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Degradation of human hair by three soil fungi. An electron microscopic study.

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Degradation of hair keratin has been studied in three soil fungi differing in keratinolytic ability, viz. *Keratinophyton terreum*, *Dictyoarthrinopsis kelleyi* and *Fusarium moniliforme*. All fungi attacked the hair cuticle forming specialised mycelial organs, fronds, under the scale-like cuticular cells. The cortex was attacked by very thin "boring hyphae". Their growth was intracellular and perpendicular to the hair axis. In *Keratinophyton terreum* older boring hyphae branched into complex formations, displaying clear lytic action on keratin. In *Dictyoarthrinopsis kelleyi* branching was rare and lysis of keratin weaker. In *Fusarium moniliforme*, a fungus not regarded as keratinophilic, the growth of boring hyphae ceased early and the lytic action remained minimal. All fungi digested the less keratinised parts of the hairs (endocuticle, intercellular substance, interfibrillar matrix) prior to the lysis of hard keratin fibrils.

Key words: keratinophilic fungi, keratinolysis, human hair, electron microscopy

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Rozklad vlasového keratinu byl studován u tří půdních hub (*Keratinophyton terreum*, *Dictyoarthrinopsis kelleyi*, *Fusarium moniliforme*), lišících se keratinolytickými schopnostmi. Všechny houby rozkládaly kutikulu vlasu, kde tvořily specializované myceliální orgány („fronds") pod šupinovitými kutikulárními buňkami. Kortex byl prorůstán velmi tenkými hyfami, jejichž růst byl intracelulární a kolmý k podélné ose vlasu. U *K. terreum* se starší hyfy v korte větvily a tvořily složité útvary s jasným lytickým účinkem na keratin. U *D. kelleyi* bylo větvení vzácné a lýza keratinu slabá. U houby *F. moniliforme*, která není považována za keratinofilní, se růst hyf v korte brzy zastavil a keratinolýza zůstala minimální. Všechny houby rozpouštěly méně keratinizované části vlasu (endokutikulu, intercelulární hmotu, interfibrilární matrix) dříve než napadly fibrily tvrdého keratinu.

INTRODUCTION

Keratinophilic fungi represent an ecological group of fungi that act as decomposers of keratinaceous materials of animal origin (epidermal scales, hairs,

nails or claws, hooves, feathers etc.) in soil. Whereas the term "keratinophilic" is conventionally used for all fungi found colonising keratinaceous remnants, the term "keratinolytic" should be reserved for species or strains displaying clear degradation of keratin, observable by microscopy or proven by physiological experiments (Filipello-Marchisio 2000).

The study of keratinophilic fungi was stimulated by the finding that some primitive species of dermatophytes (keratinolytic fungi causing diseases of the skin of humans and animals) may be found in soil (Vanbreuseghem 1952). Such "geophilic dermatophytes" have been isolated from human hair, feathers and other similar materials used as "bait". Microscopic observation of hair bait enhanced the knowledge of the morphology of hard keratin degradation by keratinolytic fungi. By the use of light microscopy, the decomposition of human hair by keratinophilic fungi has been most thoroughly investigated by English (1963) who described in detail the specialised organs formed by filamentous fungi for the degradation of hair cuticle ("fronds") and hair cortex ("perforating organs", "boring hyphae"). She also studied attack of non-keratinophilic fungi on human hairs and observed some morphological similarities (English 1965).

In vitro electron microscopic studies on the decomposition of human hair by the dermatophyte *Trichophyton mentagrophytes* were published by Mercer & Verma (1963), who observed only the first five days of the growth. The results of the above authors were expanded by Baxter & Mann (1969), Hsu & Volz (1975), Kunert & Krajčí (1981), Kaaman & Forslind (1985) and specifically by detailed studies of Kanbe & Tanaka (1982) and Kanbe et al. (1986). Scanning electron microscopy was also used for the study of keratinolysis by dermatophytes (Kunert & Hejtmánek 1976, Kanbe & Tanaka 1982, Kaaman & Forslind 1985, Filipello-Marchisio et al. 1994, Rashid et al. 1995, Wawrzkiewicz et al. 1998, Filipello-Marchisio et al. 2000). This method is especially suitable for illustrating the decomposition of the surface layers of hairs or nails.

In comparison to the dermatophytes, less attention has been paid to the degradation of hard keratin by other keratinophilic fungi. Cano et al. (1991) investigated three species of the genus *Aphanoascus* which attack human hair from the surface or cutting edges without forming perforating organs or the typical fronds. Fusconi & Filipello-Marchisio (1991, see also Filipello-Marchisio 2000) described the degradation of human hair by *Chrysosporium tropicum*. This fungus initially attacks the cortex of hairs by thin and long unbranched hyphae (boring hyphae) growing from appressoria-like cells found in the cuticle. Later on, the hyphae grow thicker, become septated and branched and turn into complex formations similar to true perforating organs. The lytic action of these formations on keratin is evident. A similar type of hair degradation was observed by light microscopy in other species of *Chrysosporium* (English 1969, 1976) and in *Keratinophyton terreum* (Kunert 1967). In *Scopulariopsis brevicaulis* only three

out of nine studied strains degraded the cuticle and cortex of hairs to some extent and sent off boring hyphae into the cortex. These hyphae degraded the structures of keratinized cells in their surroundings but did not swell and did not produce large lytic channels (Filipello-Marchisio et al. 2000). At the level of light microscopy, conventional (thin) boring hyphae and various types of "wide" or "swollen" boring hyphae have been repeatedly described in keratinophilic fungi with limited keratinolytic ability (see e.g. Filipello-Marchisio 1986, Filipello-Marchisio et al. 1994).

In the present study three species of soil fungi, displaying moderate to weak keratinolytic ability, were studied by means of transmission and scanning electron microscopy (TEM, SEM). In *Keratinophyton terreum*, light microscopy (LM) showed the presence of swollen boring hyphae that turned into formations resembling true perforating organs (Kunert 1967). *Dictyoarthrinopsis kelleyi* produced numerous boring hyphae which only rarely thickened and branched. *Fusarium moniliforme* represents a fungus that is not regarded as keratinophilic. However, our strain was able to grow on human hairs and degrade them partially by fronds and boring hyphae.

MATERIAL AND METHODS

Three species of soil fungi from the collection of dermatophytes and keratinophilic fungi at the Department of the first author were used: *Keratinophyton terreum* Randhawa et Sandhu (1963, type strain), *Dictyoarthrinopsis kelleyi* Dominik et Majchrowicz (1966, type strain) and *Fusarium moniliforme* Sheldon. The first two fungi were found on keratinaceous substances, the third one was isolated directly from agricultural soil. All strains were kept on 4 % glucose – 1 % peptone agar slants at 28 °C in the dark.

Fair children's hairs from a barber's were washed with warm water containing detergent, then washed repeatedly with distilled water, dried at room temperature and cut into pieces approximately 2 cm long. The hairs were sterilised by propylene oxide (6 hours at room temperature in an atmosphere saturated with vapourised oxide, Kunert & Krajčí 1981), placed on a layer of 1.5 % water agar in Petri dishes and inoculated with spores from the surface of 10-day old cultures on the glucose-peptone medium. In preliminary experiments with *F. moniliforme*, hairs sterilised by mild autoclaving (110 °C, 15 min.) and non-sterile hairs were also used.

After one to seven weeks of growth at 28 °C hairs at various stages of degradation were fixed in vapours of glutaraldehyde in Petri dishes for 24 hours. Then they were fixed in 2 % glutaraldehyde and 1 % formaldehyde in 0.1 M phosphate buffer pH 7.4 (24 hours) and postfixed in 1 % osmium tetroxide in the

same buffer (2 hours), dehydrated in acetone and embedded in Durcupan ACM (Fluka, Switzerland).

Thin sections were made on an Ultracut (Reichert, Austria) microtome using a glass knife, contrasted with uranyl acetate and lead citrate according to Reynolds and observed under a Zeiss Opton 109 (Germany) electron microscope operated at 80 kV. The primary magnification was within a range of 3,000 to 50,000 ×.

Fixation for SEM was performed with a mixture of 2 % glutaraldehyde and 1 % formaldehyde in 0.1 M phosphate buffer pH 7.4 (24 hours) and the samples were dehydrated in a graded acetone series. The samples were then dried using the critical point dryer CPD-030 (Bal-Tec, Liechtenstein), coated with a 5 nm layer of gold and palladium in the sputtering device Polaron E 5100 (Great Britain), and observed under a Tesla BS 340 (Czech Republic) scanning electron microscope.

