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# Autecology of *Scleroconidioma sphagnicola* particularly in Šumava National Park (Czech Republic)

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The anamorphic fungal species *Scleroconidioma sphagnicola* was repeatedly isolated from spruce and pine litter needles in Šumava National Park. The morphology of thirteen strains of this fungal species was compared; oxidative enzymes and possible parasitism on *Sphagnum* in vitro were tested. Our results showed that all of the strains differed from the original description in only one characteristic – microsclerotia lacked conidiogenous cells on their surface. All strains produced laccase and peroxidase, eleven strains produced polyphenol oxidases. Inoculation of *Sphagnum* species resulted in only negligible colonisation. We suppose that *Scleroconidioma sphagnicola* in the studied area does not parasite on *Sphagnum*. This conclusion is supported by the fact that strains of *Scleroconidioma sphagnicola* were isolated from needles lying among *Sphagnum*, but no diseased plants were found. Results of our previous experiments with *Scleroconidioma sphagnicola* dealing with decomposition of spruce litter needles and competition with other fungal species are summarised.

**Key words:** *Scleroconidioma sphagnicola*, *Dothideales*, microsclerotia, coniferous litter, *Sphagnum*

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Anamorfní askomycet *Scleroconidioma sphagnicola* byl opakovaně izolován ze smrkových a borových jehlic v opadu v NP Šumava. Morfologické znaky třinácti kmenů tohoto druhu byly porovnány s originálním popisem. Produkce oxidativních enzymů a potenciální parazitizmus na rašeliníku byly ověřeny in vitro. Z našich výsledků je patrný jediný rozdíl v morfologii pozorovaných kmenů oproti originálnímu popisu. Nebyly nalezeny konidiogenní buňky na povrchu mikrosklerocií. Všechny kmeny produkovaly lakázu a peroxidázu, jedenáct kmenů produkovalo polyfenol oxidázu. Testované kmeny nebyly schopny kolonizovat rašeliník v experimentálních podmínkách. Domníváme se, že populace druhu *S. sphagnicola* ve sledované oblasti neparazituje na rašeliníku, neboť pět kmenů druhu *S. sphagnicola* bylo izolováno z jehlic ležících v rašeliníku, bez symptomů napadení. Výsledky našich předchozích prací zaměřených na dekompozici smrkových jehlic v opadu druhem *S. sphagnicola* a jeho kompetici s ostatními druhy hub jsou shrnuty společně s dalšími literárními údaji týkajícími se rozšíření a autekologie druhu *S. sphagnicola*.

## INTRODUCTION

Mycobiota of the Šumava National Park (NP) in the mountain range in the south-western part of the Czech Republic is particularly rich due to the high diversity of well-preserved natural and semi-natural ecosystems and specific microclimatic conditions (especially high humidity). Records of rare macro– and micromycetes from this area have been regularly published (e. g. Holec 2000, Holec 2004, Holec et al. 2002, Tomšovský 2002, Kubátová et al. 1998) including new fungal species and genera (e. g. Réblová and Seifert 2004). However, micromycete isolations were adopted only sporadically and large areas of the Šumava NP may still supply intriguing data. We carried out regular biannual isolations of saprotrophic fungi from litter needles collected in one of these areas, in the ecotone of spruce forest and swamp on the southern edge of the "Novohuťský močál" swamp, beginning in 2002. Our isolations often yielded sterile strains or strains with insufficient morphological features enabling their identification. Thus, sequencing of the internal transcribed spacer region (ITS) of the nuclear ribosomal DNA was adopted and strains were identified based on the alignment with known sequences (Koukol et al. 2006a).

Comparison of our ITS rDNA sequences with those published in the GenBank revealed that several of our sequences were identical to *Scleroconidioma sphagnicola* Tsuneda, Currah & Thormann, an anamorphic species parasiting on *Sphagnum fuscum* (Schimp.) Klinggr. in Canada (Tsuneda et al. 2000). Tsuneda et al. (2001a) verified the parasitic status of the fungus after further collections of diseased plants and examination under light and electron microscopes. Moreover, artificial inoculation of healthy plants resulted in the same symptoms.

In their further study dealing with the conidiogenesis of *Scleroconidioma sphagnicola*, Tsuneda et al. (2001b) distinguished the terms microsclerotium and conidioma. The former refers to multicellular sterile structures whose primary function is survival while the latter refers to stromatic bodies bearing conidiogenous cells. They also provided a detailed description of the pleomorphic conidiogenesis of *S. sphagnicola*. Juvenile hyphae of *S. sphagnicola* produced conidia percurrently (occasionally sympodially). Microsclerotia arose from older hyphae and developed into conidiomata as their surface cells converted into annellidic and phialidic conidiogenous cells.

