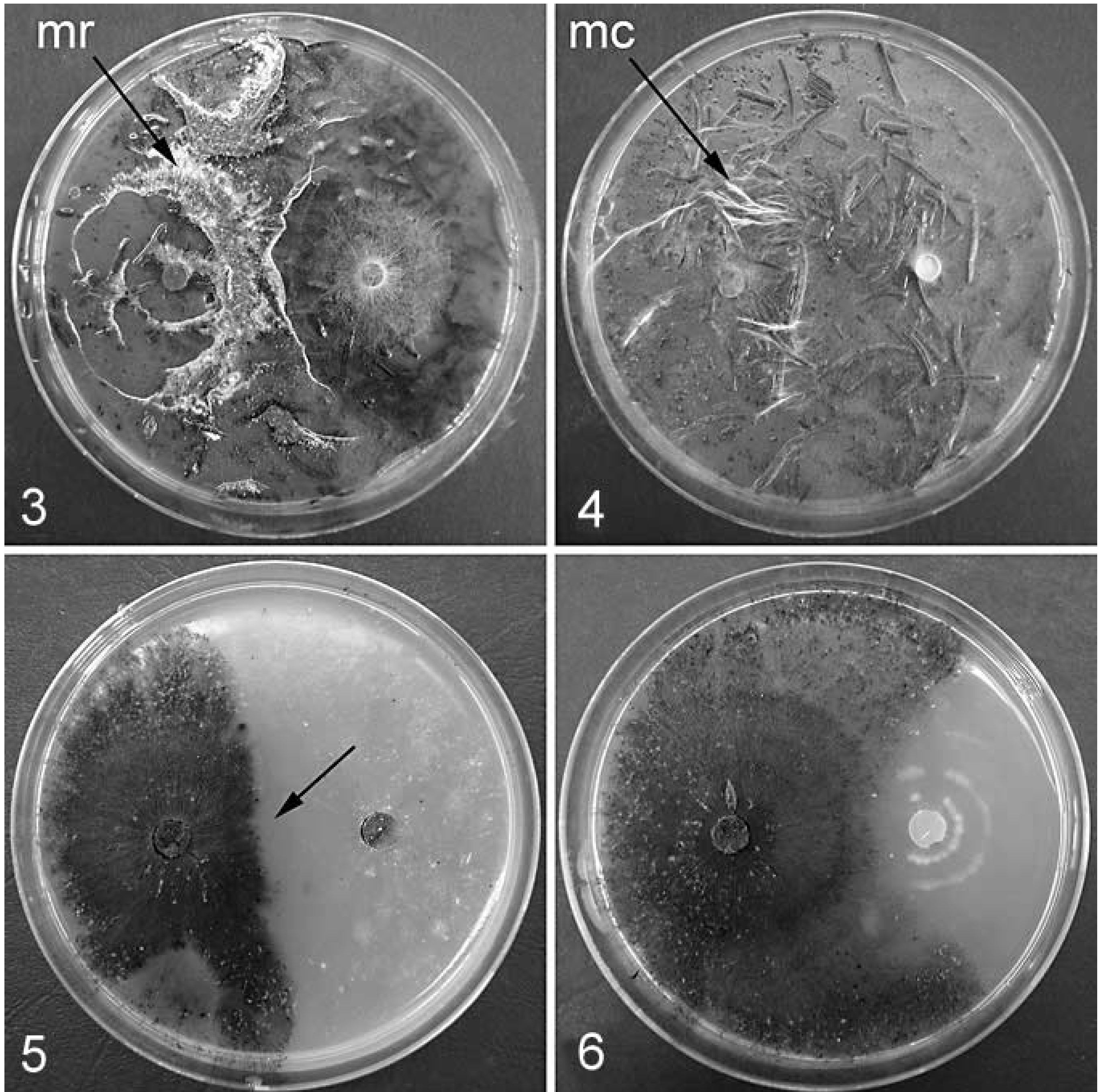
Figs. 3–6. Mycelial competition between strains of litter needle colonizers and wood-decaying fungus in agar plates (9 cm diameter). The fungus mentioned first was inoculated on the left side of the plate and that mentioned second on the right side. Fig. 3 *Setulipes androsaceus* replacing *Mollisia minutella* on SPNA medium after 45 d; note the abundant mycelial ridges (mr) of *Setulipes androsaceus*. Fig. 4 *Ceuthospora pinastri* being replaced by *Hypholoma fasciculare* producing branched mycelial cords (mc) after contact with the mycelium of *Ceuthospora pinastri* on SPNA medium after 45 d. Fig. 5 Noncontact deadlock between *Scleroconidioma sphagnicola* and *Ceuthospora pinastri* on SPEA medium after 60 d; the zone between mycelia is indicated by an arrow. Fig. 6 *Scleroconidioma sphagnicola* partially replacing *Setulipes androsaceus* on SPEA medium after 85 d.