RESULTS

The morphology of keratin degradation by the fungus *Keratinophyton terreum* was, in most respects, similar to that of *Chrysosporium tropicum,* which was described by Fusconi & Filipello-Marchisio (1991). Its description and illustration is therefore presented in an abbreviated form. The degradation started at the hair surface, where the hyphae grew along the edges of cuticular cells and, thereafter, penetrated under the cuticular scales. There the hyphae were densely branched and composed of flat and lobose cells ("fronds", "fronded mycelium" – English 1963) (Fig. 1a, e). In advanced stages the hyphae were thick and circular in cross-section and evidently contributed to the mechanical decomposition of cuticular layers (Fig. 1d). The most easily decomposed layer of the cuticle cells was the endocuticle, an inner layer containing transformed remnants of the cytoplasm of keratinised cells. Its degradation could be observed up to several micrometers from the nearest hypha. Later on, blocks of amorphous keratin in the exocuticle (outer layer) were gradually digested. The most resistant layer was the A-layer of the exocuticle under the outer cytoplasmic membrane and a similar thinner layer facing the inner membrane. These layers remained mostly undegraded even in advanced stages of keratinolysis (Fig. 1d, e).

The cortex was attacked by hyphae growing perpendicularly to the longitudinal axis of the hairs. They arose by an inward growth of some cells of fronds at the border between cuticle and cortex. These hyphae ("boring hyphae", "borers" – English 1963) were very thin (under 0.5 μm) and penetrated the hair intracellularly across the cortical cells. At first, the boring hyphae were fully embedded in the keratinaceous mass of penetrated cells but, later on, lytic channels were visible around them (Fig. 1c). The hyphae then grew thick and divided into columns of short cells, similar to true perforating organs of dermatophytes. The component of the cortex degraded first was the complex of cytoplasmic membranes and

intercellular cementing substances between adjacent cells. The inter-macrofibrillar matrix inside the cells was digested next, followed by the keratin macrofibrils themselves. The latter were digested both from the surface and from the centre, where the packing of keratin microfibrils was less dense. In advanced stages of cortex degradation many macrofibrils disappeared, giving rise to extended lytic channels. In these channels a new type of mycelium ("erosive mycelium") was observed, produced by branching of swollen boring hyphae. It grew in parallel with the longitudinal axis and reminded of the fronds in the cuticle by its primarily intercellular growth and its morphology. The only undegraded component of the cortex was the melanin granules.

In the cytoplasm of hyphal cells nuclei with nucleoli, mitochondria, vacuoles with granules and membrane debris, endoplasmic reticulum, lipid drops and various vesicles and granules were observed (Fig. 1a). Some vesicles showed a reticular structure at their surface (Fig. 1b).

Hair degradation by *Dictyoarthrinopsis kelleyi* was slower and its course indicated a weaker keratinolytic ability of this fungus. Typical fronds, proliferating in place of the digested endocuticle, were observed in the cuticular cells. The exocuticle was digested in some places. However, its overall degradation was slow and the A-layer remained mostly intact (Fig. 2a). The organs of cortex degradation were frequent and typical borers. They originated from swollen, appressoria-like cells of fronds in or under the cuticle (Fig. 2b, c) and penetrated the cortex directly, more or less perpendicularly to the long axis of the cells and their keratin fibrils. The exocuticle of the cuticular cells (Fig. 2b) and the elements of the cortex (Fig. 2c) looked like drilled through by the boring hyphae, in the vicinity of which no signs of mechanical deformation (e.g. bending and displacing of the fibrils) were observed. Young boring hyphae filled tightly the holes they produced but in later stages the lytic action on less keratinised elements (transformed cytoplasmic membranes with intercellular cement, cytoplasmic remnants, inter-macrofibrillar matrix) was evident. Because these parts were degraded faster than the hard keratin of macrofibrils, the line of lysis along the hypha was irregular and typically "festooned" (Fig. 2c, d). The keratin itself was also attacked, as demonstrated by the separation of keratin microfibrils and an increased osmiophilia of the attacked parts. However, the lytic holes around the boring hyphae increased only slowly. An erosive mycelium produced by the branching of swollen boring hyphae remained rare and the hair degradation stopped after six to eight weeks without decomposing most of the hair substance both in the cuticle and the cortex.

Hair degradation by the strain of *Fusarium moniliforme* capable of growing on human hairs was in most aspects similar to that of *Dictyoarthrinopsis kelleyi*. The cuticle layer was attacked first by morphologically normal hyphae, growing along the edges and penetrating into the spaces between cuticular scales (Fig. 3a). Later on, typical fronds were formed that spread in place of the digested endocuticle.

The exocuticle was degraded only slowly and its A-layer remained intact even in advanced stages of the growth on hairs. The main organs of hair degradation were numerous boring hyphae (Fig. 3b). They arose from swollen, appressoria-like cells of fronds found mainly at the border between cuticle and cortex and penetrated it mostly perpendicularly to the long axis of the hairs (Fig. 3c, d). The boring hyphae were thin and non-branched and their course was often tortuous due to changes of direction at the borders of cortical cells and layers. Young borers fitted tightly into the holes they produced in the resistant layers of the cuticle or the cortex. Later on, lytic action on less resistant components of the cortex was evident and typical "festooned" lines of lysis were observable both on cross and longitudinal sections (Fig. 4 a, b). Hard keratin fibrils were also attacked, as shown by desintegration of macrofibril margins and an increased osmiophilia of the attacked areas (Fig. 4c). However, no large lytic channels (observable by light microscopy) were formed and the boring hyphae remained thin. An eroding mycelium was formed only rarely. This was found predominantly in intercellular spaces and its effect remained limited to the less keratinised components of the cortex (the complex of plasmatic membranes and the inter-macrofibrillar matrix, Fig. 4d). After six to eight weeks further growth on hairs and their degradation ceased. Even in the regions of hairs attacked most strongly only approximately 20 % of the hair substance was digested.

In preliminary experiments hair degradation by *Fusarium moniliforme* was studied under different conditions of cultivation. The degradation was slightly stronger with autoclaved hairs compared to hairs sterilised by propylene oxide and non-sterile hairs. It was also faster with hairs on glucose-peptone agar than on agar without the added nutrients. However, the course and morphology of hair degradation was essentially the same under all conditions.

DISCUSSION

Degradation of human hair was studied in three soil fungi differing in keratinolytic ability. Human hairs, belonging to the most resistant of mammalian hairs (Wawrzkiewicz et al. 1998) were chosen as the substrate. These hairs were also used in most of the previous studies.

In all three species the cuticle of the hairs was attacked first. Specialised hyphal organs were observed, described as "fronds" by English (1963) and found later by many authors studying hair degradation by keratinophilic fungi in vitro (see Introduction). Their morphology probably reflects an adaptation to growth in the flat and thin spaces formed by the degradation of the least resistant parts of cuticular cells (scales). Filipello-Marchisio et al. (1994) suggested that fronds are similar to the hyphopodia of phytopathogenic fungi formed at plant surfaces. The fronded mycelia grow oriented under the cuticular scales at the hair surface. They rapidly digest the complex of cytoplasmic membranes of adjacent cells and their