The morphology of *S. sphagnicola* alone did not allow its phylogenetic placement, until Hambleton et al. (2003) provided the phylogenetic position of *S. sphagnicola* based on the analyses of the rDNA. The analyses suggested that *S. sphagnicola* is closely related to the *Dothideales*. According to the analysis of the SSU rDNA, it seems to be closely related to *Aureobasidium pullulans* (de Bary) G. Arnaud, a cosmopolitan saprotrophic coloniser of phylloplane, soil and plant debris including that of coniferous origin (Domsch et al. 1980, Tokumasu 1996).

The analysis of the ITS rDNA revealed that it clustered with *Rhizosphaera kalkhoffii* Bubák and *Rhizosphaera pini* (Corda) Maubl., two parasites of conifers.

The only other record of *S. sphagnicola* was provided by Vasiliauskas et al. (2005), who isolated *S. sphagnicola* from spruce woody debris in Sweden. Their study on airborne fungal colonisers revealed a surprisingly high frequency of *S. sphagnicola* colonising freshly cut sections of spruce stems.

Our isolates represent the third record of this species in the world and the first isolation in the Czech Republic. Furthermore, they were all obtained from a new niche of the species, coniferous litter needles. Strain *S. sphagnicola* NK08 (CCF 3545) was used in a series of laboratory experiments and its ability to decompose litter needles, participate in organic phosphorus transformation and compete with other fungal strains was described in earlier works of the first author (Koukol et al. 2006a, Koukol et al. 2006b). Results of these experiments suggest that the autecology of strains of *S. sphagnicola* from the Šumava NP is strikingly different from Canadian peat bogs. Therefore, we wanted to test the hypothesis that our strains are able to parasite on local populations of *Sphagnum* species as well. We also increased the present knowledge of the enzymatic abilities of *Scleroconidioma sphagnicola*, especially the production of oxidative enzymes. Finally a morphological comparison of 13 strains was carried out, as the morphology of *S. sphagnicola* is also extremely variable and various strains may exhibit substantial differences in conidiogenesis. This paper summarises our original data and literature records regarding morphology and autecology of *S. sphagnicola*.

## MATERIALS AND METHODS

**Fungal strains collection.** Strains of *S. sphagnicola* were isolated from litter needles collected regularly at the beginning of November between the years 2002 and 2005. The sampling site in the Šumava NP (Czech Republic) was situated 6 km S of the village of Modrava. The site (approx. 30 $m^2$) included an 80–100 year old unmanaged monoculture of *Picea abies* (L.) Karst. (*Calamagrostio-villosae Piceetum* ass.) and the "Novohuťský močál" swamp with growth of *Pinus ×pseudopumilio* (Willk.) Beck (*Sphagno-Pinetum mughi* ass.). Coordinates of the centre of the site were as follows: 48° 59.11 N / 13° 26.86 W, elevation approx. 1220 m above sea level. Although the forest was originally man-made, together with the swamp it belongs to zone 1 (strictly protected) of the Šumava NP. Limited access and lack of forestry management makes natural processes in the litter likely.

Single spruce needles were sampled by hand at several microlocalities within the sampling site with clearly distinguished $O_l$ and $O_f$ soil horizons (in both the forest and the swamp). Pine needles were sampled from the surface of the *Sphagnum* cover. Needles were put into sterile polyethylene bags. Codes of all 13 strains, habitats and dates of isolation are summarised in Tab. 1.

In the laboratory, individual needles were surface sterilised for 30 s with hydrogen peroxide (30 %) and washed in sterile water. Washed needles were incubated in Petri dishes containing malt extract agar (1.5 % MEA, Sigma-Aldrich Co., St. Louis, Missouri) at room temperature. Growing mycelia were

transferred to half strength potato dextrose agar (50 % PDA, Sigma-Aldrich Co.) and preserved in the first author's fungal collection and in the Culture Collection of Fungi (CCF, Prague). One strain was isolated from *Cephalcia abietis* L. (Hymenoptera: *Pamphiliidae*) frass pellets caught in litter containers (Kovářová and Vacek 2003). One strain was obtained from a personal collection of Rimvydas Vasiliauskas (SLU, Sweden).