tion was recorded also after *Scleroconidioma sphagnicola* partially replaced *Setulipes androsaceus* (Fig. 6).

Enzyme production

The intensity of colorization for most of the tested fungal strains differed among nutritionally poor SPEA and richer PDA and MEA, and the results were mostly species dependent (Table 3). No general conclusion about the induction of enzymatic activity on nutritionally poor media could be derived. The mycelium of *Scleroconidioma sphagnicola* produced two of the tested oxidative enzymes. The presence of guaiacol gum resulted in weak activity of polyphenol oxi-

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Table 2. Scores of reisolation from individual pairings on SPEA and SPNA media.

	Scleroconidioma sphagnicola	*Mollisia minutella*	*Setulipes androsaceus*	*Hypholoma fasciculare*	*Chalara longipes*	*Ceuthospora pinastri*
SPEA						
Ceuthospora pinastri	0.2/5[a]	3/5[a]	5/0	0/5	2/1.4	5
Chalara longipes	0/5	1.6/5[a]	3.8/0.2	0/5	5	
Hypholoma fasciculare	5/1.2[a]	5/0	5/0	5		
Setulipes androsaceus	1/5[a]	0/5	5			
Mollisia minutella	3.4/5[a]	5				
Scleroconidioma sphagnicola	5					
SPNA						
Ceuthospora pinastri	0.4/5[a]	0/5	0/5	0/5	5/2[a]	5
Chalara longipes	0/5	0/5	0/5	0/5	5	
Hypholoma fasciculare	5/0	5/0	5/0	5		
Setulipes androsaceus	3.4/3.6[a]	4/1	5			
Mollisia minutella	2.8/5[a]	5				
Scleroconidioma sphagnicola	5					

Note: Numbers refer to the mean of reisolated discs (n = 5) by individual fungi in particular interacting pairs; the first number refers to the fungal species in the left column and the second to the upper one.

[a]Both species grew from one reisolation disc.

dase on SPEA and 1.5% MEA with no reaction on 50% PDA. The pyrogallol droplet induced a medium to strong reaction of peroxidase on all three agar media. The presence of *p*-cresol caused only a weak reaction on 1.5% MEA and 50% PDA. All reactions progressed slowly, reaching maximal intensity after 24 h. *Hypholoma fasciculare* showed a strong to medium reaction with all substrates tested, having the strongest enzymatic activity of all tested fungi.