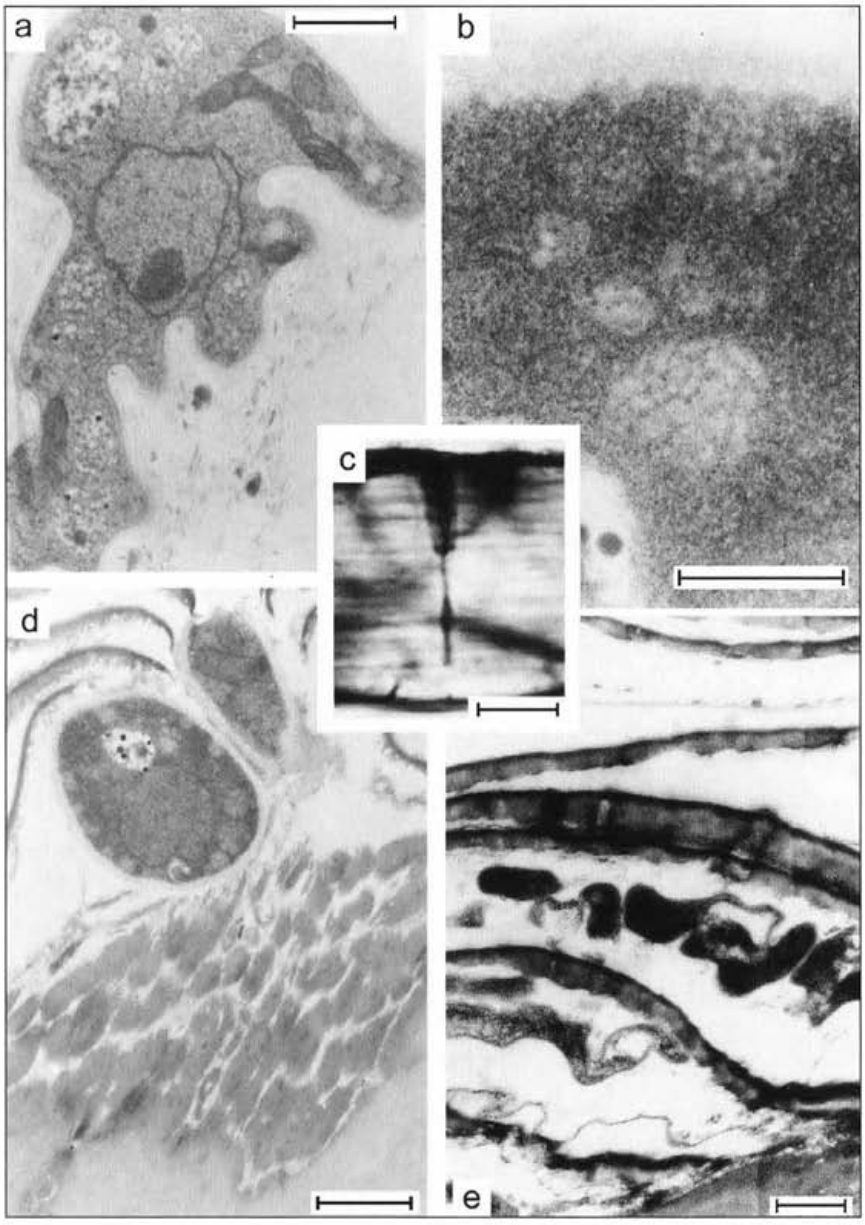

Fig. 1. *Keratinophyton terreum*. **a** Lobose cells ("fronds") in the cuticle. Tangential section of the hair. TEM, scale bar = 1 μm; **b** Coated vesicles in the cytoplasm of older hyphal cells. TEM, scale bar = 0.2 μm; **c** "Boring hypha" in the hair cortex. Note the lytic hole around the older (upper) part of the hypha. LM, scale bar = 20 μm; **d** Older hyphae in the cuticle. Cross section of the hair. Partly digested cuticle (upper part) and cortex (lower part of the picture). TEM, scale bar = 1 μm; **e** Cross sections of lobose cells in the cuticle. TEM, scale bar = 1 μm.

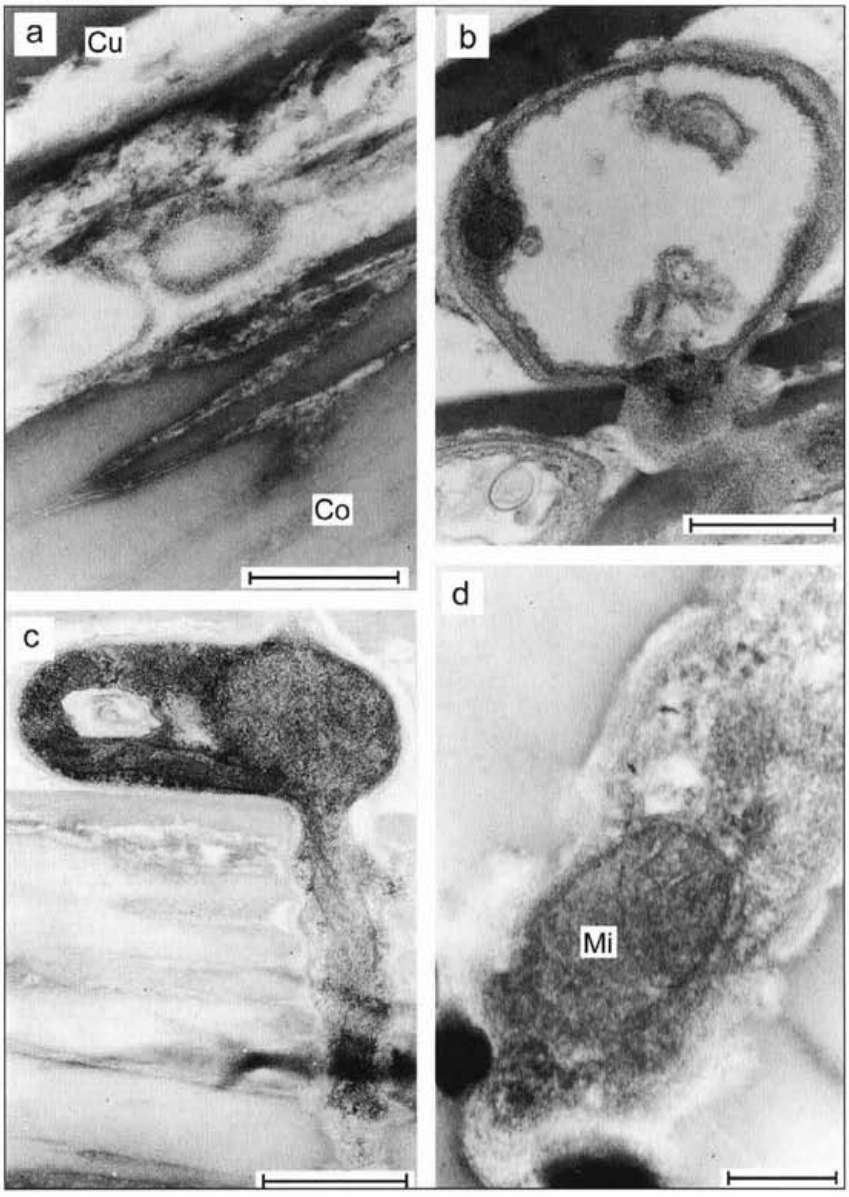

Fig. 2. *Dictyoarthrinopsis kelleyi*, TEM. **a** Cross sections of hyphae in the cuticle (Cu). Degradation of endocuticle and exocuticle. Lower part: lytic action on intercellular substance in the cortex (Co). Scale bar = 0.5 μm. **b** Appressorium-like cell in the cuticle with a boring hypha penetrating innermost cuticle cells. Scale bar = 0.5 μm. **c** Appressorium-like cell under the cuticle with a boring hypha penetrating the hair cortex. Scale bar = 1 μm. **d** Part of a boring hypha in the cortex. Note the signs of lysis of surrounding keratinaceous substance. Mi = mitochondrion. Scale bar = 0.2 μm.