Fungal morphology and microscopic observation. The fungal strains were cultivated on corn-meal agar medium with glucose (CMAD, HiMedia, India) prior to the observations under both light and scanning electron microscopes (SEM). This procedure eliminated the production of vegetative mycelia and enhanced conidia production (Tsuneda et al. 2000). Additional cultivations were performed on PDA and spruce extract agar (SPEA). The SPEA was prepared as follows: 25 g of $O_f$ spruce needles were extracted in 1 litre of deionised water overnight and the extract was filtered through filter paper. Finally, 15 g of agar was added to 1 litre of the extract and the medium was sterilised in an autoclave for 15 minutes (121 °C, 103 kPa).

The agar media in the Petri dishes were covered with sterilised discs of cellophane and the fungi were inoculated on the cellophane. This method simplified further preparation of the samples for microscopic observation (Kolařík, pers. com.). Pieces of cellophane overgrown by mycelium were cut with sterilised scissors and transferred together with mycelia onto a microscope slide or into an SEM observing chamber. Samples were prepared in Meltzer's reagent for light microscopy. Microscopic observations were performed with an Olympus BX61 scientific microscope. Pictures were taken with an Olympus DP70 camera. SEM photographs were taken with an FEI Quanta 200 ESEM environmental scanning electron microscope at the Environmental Mode at 25 kV (220 Pa and –12 °C). Pieces of cellophane with mycelium were observed directly after removal from the Petri dish, as this type of observation did not require fixation and metallic coating. Digital pictures were processed using the QuickPhoto Micro 2.0 software (Olympus, Hamburg, Germany).

**Tab. 1.** Detailed habitats and dates of isolation of strains of *S. sphagnicola*.

| Strain | Substrate | Habitat | Date of isolation |
|---|---|---|---|
| NK01 | *Picea abies* | $O_l$ litter needles | 5. 10. 2002 |
| NK02 | *Picea abies* | $O_f$ litter needles | 22. 9. 2003 |
| NK04 | *Picea abies* | $O_f$ litter needles | 22. 9. 2003 |
| NK05 | *Picea abies* | $O_f$ litter needles | 22. 9. 2003 |
| NK06 | *Picea abies* | $O_l$ litter needles | 22. 9. 2003 |
| NK08[1] | *Picea abies* | $O_f$ litter needles | 5. 10. 2002 |
| 3–3 | *Picea abies* | $O_f$ litter needles within *Sphagnum* sp. | 2. 11. 2005 |
| 4–1 | *Picea abies* | $O_f$ litter needles within *Sphagnum* sp. | 2. 11. 2005 |
| 5–1 | *Picea abies* | $O_f$ litter needles within *Sphagnum* sp. | 2. 11. 2005 |
| 9–1 | *Pinus* ×*pseudopumilio* | $O_l$ litter needles within *Sphagnum* sp. | 2. 11. 2005 |
| 10–2 | *Pinus* ×*pseudopumilio* | $O_l$ litter needles within *Sphagnum* sp. | 2. 11. 2005 |
| TVIII 03 | *Picea abies* | *Cephaltis abietis* frass pellets | 2. 11. 2005 |
| Olrim 428[2] | *Picea abies* | stem section | summer 2001 |

[1] CCF 3545, GeneBank Accession number DQ182416
[2] isolated by R. Vasiliauskas, GeneBank Accession number AY805592

Oxidative enzyme assessment. The production of four oxidative enzymes was estimated using "spot tests" described by Gramss et al. (1998). These tests enable a relatively fast semi-quantitative estimation of laccase, peroxidase, tyrosinase and polyphenol oxidase on the basis of colour changes of specific compounds added directly to the mycelium on the agar plate (naphthol, pyrogallol, p-cresol and guaiacol, respectively). Tests were performed on three agar media: 50 % PDA, SPEA and artificial medium with glucoses (AM, according to Anderson et al. 1999).

Artificial inoculation of *Sphagnum*. Possible parasitism of *Scleroconidioma sphagnicola* on local *Sphagnum* species was tested using the method described by Tsuneda et al. (2001b). In short, surface sterilised whorls of *Sphagnum* leaves were placed on 20-day old colonies of *Scleroconidioma sphagnicola* growing on PDA. Five whorls were placed on one Petri dish. Dishes were then kept at room temperature and observed regularly for 2 months. Five strains of *S. sphagnicola* were used (codes NK01, NK08, 9–1, Olrim428 and TVIII03) in combination with three *Sphagnum* species, *S. rubellum* Wils., *S. girgensohnii* Russ. and *S. russowii* Warnst. collected in the Březník valley (Šumava NP). Petri dishes were observed under a dissecting microscope regularly for 2 months and their colonisation was recorded.