Discussion

The anamorphic ascomycete *Scleroconidioma sphagnicola* was described as a necrotrophic parasite of *Sphagnum fuscum* growing in boreal peat bogs in Canada (Tsuneda et al. 2000). However, Vasiliauskas et al. (2005) recently isolated this species from freshly cut spruce stems in Sweden. Six strains of *Scleroconidioma sphagnicola* isolated during our study from spruce needle litter confirmed the saprotrophic ability of this fungus. The ability of *Scleroconidioma sphagnicola* to withstand adverse abiotic conditions seems to be valuable also during fungal competition. The strain selected for our competition study revealed its ability to grow in low-nutrient media and was not substantially affected by lack of nutrients during competition, which is of particular importance, as factors such as diminishing content of nutrients during needle decomposition, leaching with percolated water (Hongve et al. 2000), and the grazing of soil fauna, namely oribatid mites (Kaneko et al. 1995), affect fungal colonization and interactions. Compared with other strains, *Scleroconidioma sphagnicola* formed predominantly a substrate mycelium composed of thick and strongly melanized cell walls. Together with the formation of microsclerotia, these features were already suggested by Hambleton et al. (2003) to play a key role during interaction with other fungi and for survival in unfavourable microclimatic conditions. We suggest that these features served as a defensive reaction against the approaching mycelium of the competitor. Mycelium of *Scleroconidioma sphagnicola* remained viable and grew from reisolation discs even after being completely replaced by *Mollisia minutella* and *Hypholoma fasciculare* (Fig. 2*a*). The latter is of great importance, as the strain of wood-decaying fungus exceeded all other tested strains in both growth rate and enzyme production.

Setulipes androsaceus, known as one of the major colonizers of spruce, pine, and fir needles (Mitchell and Millar 1978; Gourbière and Corman 1987), dominated among strains of litter colonizers in SPNA medium. Mycelial ridges, rhizomorphs, and even fruit bodies were also observed on inoculated sterile litter needles where temperature and humidity were favourable for fungal growth (O. Koukol, data not included).

Although *Scleroconidioma sphagnicola* survived in competition and *Setulipes androsaceus* often replaced other strains, litter colonizers were weaker competitors compared with the strain of wood-decaying fungus tested. The fact that *Hypholoma fasciculare* was able to outcompete the other strains suggests that litter-colonizing fungi probably invested less energy in direct mycelial competition. Data from this study are difficult to compare with the literature, as only a few studies have considered competition in forest litter with ascomycetes as model species. Wardle et al. (1993) assessed the competition of *Mucor hiemalis* Wehmer with *Trichoderma harzianum* Rifai, and *Trichoderma polysporum* (Link) Rifai in agricultural soil and forest litter based on propagule production. Distribution and colonization of these fungi were driven mostly by their enormous production of spores and conidia. Hundreds of ascomycetes, which do not produce fertile structures (fruit bodies, conidiomata) or produce fewer propagules, occupy the needle litter as well. Tokumasu (1998) recorded a decrease in frequency of occurrence of the fungus *Selenosporella curvispora* G. Arnaud ex MacGarvie during fungal succession on *Pinus densiflora* Siebold & Zucc. needles in Japan. He attributed this fact to the competition with *Verticicladium trifidum* Preuss. (anamorph of *Desmazierella acicola* Lib.), as the occurrence of *Selenosporella curvispora* considerably increased in the absence of *Verticicladium trifidum*.

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Table 3. Reaction of fungal strains to droplets of guaiacol, pyrogallol, and *p*-cresol testing for the presence of polyphenol oxidase, peroxidase, and tyrosinase, respectively.

	SPEA		1.5% MEA		50% PDA	
	1 h	24 h	1 h	24 h	1 h	24 h
Guaiacol – polyphenol oxidase						
Ceuthospora pinastri	–	+	–	–	+++	+++
Chalara longipes	0	0	–	–	–	–
Hypholoma fasciculare	+	+++	++	+++	+	++
Setulipes androsaceus	+	+	–	+	++	++
Mollisia minutella	++	+++	+	+	++	+++
Scleroconidioma sphagnicola	+	++	+	+	–	–
Pyrogallol–peroxidase						
Ceuthospora pinastri	+	++	–	–	++	++
Chalara longipes	0	0	+	+	+	+
Hypholoma fasciculare	++	+++	++	++	+	++
Setulipes androsaceus	+	+	–	+	+	++
Mollisia minutella	+	+++	–	++	+	++
Scleroconidioma sphagnicola	+	+++	++	++	++	+++
***p*-Cresol–tyrosinase**						
Ceuthospora pinastri	–	–	–	–	–	–
Chalara longipes	0	0	–	–	–	–
Hypholoma fasciculare	+	++	++	++	+	++
Setulipes androsaceus	–	–	–	–	+	+
Mollisia minutella	–	–	–	–	+	+
Scleroconidioma sphagnicola	–	–	–	+	–	+

Note: Intensity of reaction: –, negative; +, weak; ++, medium; +++, strong reaction; 0, not tested.