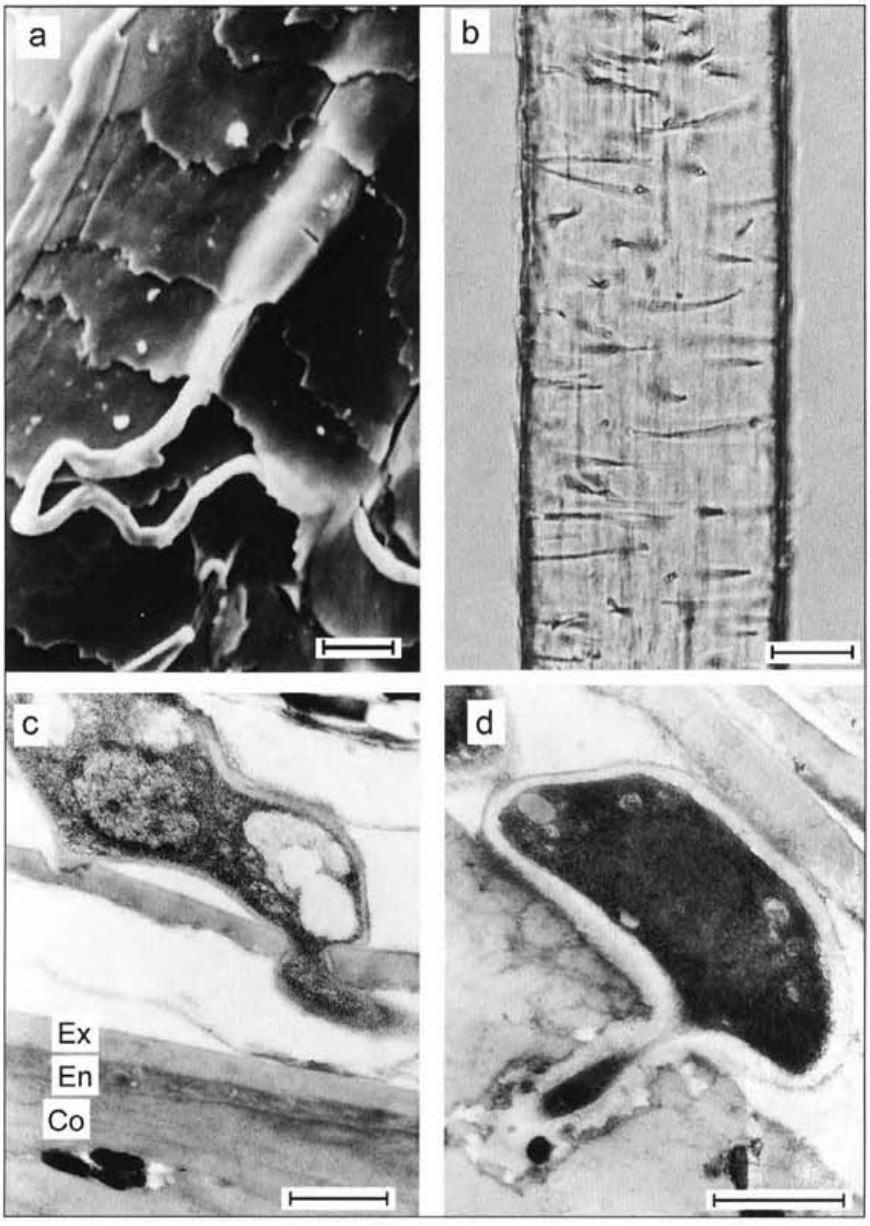

Fig. 3. *Fusarium moniliforme*. **a** Hyphae penetrating under the cuticular cells of the hair. SEM, scale bar = 5 μm. **b** Numerous boring hyphae in the attacked human hair. LM, scale bar = 20 μm. **c** Appressorium-like cell with a boring hypha penetrating the exocuticle. In the intact cuticle cell endocuticle (En) and exocuticle (Ex) are visible. Co = cortex. TEM, scale bar = 1 μm. **d** Appressorium-like cell with a boring hypha penetrating the hair cortex. Note the signs of cortex degradation around the boring hypha. TEM, scale bar = 1 μm.

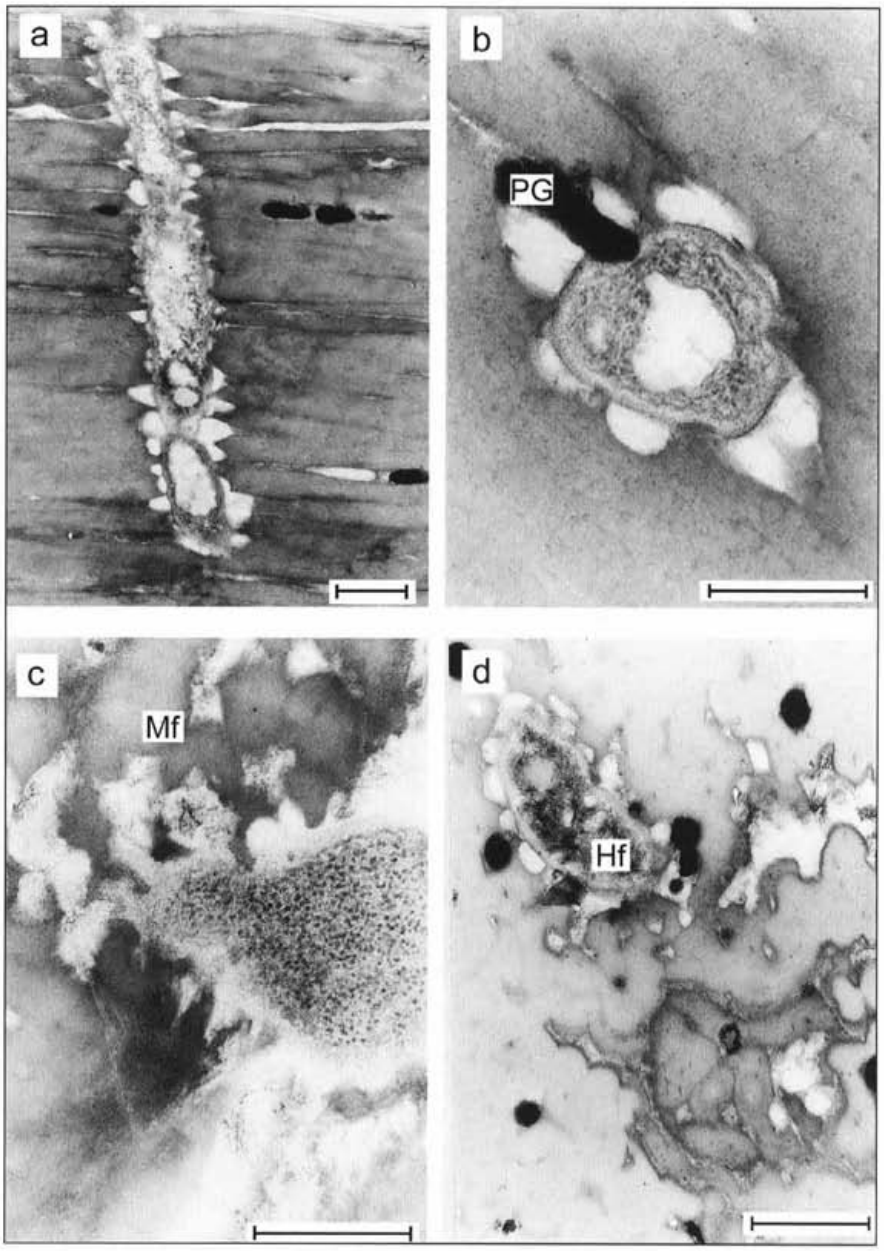

Fig. 4. *Fusarium moniliforme*, TEM. **a** Boring hypha in the hair cortex with a "festooned" line of lysis around it. Longitudinal section of the hair. Scale bar = 1 μm. **b** Cross-section of a boring hypha in the cortex with a surrounding irregular zone of lysis. PG = pigment granule. Scale bar = 0.5 μm. **c, d** Older boring hyphae (Hf) in the cross-sectioned hair cortex. Degradation of interfibrillar matrix separating individual hair keratin macrofibrils (Mf). Scale bar = 0.5 μm.

growth becomes intracellular. The sequence of degradation of cellular components corresponds to their degree of keratinization, paralleled by the content of sulphur (cystine). The endocuticle, containing remnants of cellular cytoplasm, is digested first, followed by the exocuticle, containing blocks of amorphous keratin. The A-layers, which are very rich in cystine (and perhaps also in other cross-linking bonds, e.g. isopeptides, Rice et al. 1994), remain mostly undegraded, especially in the less keratinolytic species, *Dictyoarthrinopsis kelleyi* and *Fusarium moniliforme*.

Whereas the hyphae grew parallel to the long axis of hairs in the cuticle, in the cortex the growth was perpendicular to this axis and intracellular from the very beginning. This is typical of all keratinophilic fungi. In the dermatophytes and other strongly keratinolytic species the organs of cortex degradation are perforating organs, columns of short cells producing rapidly large lytic channels and branching soon into the complex formations, described in detail by Kanbe & Tanaka (1982). Weakly keratinolytic fungi form only thin and unbranched boring hyphae (English 1963, 1965), usually without lytic channels discernible under the light microscope. Their ultrastructure was studied in detail by Fusconi & Filipello-Marchisio (1991) in *Chrysosporium tropicum* and by Filipello-Marchisio et al. (2000) in *Scopulariopsis brevicaulis*. Our results are in agreement with those of the above authors: the formation of boring hyphae from appressoria-like cells, their passage through tight holes in the exocuticle and cortex, and "festooned" lines of hair substance degradation around them were observed. The presence of appressoria-like cells suggests the concerted action of mechanical pressure and enzymatic lysis in cortex penetration. The lysis is probably more important, as shown by the absence of keratin fibril deformation in the surroundings of boring hyphae including their apices. In *Keratinophyton terreum* older boring hyphae produced large lytic channels, grew thick and densely septated, and were transformed into formations similar to perforating organs. This intermediary type (present also in species of the genus *Chrysosporium*, see Introduction) probably demonstrates the origin of perforating organs from boring hyphae in the evolution of keratinophilic fungi.