## RESULTS

### Morphological characteristics

Greenish black colonies growing on CMAD grew slower compared to the original description and only strain 4–1 reached a diameter of 50 mm in 10 days as described by Tsuneda et al. (2000). Differences in growth rates between strain 4–1 and TVIII 03 were almost two-fold (Tab. 2). No tendency to grow more slowly was evident in strains kept longer in the culture collection. A thin white zone along the margin as mentioned in the description was observed only in colonies growing on PDA.

On CMAD all strains produced predominantly mycelium consisting of septate, melanised hyphae embedded in the agar. Aerial hyaline hyphae were also present and usually formed whitish tufts on the surface of the colony. Density of the colonies declined in the following order: PDA > CMAD > SPEA. Microsclerotia frequently formed on SPEA were both embedded in the agar and grew on its surface. They were made up of irregular cells with thick walls (Figs. 1–3). Although a substantial number of microsclerotia was observed, no conidiogenous cells on their surface were recorded. Samples of mycelium were observed under the electron microscope at regular intervals (for more than one month), but no agar medium including CMAD induced conidiogenous cell production. When growing on PDA, the formation of microsclerotia was suppressed and dense mycelium dominated.

Our strains produced conidia only from one type of intercalary conidiogenous cells in the vegetative hyphae. Conidia rested solitarily on the hyphae or were aggregated into clusters, especially in young colonies growing on CMAD (Figs. 4–7). The peg-shaped conidiogenous locus was visible under the light microscope as a thin-walled prominence (Fig. 4, arrow). Conidia were hyaline to slightly darkened, fusiform to spathulate, 1.5–3.4 µm wide and 5.5–10.5 µm long. This range is within the values described by Tsuneda et al. (2000), which were 1–3 × 6.5–9 µm.

**Tab. 2.** Morphological characteristics and growth rates of studied strains of *S. sphagnicola* (colony diameter measured after 10 days at 25 °C on CMAD, values of morphological characteristics represent means of 10 measurements ± S.E.).

| | Conidia size (µm) | | Mycelium width (µm) | | Growth (mm) |
|---|---|---|---|---|---|
| Strain | Length | Width | Melanized | Hyaline | |
| NK01 | 10.5 ± 0.60 | 2.1 ± 0.09 | 4.3 ± 0.09 | 1.8 ± 0.03 | 41 |
| NK02 | 7.3 ± 0.64 | 1.8 ± 0.04 | 3.8 ± 0.09 | 1.4 ± 0.11 | 41 |
| NK04 | 8.6 ± 0.76 | 2.2 ± 0.09 | 4.1 ± 0.13 | 1.6 ± 0.04 | 42 |
| NK05 | 9.8 ± 0.57 | 1.8 ± 0.13 | 4.4 ± 0.25 | 1.3 ± 0.04 | 36 |
| NK06 | 6.7 ± 0.25 | 1.5 ± 0.03 | 4.1 ± 0.13 | 1.2 ± 0.07 | 44 |
| NK08 | 9.2 ± 0.82 | 2.6 ± 0.19 | 3.6 ± 0.09 | 1.6 ± 0.11 | 45 |
| 3–3 | 10.3 ± 0.87 | 2.7 ± 0.16 | 4.7 ± 0.41 | 1.8 ± 0.09 | 44 |
| 4–1 | 7.4 ± 0.49 | 2.1 ± 0.10 | 3.2 ± 0.16 | 1.2 ± 0.09 | 50 |
| 5–1 | 7.8 ± 0.35 | 2.3 ± 0.09 | 4.3 ± 0.19 | 1.6 ± 0.07 | 35 |
| 9–1 | 7.4 ± 0.44 | 1.7 ± 0.06 | 4.4 ± 0.13 | 1.4 ± 0.06 | 40 |
| 10–2 | 8.6 ± 0.48 | 2.3 ± 0.18 | 3.8 ± 0.20 | 1.8 ± 0.14 | 38 |
| TVIII 03 | 5.5 ± 0.16 | 1.8 ± 0.06 | 3.6 ± 0.09 | 1.6 ± 0.09 | 26 |
| Olrim 428 | 7.3 ± 0.51 | 3.4 ± 0.09 | 4.6 ± 0.29 | 2.1 ± 0.27 | 36 |

## Enzymatic screening

Most of the strains produced laccase, polyphenol oxidase and peroxidase on at least one agar medium (Tab. 3). The strains NK05 and 9–1 did not produce polyphenol oxidase. All the reactions had a slow progress, reaching maximal intensity after 24 hours. Remarkable differences were found in the reaction detecting polyphenol oxidase. The strains NK05 and 9–1 failed to react with guaiacol on any of the tested media. None of the tested strains produced tyrosinase.