Competition of species that fruit and form distinct conidiophores such as *Lophodermium pinastri* (Schrad.) Chevall. and *Verticicladium trifidum*, respectively, was detected directly in the field or in the laboratory (Gourbière et al. 2001). In our experience, the results of competition among our model strains were not really detectable by direct observation. The reisolation tests offered a reliable tool to confirm the success of individual strains after contact with a competitor's mycelium.

The life strategies of *Scleroconidioma sphagnicola*, including necrotrophic parasitism on peat moss and saprotrophic colonization of spruce needles, require several similar characteristics. Both *Sphagnum* cell walls and spruce litter needles have a high C:N ratio and contain substantial amount of polyphenols, specifically lignin and tannins. *Sphagnum* cells are also resistant to microbial degradation owing to the microstructure of the cell wall (Tsuneda et al. 2001*a*). Tsuneda et al. (2001*b*) observed that the mode of colonization of *Sphagnum* cells by *Scleroconidioma sphagnicola* and their utilization were similar to those of soft rot fungi. A key role in wood decay and decomposition of recalcitrant substances, such as *Sphagnum* cell wall and spruce litter, is attributed to oxidative enzyme production (Tuomela et al. 2002). Their detection is of importance, as they can reveal the capabilities of particular fungi. Polyphenol oxidase, peroxidase, and tyrosinase were detected in a wide variety of litter-degrading fungal strains (Gramss et al. 1998; Ghosh et al. 2003). These enzymes are also active during competition with other fungi (Boddy 2000). Our enzymatic assay proved the production of polyphenol oxidase and peroxidase by most of the strains including *Scleroconidioma sphagnicola*. Tyrosinase was detected only in *Hypholoma fasciculare*. Tyrosinase was observed to act contradictory to laccase, which induced production of mycelial cords in *Hypholoma fasciculare* (Griffith et al. 1994*a*). Activity of laccase was thus probably responsible for the production of mycelial cords during pairing of *Hypholoma fasciculare* and *Ceuthospora pinastri* (Fig. 4). Why the cords were not produced during other pairings remains unanswered.

The use of pairings on agar media or natural substrate has its limitations. Several studies showed that the outcome of mycelial competition on rich artificial agar media differed from that on the natural substrate, i.e., wood (Holmer and Stenlid 1993) or soil (Whipps 1987). The success of a particular species was often dependent on the inoculum size of the fungus and the quality of the substrate as well (Boddy 2000). However, as we consistently used the same quality and size of inoculum, we eliminated this problem.

To conclude, the role of *Scleroconidioma sphagnicola* in the ecosystem is not yet fully understood. It seems that this species has a broad distribution and a rather high ecological plasticity. At the present state of our knowledge, two ecological strategies of *Scleroconidioma sphagnicola* have been identified: necrotrophic parasite on moss and saprotrophic colonizer of wood and litter needles. Further research using systematic sampling of litter needles over the seasons might show whether *Scleroconidioma sphagnicola* belongs to rare species occurring only during specific conditions or is able to form a substantial part of the litter mycoflora. At present,

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the seasonal dynamics are uncertain. Colonization of woody stems in Sweden was significantly higher during summer months compared with autumn (Vasiliauskas et al. 2005). On the other hand, our original strains were isolated in late autumn (2002 and 2003). Recently, isolation from October 2005 yielded five more strains of *Scleroconidioma sphagnicola* from spruce litter needles and pine (*Pinus mugo* Turra) litter as well.

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