Also in the cortex, the first attacked elements are those that do not represent a true keratin (intercellular complex, remnants of cell organelles). Hard alpha-keratin is digested later and keratinaceous intermediate filaments are probably dissolved faster than the osmiophilic matrix found among them (Kunert & Krajčí 1981, Fusconi & Filipello-Marchisio 1991, Filipello-Marchisio 2000). This corresponds again to their content of sulphur (cystine). The sulphur content reflects the amount of disulphide (cystine) bridges that are the main source of keratin resistance. Truly keratinolytic fungi are able to cleave these cross-links by means of excretion of sulphite formed during intracellular oxidation of cystine sulphur (sulphitolytic theory of keratinolysis – Kunert 1972, for review see

Kunert 1995, 2000). At the level of electron microscopy sulphitolysis may be the cause of an increase in osmiophilia of the attacked keratin, observed by several authors (Kunert & Krajčí 1981, Kanbe & Tanaka 1982, Kanbe et al. 1986, Fusconi & Filipello-Marchisio 1991) and also in this study. Wickett & Barman (1985) described namely similar effects during the reduction of disulphide bonds of the hairs by dithiols.

The only component of the hairs that remains completely intact during hair degradation by keratinolytic fungi are the non-proteinaceous pigment granules (Mercer & Verma 1963, Hsu & Volz 1975, Kunert & Krajčí 1981, Cano et al. 1991, Fusconi & Filipello-Marchisio 1991).

Some fungi of the genus *Fusarium* are capable of growing on keratinaceous substrates and are mentioned among primary colonisers of such substrates in soil. These members of fungal succession were thought to use only non-keratins of hairs, nails etc. However, in the experiments of Oyeka & Gugnani (1998) a strain of *Fusarium solani* digested up to 20 % of powdered keratin suspension. Among eight strains of *Fusarium* spp. only one isolate of *F. moniliforme* attacked human hairs in a way comparable to keratinophilic fungi (Kunert, non-published results). In the present study it degraded the hairs nearly as intensively as *Dictyoarthrinopsis kelleyi*, originally described as a keratinophilic fungus (Dominik & Majchrowicz 1966). In contrast to *D. kelleyi* cuticle decomposition was slower and more incomplete, and the boring hyphae stopped grow earlier, mostly without branching and producing further mycelia. However, even here the boring hyphae in the cortex displayed a clear lytic, obviously enzymatic, activity. It is therefore highly probable that even the most weakly keratinophilic fungi penetrate keratinised tissues by a lytic action and cannot solely use mechanical pressure.

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CZECH MYCOL. 53 (3), 2001

Diplogelasinospora princeps (Sordariales), the first record in the Czech Republic

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Novotný D. (2001): Diplogelasinospora princeps (Sordariales), the first record in the Czech Republic. – Czech Mycol. 53: 203–209

Diplogelasinospora princeps, *Sordariales*, was isolated from the root of a sessile oak (*Quercus petraea*) in the Křivoklátsko region (Czech Republic) during a study of the endophytic mycoflora of roots. This is not only the first record in the Czech Republic, but probably the first record from Europe, too. Growth of the isolated strain under different conditions was tested.

Key words: roots, bark, *Diplogelasinospora princeps*, Křivoklátsko region, endophytes, *Quercus petraea*, oak

Novotný D. (2001): Diplogelasinospora princeps (Sordariales), první nález v České republice. – Czech Mycol. 53: 203–209

Diplogelasinospora princeps, patřící do řádu *Sordariales*, byla izolována při studiu endofytické mykoflóry kořene dubu zimního (*Quercus petraea*) na Křivoklátsku v České republice. Tento nález je první nejen z České republiky, ale pravděpodobně i z Evropy. U nalezeného kmene byla testována růstová rychlost při různých teplotách a na různých agarových médiích.

INTRODUCTION

Diplogelasinospora princeps Cain 1961 was the first described species of *Diplogelasinospora* (Cain 1961). This genus is classified in the family *Sordariaceae*, order *Sordariales* (Hawksworth et al. 1995). So far three species of this genus are known: *D. grovesii*, *D. inaequalis*, *D. princeps* (Udagawa et al. 1973). Probably all strains of these species isolated and deposited by other mycologists were found in North America, Japan or South-east Asia. They were obtained from soil or flax seed (Cain 1961, Udagawa and Horie 1972, Udagawa et al. 1973, Anonymus 1996, Huang and Schmitt 1975).

MATERIALS AND METHODS

This species was isolated in October 1997 from peridermal bark of a 3 cm thick root of sessile oak (*Quercus petraea*) in an oak wood near the village of Nižbor in

the Křivoklátsko region, Central Bohemia, Czech Republic. Roots were studied in order to learn their endophytic mycoflora.

The roots were brushed under running water, their surface sterilised (96% ethanol 1 min., sodium hypochlorite (NaClO) 3 min., 96% ethanol 0.5 min.), cut and separated into wood, subperidermal bark and peridermal bark. Pieces of tissue were laid on 2% malt extract agar and incubated at room temperature for four weeks.

Growth of the isolated strain was tested on 2% malt extract agar (MA2), potato-dextrose agar (PDA), potato-carrot agar (PCA) and oat-meal agar (OA). Incubation on MA2 was conducted at seven different temperatures (5, 15, 25, 30, 37, 42 and 45 °C). Mycelium of the tested strain was transferred to three Petri dishes per medium and temperature.

RESULTS AND DISCUSSION

Description of *Diplogelasinospora princeps* CCM 8255

Only a single strain of this fungus with the working designation DA/T/V2 was isolated. This strain was freeze-dried and also preserved under mineral oil. It was deposited as CCM 8255 in the Czech Collection of Microorganisms (CCM), Faculty of Science, Masaryk University, Brno, Czech Republic and as CCF 3188 in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague, Czech Republic.

Macroscopic description

(Comparison of growth rates under different conditions are given in the tables 1 and 2)

MA2, 25 °C: colonies white-brown to brown, low, cottony, exudate light brown to brown, reverse dark grey-blue in the centre and dark brown on margins. Perithecia soon formed.

PDA, 25 °C: colonies brown, low, cottony, exudate absent, reverse dark grey-blue in centre and dark brown on margins. Perithecia formed sooner than on MA2, but later than on OA.

PCA, 25 °C: colonies green-brown, low, cottony, exudate absent, reverse green-brown. Perithecia formed sooner than on MA2, but later than on OA. Conidia abundant.

OA, 25 °C: colonies white-grey brown to brown, low, cottony, exudate clear, reverse dark grey-blue to brown. Perithecia appeared early, abundant.

MA2, 5 °C: colonies white to white brown, low, cottony, exudate absent, reverse green-brown. Perithecia were not observed during this study.