## Parasitism on bryophytes

Artificial inoculation of whorls of three *Sphagnum* species with five strains of *Scleroconidioma sphagnicola* resulted in a negligible fungal colonisation of the bryophyte tissue. Leaves remained sterile with the exception of minute areas where the leaves directly touched the mycelia on the agar medium. However, colonisation was limited only to these spots and consisted of dark sterile hyphae as observed under the light microscope. Fungal colonisation did not spread across the leaves and no conidiogenous structures including microsclerotia (conidiomata) were formed.

**Tab. 3.** Enzymatic screening of studied strains of *S. sphagnicola* on three oxidative enzymes on three different agar media. Reactions were observed 2 and 24 hours after addition of reactants, respectively. For abbreviations of agar media, see text; – no reaction; + weak reaction; ++ moderate reaction; +++ strong reaction (not recorded).

| | Laccase | | | | | | Polyphenol oxidase | | | | | | Peroxidase | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SPEA | | PDA | | AM | | SPEA | | PDA | | AM | | SPEA | | PDA | | AM | |
| Strain | 2 h | 24 h | 2 h | 24 h | 2 h | 24 h | 2 h | 24 h | 2 h | 24 h | 2 h | 24 h | 2 h | 24 h | 2 h | 24 h | 2 h | 24 h |
| NK01 | ++ | ++ | + | + | + | ++ | + | ++ | + | + | – | – | + | + | + | + | + | + |
| NK02 | ++ | ++ | + | + | – | ++ | + | + | + | + | – | – | + | ++ | + | + | + | + |
| NK04 | – | ++ | + | + | – | ++ | + | ++ | – | – | – | – | + | + | + | + | + | ++ |
| NK05 | ++ | ++ | + | + | – | + | – | – | – | – | – | – | + | + | + | + | + | + |
| NK06 | ++ | ++ | + | + | – | ++ | – | + | + | + | – | – | + | ++ | + | + | + | + |
| NK08 | + | ++ | – | + | + | ++ | + | + | – | – | – | – | + | + | + | + | + | ++ |
| 3–3 | ++ | ++ | + | + | – | + | + | ++ | – | – | – | – | + | + | + | + | + | ++ |
| 4–1 | ++ | ++ | + | ++ | + | ++ | – | + | – | – | – | – | + | + | + | + | + | + |
| 4–2 | ++ | ++ | + | + | – | + | – | + | – | – | – | – | + | ++ | + | + | – | + |
| 5–1 | ++ | ++ | – | + | – | + | – | – | + | + | – | – | + | + | + | + | + | + |
| 9–1 | ++ | ++ | – | ++ | – | + | – | – | – | – | – | – | + | + | – | – | + | ++ |
| 10–2 | ++ | ++ | – | ++ | – | + | + | + | – | – | – | – | + | + | + | + | + | + |
| T VIII 03 | ++ | ++ | + | + | – | ++ | – | + | – | – | – | – | + | ++ | + | + | – | + |

## DISCUSSION

### Morphology

In total, thirteen strains of *Scleroconidioma sphagnicola* were investigated on their growth and morphological characteristics, and enzymatically screened. According to Tsuneda et al. (2000), the pleomorphic conidiogenesis of *S. sphagnicola* includes four types of conidiogenous cells, two types formed on vegetative hyphae and two on conidiomata. Neither the observations under the light nor those under the electron microscope could distinguish whether the conidiogenous cells on the vegetative hyphae were annelidic or of a different type. Especially the use of an environmental scanning electron microscope brought rather disappointing results. When observed under the light microscope, the conidiogenous loci were visible as pale prominences growing from dark mycelium (Fig. 4). The loci were less distinct under the electron microscope (Fig. 5) and newly produced conidia were hardly distinguishable from conidia attached accidentally to the hyphae (Fig. 7). The use of an environmental scanning microscope markedly simplified the preparation of samples, but on the other hand, the resolution was insufficient to reveal morphological details of the conidiogenous cells.

Rather surprising was the absence of microsclerotia converted into conidiomata, which gave its name to the genus *Scleroconidioma*. Conidiomata were not observed on any of the agar media used, although the colonies were examined at regular intervals for several weeks. According to the sequence of conidiogenesis described by Tsuneda et al. (2001b), conidiomata should have been formed after no later than one week of incubation. Thus, we are using the term microsclerotia throughout this paper, as Tsuneda et al. (2001b) delimited this term to sterile stromatic bodies produced to survive harsh microclimatic conditions.

There are several species and genera of dematiaceous hyphomycetes producing multicellular bodies resembling *S. sphagnicola*. Species of the genus *Phaeotheca* Sigler, Tsuneda & Carmichael may be distinguished according to the production of endoconidia within a mother cell. The species *Phaeosclera dematioides* Sigler, Tsuneda & Carmichael forms cerebriform, dry colonies and does not produce conidia (Sigler et al. 1981). *Capnobotryella renispora* Sugiyama, another stromatic hyphomycete colonising *Sphagnum* species, can be distinguished according to chains of globose cells forming the microsclerotia. One could also misidentify *Scleroconidioma sphagnicola* as *Aureobasidium pullulans*, which produces ellipsoidal conidia, but never forms stromatic bodies. However, when *Scleroconidioma sphagnicola* is cultivated on nutrition-rich media such as MEA, the production of microsclerotia is suppressed.

We suppose that colony features (greenish colour, pale tufts of aerial mycelium and numerous stromatic bodies on CMAD and SPEA) and microscopic characteristics (one-celled conidia on vegetative hyphae) are sufficient and enable reliable identification without the need of a DNA analysis. The above-mentioned features may be used for identification even if the prominent structures of *S. sphagnicola*, the conidiomata, are not produced.

## Autecology

The autecology of *S. sphagnicola* has been thoroughly investigated by several authors in relation to i) its role in the habitat; ii) local climatic conditions, and iii) interactions with other fungi. Therefore firstly, the effect of *S. sphagnicola* on