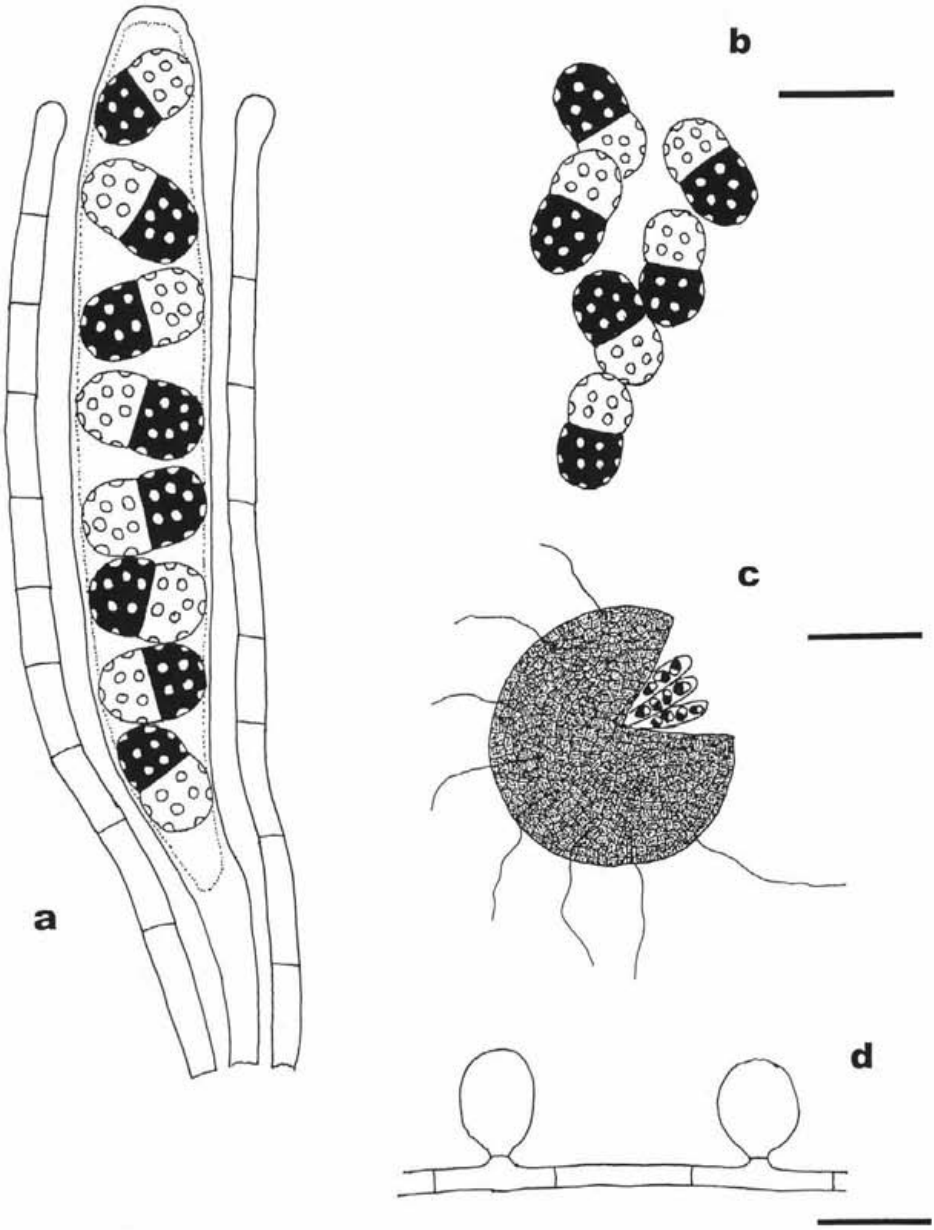

Fig. 1. *Diplogelasinospora princeps* – A: ascus with ascospores and paraphyses. B: two-celled ascospores. C: ascoma. D: conidia. Scale bar for A, B = 20 μm, for C = 150 μm, for D = 10 μm.

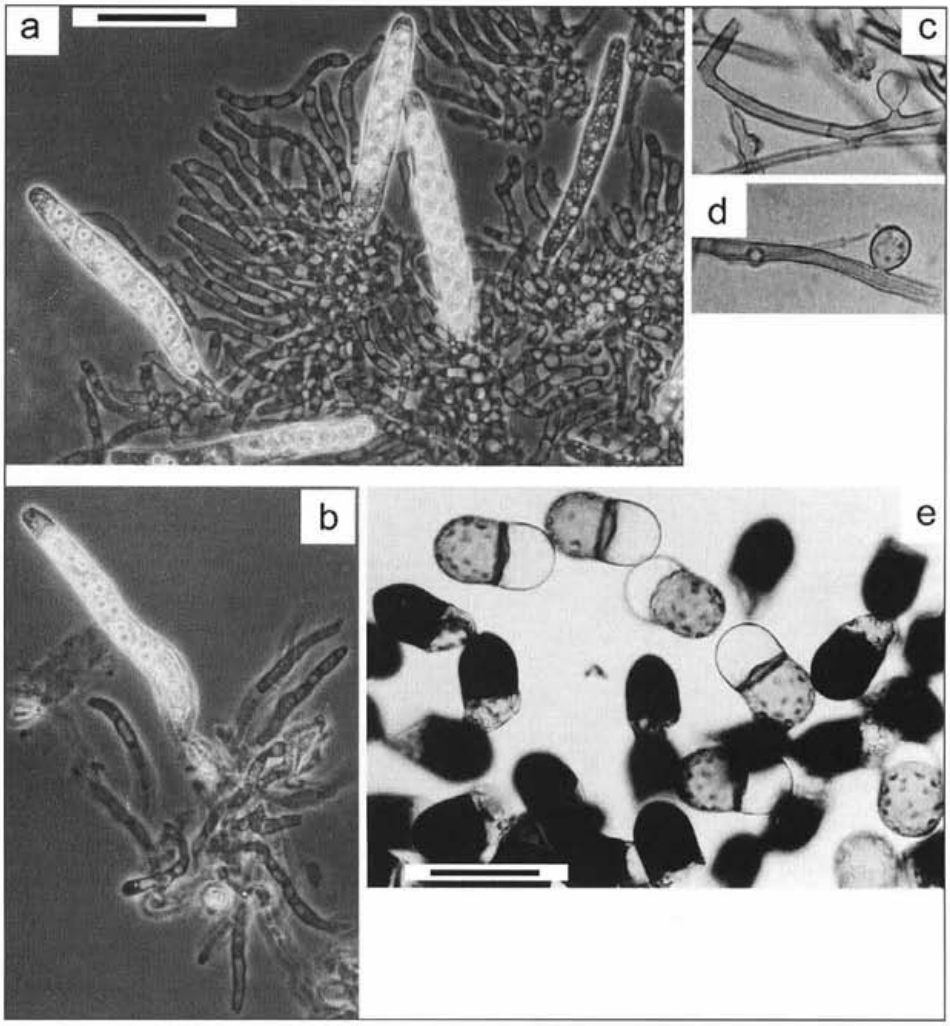

Fig. 2. *Diplogelasinospora princeps* – A, B: young asci with ascospores. C, D: conidia, E: two-celled ascospores. Scale bar for A, B = 50 μm, for C-E = 20 μm.

MA2, 15 °C: colonies white to white-brown, low, cottony, exudate absent, reverse dark grey-blue in the centre and green-brown on the margins. Perithecia were not observed during this study.

MA2, 30 °C: colonies grey-brown to brown, cottony with elevated central circle, exudate absent, reverse dark grey-blue in the centre and dark brown on the margins. Perithecia few.

MA2, 37 °C: colonies white-brown to brown, cottony with elevated central circle, exudate pale to yellow, reverse dark grey-blue in the centre and dark brown on the margins. Perithecia scarce.

MA2, 42 °C: colonies light brown, cottony with elevated central circle, exudate absent, reverse light brown, dark on the margins. Perithecia were not observed during the study.

MA2, 45 °C: growth nil.

Table 1. – Growth on different media at 25 °C.

Medium	Colony diameter		
	7 days (mm)	10 days (mm)	14 days (mm)
MA2	15–21	26–31	42–47
OA	20–24	32–34	55–57
PDA	26–28	43–46	69–72
PCA	34–37	57–61	70–74

The studied strain grows fastest on PCA medium at 25 °C and on MA2 at a temperature 30 °C. No growth was recorded at a temperature of 45 °C. Perithecia develop earliest and most abundant on OA medium.

Table 2. – Growth on MA2 at different temperatures.

Temperature	Colony diameter		
(°C)	7 days (mm)	10 days (mm)	14 days (mm)
5	0–2	1–4	4–8
15	12–13	25–27	42–45
25	15–21	26–31	42–47
30	73–78	>90	>90
37	48–49	72–80	>90
42	3–4	6–9	14–18
45	0	0	0

Microscopic features

Hyphae pale brown, 2–4 μm wide, smooth, with cells 13–35 μm long. Ascomata (Fig. 1c) dark brown, globose or subglobose, 250–385 μm in diam. Ostiolum not observed. Peridium dark brown, with angular cells 4–10 × 4–10 μm. Depending on medium used, the first ascomata appear after 14 days of incubation, but most frequently after 4–6 weeks.

Asci (Fig. 1a, 2a, b) cylindrical, 150–170 × 10–17 μm, 8-spored, non-amyloid, broadest in the upper part, soon evanescent. Paraphyses hyaline, cylindrical, 120–150 × 3.5–5 μm, swollen up to 7 μm. Ascospores (Fig. 1b, 2e) at first

hyaline, ovate, one-celled, later becoming two-celled. Mature ascospores pitted (pits 1–1.5 μm in diam.), 19–23 × 11–13 μm, one cell is dark, semiglobose to semisubglobose, 11–15 × 11–14 μm, the other cell is hyaline, semiglobose to semisubglobose, 7–10 × 11–14 μm.

Only aleurioconidia (sessile blastoconidia) (Fig. 1d, 2c,d) were observed. These are elliptical, hyaline to pale brown and measure 8–11 × 4–5 μm. They arise singly, terminal or lateral on hyphae. Arthroconidia were not found.

Occurrence and discussion

Udagawa and Horie (1972) observed luxuriant growth in their isolate on PCA at 37 °C. They did not study the growth at different temperatures. In the present study the fastest growth of the isolated strain was recorded at 30 °C.

All records of this species known to the present author are hitherto from soil or plant plant (Anonymus 1996, Huang and Schmitt 1975). The studied strain was found in peridermal bark of an oak (*Quercus petraea*) root. It is part of the inner (endophytic) mycoflora of a plant, but it is also close to rhizosphere and to soil. During the author's study it was isolated only once. It is not a typical endophyte, but is probably capable of endophytic life.

This fungus was recorded in North America and Japan (Cain 1961, Udagawa and Horie 1972, Anonymus 1996, Huang and Schmitt 1975). Besides, *Diplogelasinospora grovesii* was found in Japan (Udagawa and Horie 1972), and *D. inaequalis* in New Guinea (Udagawa et al. 1973). The present author has not found any record of the occurrence of *Diplogelasinospora princeps* in the Czech Republic nor Europe.

There were observed differences in presence and size of some morphological structures between the strain from the Czech Republic and the strains found in Canada and in Japan (Table 3). The ascomata of the strain from the Czech Republic are similar in size to the ascomata from Canada (Cain 1961) and from Japan (Udagawa and Horie 1972). However, the asci of the strain from the Czech Republic are shorter and narrower than the asci of the strains from Canada (Cain 1961) and from Japan (Udagawa and Horie 1972). The ascospores are shorter than in non-European strains. Udagawa and Horie (1972) observed two types of conidia (aleurioconidia and arthroconidia). Cain (1961) did not observe any of them. In the present study aleurioconidia were observed. They are similar in shape and in size to the conidia of the Japanese strain. Sigler and Carmichael (1983) did not observe arthroconidia during their study of the strain NHL 2504 from Japan. The author of the present study did not observe this type of conidia either.

Table 3. – Comparison of the size of some morphological structures in three strains of *Diplogelasinospora princeps* (in μm)

Morphological structure	strain from Canada Cain (1961)	strain from Japan Udagawa and Horie (1972)	strain from the Czech Republic (present study)
Size of perithecia	250–400	325–400	248–387
Length of asci	180–220	160–200	150–170
Width of asci	18–21	16–20	10–17
Length of ascospores	19–25	20–27	19–23
Width of ascospores	10–14	10–15	11–13
Length of aleurioconidia	*	8–12	8–11
Width of aleurioconidia	*	3,6–5	4–6
Length of arthroconidia	*	7–14	absent

* – not recorded

ACKNOWLEDGEMENTS

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Book Review

MIGUEL ULLOA AND RICHARD T. HANLIN (2000):

Illustrated dictionary of mycology. –
448 p., APS Press, St. Paul, Minnesota.
ISBN 0–89054–257–0
(The book is deposited in the library of the Society)

This comprehensive book is meant as a dictionary for students of mycology. More than 3800 terms are included and supplemented with 774 black-and-white photographs and 548 drawings. This makes the book very attractive for all readers. A complete etymology for all of the terms explained in the dictionary is another unique and remarkable feature of this book. Readers will thus be able to understand the origin of mycological terms which are usually used without knowing their linguistic history. I consider this fact one the most important features of this dictionary, especially in a time characteristic of a decreasing knowledge of Greek and Latin. The dictionary is not taxonomically oriented, but an outline of the classification of included taxa to genus level is added at the end.

The texts are easy to read, exact and rather detailed, which certainly is an advantage when compared e.g. with the brief explanations in Ainsworth & Bisby's Dictionary of Fungi (8th edition). In some cases specifications could be added (e.g. bitunicate ascus – no mention of the fact that the wall is not two-layered but multilayered; p. 193: photograph described as hymenium shows in fact a cross-section of the whole lamella with trama, subhymenium and hymenium). Numerous instructive illustrations help to understand the text better and give the book a very attractive layout. The photographs are usually of good quality and characteristic. On the other hand, the drawings are sometimes slightly atypical (e.g. flabellate fruiting body of *Sparassis crispa*), inaccurate or too schematic (e.g. p. 186: fertile basidium of *Galerina phillipsii* – in fact, the spores are not oriented along the axis of the sterigma and the basidium is not so clavate; p. 34: the spores of *Coprinus comatus* are not a good example of amygdaloid spores). These shortcomings slightly lower the value of the book as accuracy of illustrations should be self-evident in such a compendium. It is not possible here to discuss the explanations of so many terms, but in some cases the selection of examples is disputable. For example, "exannulate" (lacking a ring) is characterised as follows: "like the stipe of *Russula brunneoviolacea*". This character is typical of many agarics, not only of *R. brunneoviolacea*. In the references some important mycological handbooks should have been mentioned, e.g. the mycological dictionary in 8 languages (Berger et al. 1980, Gustav Fischer Verlag Jena), "Lexikon der Mykologie" (Dörfelt et al. 1988) and "Anatomie der Hymenomyceten" (Clémençon 1997).

The shortcomings mentioned above are quite negligible in comparison with the benefit of the whole book. The "Illustrated dictionary of mycology" will certainly be used not only by students but also by mycologists for an orientation in the terminology of fungal groups and terms which are not in the centre of study. The book can be used as a standard for the correct usage of English terms derived from Latin and Greek, especially for mycologists outside the Anglo-American language area. Finally, the "Illustrated dictionary of mycology" will certainly become a standard handbook for all people dealing with fungi.

Jan Holec

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Contribution to the knowledge of the mycoflora in roots of oaks with and without tracheomycotic symptoms

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Novotný D. (2001): Contribution to the knowledge of the mycoflora in roots of oaks with and without tracheomycotic symptoms. – Czech Mycol. 53: 211–222

The mycoflora of roots of three species of oak (*Quercus robur, Q. petraea, Q. rubra)* from two localities near Moravské Budějovice (southwest Moravia, Czech Republic) with and without tracheomycotic symptoms was studied. Fifty-seven species of fungi were identified from the samples. The most frequently isolated were *Fusarium solani, Penicillium glandicola, P. glabrum, P. simplicissimum* and *Acremonium curvulum*. In the roots of trees with tracheomycotic symptoms two species of ophiostomatoid fungi were recorded. Abiotic condiontions (insufieciency of precipitation and higher average temperature) are considered to be primary reasons of oak decline.

Key words: *Quercus robur, Q. petraea, Q. rubra*, oak decline, ophiostomatoid fungi, *Penicillium*, Czech Republic, Moravia

Novotný D. (2001): Příspěvek k poznání mykoflóry kořenů dubů s tracheomykózními příznaky a bez nich. – Czech Mycol. 53: 211–222

V roce 1994 byla na dvou lokalitách v blízkosti Moravských Budějovic zkoumána mykoflóra kořenů tří druhů dubů (*Quercus robur, Q. petraea, Q. rubra*). Bylo studováno 16 stromů se symptomy onemocnění s tracheomykózními příznaky a 3 stromy bez nich. Celkově bylo v kořenech zjištěno 57 druhů hub. Nejčasteji se vyskytujícími byly *Fusarium solani, Penicillium glandicola, P. glabrum, P. simplicissimum* a *Acremonium curvulum*. Ophiostomatální houby byly zaznamenány pouze na kořenech prosychajících stromů. Za primární příčinu hynutí dubů jsou považovány abiotické podmínky (nedostatek srážek a zvýšenná průměrná roční teplota).

INTRODUCTION

Oak decline is a serious problem in oak stands of many parts of Europe observed during the twentieth century (Anonymus 1990, Oleksyn and Przybyl 1987, Ragazzi et al. 1989, Siwecki and Liese 1991). In the Czech Republic this disease is called "tracheomycosis" or "with tracheomycotic symptoms" (Jančařík 1991, 1995). The reasons or its ethiology are not fully explained, but it is caused by a complex of abiotic and biotic factors (Oleksyn and Przybyl 1987). Fungi cause some of the symptoms, but they are not the primary reason of oak decline (Kowalski 1991, 1996, Przybyl 1995).

Typical symptoms of this disease are: yellowing, atrophy and premature fall of leaves, dying of branches, thinning of crown, creation of numerous water sprouts,

brown mucous exudation, necrotic patches in bark and phloem, discoloration of sapwood, loosening of bark (Kowalski 1991, Przybyl 1995).

Oak decline was for the first time recorded in the Czech Republic in the 1950s. It was recorded very frequently in the 1970s, 1980s and 1990s (Jančařík 1995) and characterised by yellowing and premature fall of leaves, dying of branches in the crown and creating sprouts on stems (Jančařík 1991). Similar symptoms were observed on many other trees (*Pinus, Larix, Fagus, Alnus*) (Jančařík 1992a, b, 1993a, b, Jančařík et al. 1991). Necrotic patches in bark or phloem of the oaks were not noticed.

The mycoflora associated with oak decline was investigated in many countries of Europe (e.g. Germany, Polen). Most researchers studied fungi associated with aboveground parts (stems, branches, leaves) of *Quercus robur* (Kowalski 1991, 1996, Kehr and Wulf 1993, Przybyl K. 1995, 1996). Several mycologists did research on the mycoflora of oaks from different other views, too (endophytes – Halmschlager et al 1993, Petrini and Fisher 1990, mycoflora of oaks damaged by air pollution – Kowalski 1983, mycoflora of natural pruning – Butin and Kowalski 1983).