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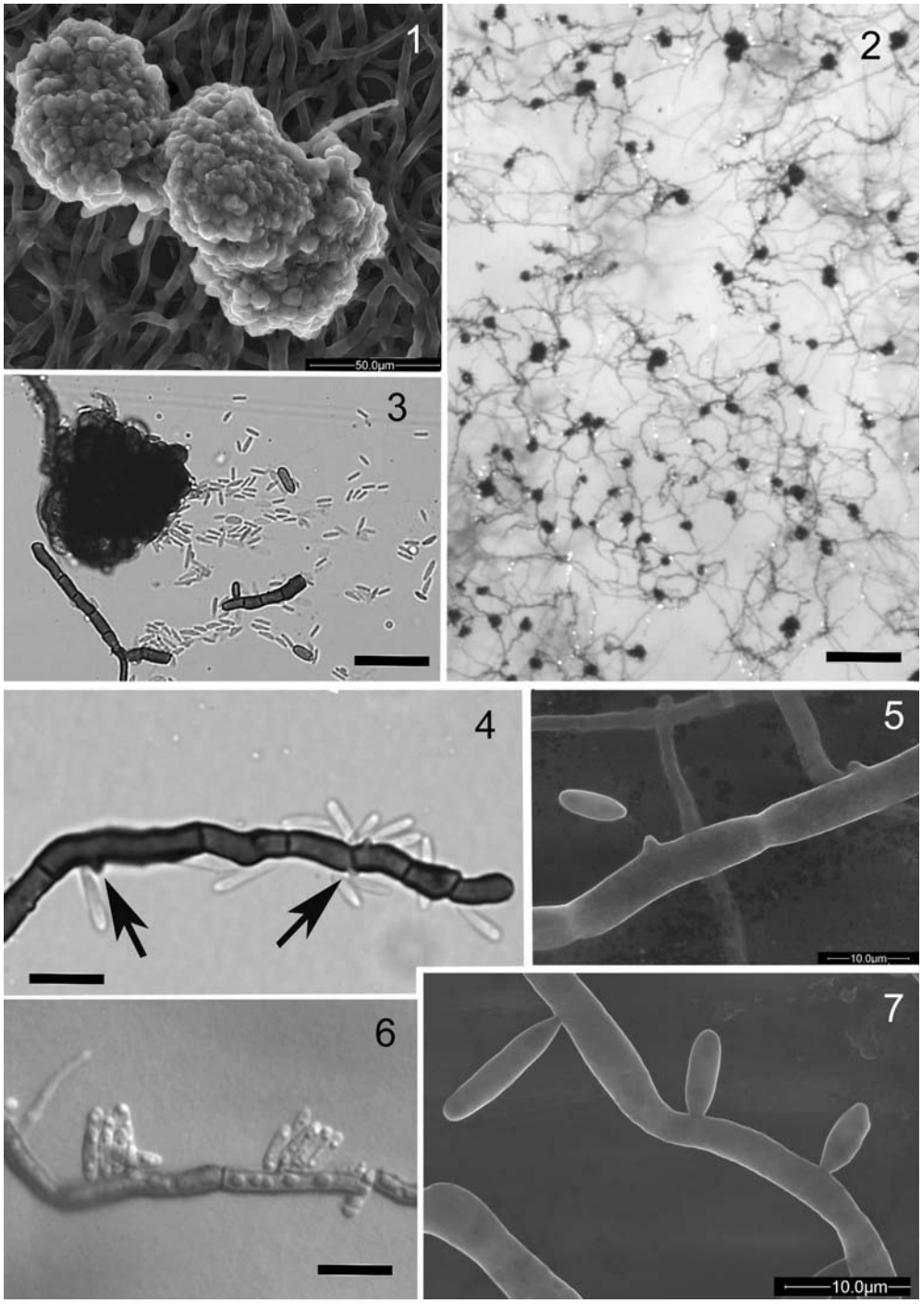
**Fig. 1.** *Scleroconidioma sphagnicola* NK08, microsclerotium (SEM, bar = 50 µm). **Fig. 2.** *S. sphagnicola* 5–1, microsclerotia (bar = 200 µm). **Fig. 3.** *S. sphagnicola* 5–1, microsclerotia and conidia (LM, bar = 50 µm). **Fig. 4.** *S. sphagnicola* 5–1, older melanised hyphae with conidiogenous loci (arrows) and conidia (LM, bar = 10 µm). **Fig. 5.** *S. sphagnicola* NK08, conidium and conidiogenous locus on hyphae (SEM, bar = 10 µm). **Fig. 6.** *S. sphagnicola* NK05, aggregated conidia most probably in the vicinity of conidiogenous loci (DIC, bar = 10 µm). **Fig. 7.** *S. sphagnicola* NK08, conidia either produced from conidiogenous loci or accidentally attached to mycelium (SEM, bar = 10 µm). All microphotographs of structures from colonies growing 10 days on SPEA at 24 °C. Abbreviations: SEM – scanning electron microscopy; LM – light microscopy in bright field; DIC – light microscopy with Nomarski contrast. 


1
50.0µm
2
3
4
5
10.0µm
6
7
10.0µm

bryophytes in Canadian peat bogs was discussed in the papers by Tsuneda et al. (2001a) and Hambleton et al. (2003). Tsuneda et al. (2001a) characterised *S. sphagnicola* as a potentially serious necrotrophic pathogen of *Sphagnum fuscum*. They observed brownish patches of diseased *Sphagnum* plants bearing numerous microsclerotia and similar symptoms after artificial inoculations of healthy *Sphagnum* plants. A detailed observation under the electron microscope revealed that the hyphae of *Scleroconidioma sphagnicola* were able to utilise both the cytoplasmic content and the cell wall. The latter is of special interest, as *Sphagnum* cell walls are highly resistant to biological degradation due to the high content of phenolic compounds (Tsuneda et al. 2001c). The fact that *Scleroconidioma sphagnicola* possesses similar enzymatic characteristics as white rot fungi do, was confirmed in our enzymatic screening. Most of the strains produced polyphenol oxidase, peroxidase and laccase. Considering these abilities, it can be no surprise that *S. sphagnicola* was isolated from spruce wood. Nevertheless, Vasiliauskas et al. (2005) did not discuss in more details the very high frequencies of *S. sphagnicola* isolated from stem cuttings exposed to airborne fungal colonisers. Consequently, the presence of *S. sphagnicola* on coniferous litter needles is neither surprising.

To conclude, the presently known three habitats of *S. sphagnicola* have common features, namely a low content of nitrogen, low availability of phosphorus and low pH together with a high amount of recalcitrant compounds, especially phenols. Therefore, it is not surprising that they are occupied by similar fungal species able to withstand these conditions.

On the other hand, populations of *S. sphagnicola* differ in their life strategies, namely parasitism and saprotrophism. It is of high interest that artificial inoculations of three *Sphagnum* species with five strains of *Scleroconidioma sphagnicola* (isolated from coniferous wood and litter) resulted in only a limited growth of *S. sphagnicola* mycelium on bryophyte leaves. Moreover, *Sphagnum* plants were regularly surveyed at the locality in Šumava NP, but no infected bryophytes were found. Apparently diseased pale whorls were collected in autumn 2005 and spring 2006, placed in damp chambers in the laboratory and cultivated. They revealed no fungal colonisation after several weeks of cultivation (data not shown). The pale colour was apparently caused by other factors than a fungal parasite.

The role of *Scleroconidioma sphagnicola* as a saprotroph participating in spruce litter needle decomposition was studied in long-term experiments (Koukol et al. 2006b). Strain *S. sphagnicola* NK08 transformed organic phosphorus from needles into diphosphates and caused negligible decrease in the C:N ratio of litter needles colonised for 6 months. This result was similar among other strains of saprotrophic ascomycetes and strongly differed from the decrease in C:N ratio caused by the basidiomycete *Setulipes androsaceus* (L.) Antonín. On the other

hand, unlike *Setulipes androsaceus*, *Scleroconidioma sphagnicola* together with *Thysanophora penicillioides* (Roum.) W. B. Kendr. and *Phialocephala fortinii* C. J. K. Wang & H. E. Wilcox, were able to colonise green spruce needles (data not published). Our preliminary results show that green needles may form up to 50 % of coniferous litter (Kovářová, unpubl.) and represent a substantial fraction of the plant debris. However, they seem to be untouchable by *Setulipes androsaceus*, the most important litter needle decomposer. We suggest that *Scleroconidioma sphagnicola* together with other anamorphic ascomycetes do not participate directly in litter needle decomposition, but precolonise green spruce needles and make them accessible for *Setulipes androsaceus*. This mechanism may include attraction of soil microedaphon causing mechanical degradation of litter needles (Maraun et al. 2003).

Climatic conditions at a particular locality may be selective apart from the quality of the substrate. Temperature fluctuation and high sun radiation in Canadian peat bog areas are selective for fungi forming microsclerotia and thick melanised cell walls. Microsclerotia serving as nutrient storage may successfully survive adverse environmental conditions. Both Tsuneda et al. (2001a,b) and Hambleton et al. (2003) found the formation of microsclerotia essential for the survival of *Scleroconidioma sphagnicola*. Conditions in the studied area of the Šumava NP are more favourable. Spruce stands in this area are less exposed to wind and sun light compared to the open Canadian peat bogs. Only the rather long lasting snow cover (usually from early November to late May) resulting in waterlogging of the litter layer may negatively affect fungal colonisers.

Microsclerotia are also effective during fungal dispersal. Tsuneda et al. (2001a) suggested that *S. sphagnicola* may disperse due to fragmentation of colonised bryophyte leaves. Thus, in open areas of peat bogs, hyphal fragments and microsclerotia may be carried to large distances by wind and water. Distribution of *S. sphagnicola* by wind power in afforested areas in Sweden was proved by Vasiliauskas et al. (2005) and is most probably important also for populations in Šumava NP. Another mechanism may be also involved. Can also edafon colonising forest litter participate in the dispersal of *S. sphagnicola*? Conidia seem to be sticky enough to be caught on the surface of oribatids and collembolans and smaller microsclerotia may survive transport through the digestive tract of mesoedafon. These points will be investigated in our future studies.

Last but not least, the coexistence of *S. sphagnicola* and other fungi should be mentioned. In their review, Butler and Day (1998) presented numerous evidences that fungal melanin plays an important role in the interaction with other microorganisms. It prevents lysis of fungal cells caused by cell wall degrading enzymes and antibiotics liberated by other fungi or bacteria. This fact was confirmed in a competition study of *S. sphagnicola* and other fungi colonising litter needles and wood (Koukol et al. 2006a). During competition on agar plates, the strongly

melanised *S. sphagnicola* remained viable although being overgrown by a stronger competitor, e.g. *Hypholoma fasciculare* (Huds.) P. Kumm. Overgrowth during mycelial competition often results in replacement of the weaker competitor (Boddy 2000). However, *Scleroconidioma sphagnicola* withstood this biological stress as mycelium or microsclerotia were not affected by the competition and were successfully reisolated from the agar plate.

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