The mycoflora of oak roots was studied by Kowalski (1983) (roots of dead oaks), Kowalski and Bartnik (1990) (occurrence of ophiostomatoid fungi), Amos and True (1967) (oaks with symptoms of oak-wilt in North America) and Jung and al. (1996) (occurrence of *Phytophtora*).

In the Czech Republic microscopic fungi of oaks with tracheomycotic symptoms were studied by Kubátová and Prášil (1995) and Fassatiová et al. (1995) only. They investigated the mycoflora of stems, branches and roots of three species of *Quercus* (*Q. petraea, Q. pubescens* and *Q. robur*).

Two methods are used for studying the mycoflora of trees. The wet chambers method was used by Kubátová and Prášil (1995) and Fassatiová et al. (1995). Methods based on strong surface sterilisation and incubation on agar medium were used by Amos and True (1967), Kowalski (1983, 1991, 1996), Kehr and Wulf (1993), Przybyl K. (1995) and others.

MATERIALS AND METHODS

The study was conducted in southwest Moravia (Czech Republic) near Moravské Budějovice, in two middle aged oak stands. The first stand (Bučina in Syrovický les, stand number 113C5, 57 years old) was composed of *Quercus robur* (74%), *Q. petraea* (24%) and *Q. rubra* (2%) and classified as a "fresh, oak-beech wood moderately rich in nutrients". The second stand (Na křivánkách in Černý les, stand number 104F4, 39 years old) was composed of *Quercus robur* (46%), *Q. petraea* (52%) and *Larix decidua* (2%) and classified as a "water-deficient oak-beech wood". In April and September 1994 three trees

(two *Q. robur* and one *Q. rubra*) remained without symptoms of oak decline and 16 trees (12 *Q. robur* and four *Q. petraea*) recently died or with disease symptoms in various stages were sampled. On branches, stems and roots of these trees no necrotic black or dark spots were observed. The trees were classified according to the health state of aboveground parts based on canopy cover (methods of Jančařík 1990). A tree marked 0 is healthy (without symptoms), a tree marked 4 is dead or missing at least 70% of leaves of canopy cover.

Temperature and precipitation were measured by the meteorological station at Moravské Budějovice. Over the years 1990–1994 the average temperature was 8.54 °C and the average annual precipitation 429.7 mm. Over the years 1965–1989 the average temperature was 7.61 °C and the average annual precipitation 531.7 mm per year (Dolejský 1997).

The samples were taken from two to five skeleton roots (2–7 cm thick) of each selected tree. The roots were cut into slices 0.5–2 cm thick. The slices were brushed under running water, then washed in a 0.47% solution of sodium hypochlorite (NaClO) or in 0.18% solution of peracetic acid (CH_3CO_3H) for 5 minutes and finally submerged in sterile water for 7–10 minutes. The slices were put in sterile glass wet chambers with sterile cotton wool and sterile filter paper. They were incubated at room temperature (20–25 °C) for 4–7 weeks in the dark. For identification the isolated fungi were cultivated on diagnostic agar media. During this study 443 of strains of fungi and three strains of bacteria were isolated.

RESULTS

The observed root systems of trees with symptoms were never damaged more than the aboveground parts. Root systems of trees in health categories 3 and 4 were strongly reduced. They were composed of skeleton and coarse roots. Fine roots were absent. Rhizomorphs of *Armillaria* spp. were observed on many roots. Ophiostomatoid fungi were observed more times on slices than they were succesfully isolated. They were found on roots of 81.8% of trees with symptoms and were not recorded on roots of healthy trees. Frequency of these fungi was different within the same health category in roots and aboveground parts of trees (Table 1).

Fifty-seven species of fungi (including sterile mycelia) and one species of bacteria were identified from the roots of the three species of oak. Twenty-four taxa were isolated from the roots of healthy trees of *Q. robur*, 42 taxa (including one species of bacteria) from the roots of diseased trees of *Q. robur*, 22 taxa from the roots of diseased trees of *Q. petraea* and 17 taxa from the roots of healthy trees of *Q. rubra* (Table 2).

Cylindocarpon destructans, Fusarium solani and *Penicillium glandicola* were found in roots of both healthy trees of *Q. robur* (Table 2).

Table 1. Observation of ophiostomatoid fungi in oak roots.

Health category of aboveground part	Health state of roots Health state of roots	Number of roots colonised by ophiostomatoid fungi	Trees species	Locality
0	healthy	—	*Q. rubra*	Bučina
0	healthy	—	*Q. robur*	Na křivánkách
0	healthy	—	*Q. robur*	Bučina
0–1	one root dead, other healthy	2	*Q. robur*	Bučina
1	healthy	4	*Q. robur*	Bučina
1	healthy, reduced	—	*Q. robur*	Bučina
1–2	healthy	3	*Q. petraea*	Na křivánkách
1–2	healthy	4	*Q. robur*	Na křivánkách
2	healthy	1	*Q. robur*	Bučina
2	healthy and dead	1	*Q. robur*	Bučina
2–3	healthy, reduced	—	*Q. robur*	Bučina
3	healthy	3	*Q. robur*	Bučina
3	healthy	—	*Q. robur*	Na křivánkách
3	healthy, reduced	2	*Q. petraea*	Bučina
3	healthy and dead	3	*Q. petraea*	Na křivánkách
4	dead	4	*Q. robur*	Bučina
4	healthy and dead	3	*Q. petraea*	Na křivánkách
4	healthy	2	*Q. robur*	Bučina
4	dead	3	*Q. robur*	Na křivánkách

Table 2. Fungi isolated from the roots of three different species of oak.

Species of fungi	*Quercus robur*		*Quercus petraea*	*Quercus rubra*
	Healthy (2 trees)	Diseased (12 trees)	Diseased (4 trees)	Healthy (1 tree)
Absidia cylindrospora Hagem		1		
Acremonium butyri (van Beyma) W. Gams		1		
Acremonium curvulum W. Gams		8	2	
Alternaria tenuissima (Kunze: Fries) Wiltshire	1			
Aspergillus fumigatus Fresenius		1		
Aspergillus versicolor (Vuillemin) Tiraboschi		2		
Beauveria brongniartii (Saccardo) Petch			1	
Botrytis cinerea Persoon: Fries			1	
Cladosporium cladosporoides (Fresenius) de Vries				1
Cylindocarpon destructans (Zinssmeister) Scholten	2	5	1	1
Cylindocarpon sp.				1
Doratomyces stemonitis (Persoon:Fries) Morton et G. Smith		1		
Engyodontium sp.		1		

Table 2. (Cont.)

Species of fungi	*Quercus robur*		*Quercus petraea*	*Quercus rubra*
	Healthy (2 trees)	Diseased (12 trees)	Diseased (4 trees)	Healthy (1 tree)
Epicoccum nigrum Link		1		
Fusarium oxysporum Schlechtendal: Fries	1		1	
Fusarium solani (Martius) Saccardo	2	10	3	1
Fusarium sp.				1
Gliocladium catenulatum Gilman et Abbott	1	6		1
Graphium sp.		2		
Mariannaea elegans (Corda) G. Arnaud ex Samson		1		
Mucor hiemalis Wehmer		1		
Oedocephalum glomerulosum (Bulliard: Fries)		1		
Ophiostoma piceae (Münch) H. et P. Sydow		1	1	
Ophiostoma sp. 1		5	2	
Penicillium arenicola Chalabuda		3		1
Penicillium aurantiogriseum Dierckx *		1		1
Penicillium canescens Sopp			1	
Penicillium chrysogenum Thom		1		1
Penicillium commune Thom		2		
Penicillium daleae Zalessky		6		1
Penicillium expansum Link		2		
Penicillium funiculosum Thom		1		
Penicillium glabrum (Wehmer) Westling	1	9	3	1
Penicillium glandicola (Oudemans) Seifert et Samson	2	10	2	1
Penicillium griseofulvum Dierckx		1		
Penicillium hordei Stolk		1		
Penicillium implicatum Biourge		1		
Penicillium janczewski Zalessky		1		1
Penicillium janthinellum Biourge	1	2		
Penicillium manginii Duché et R. Heim		1		
Penicillium minioluteum Dierckx	1	4		1
Penicillium roquefortii Thom	1	2	1	
Penicillium simplicissimum (Oudemans)Thom	1	9	1	1
Penicillium cf. *solitum* Westling		2		
Penicillium spinulosum Thom	1	7	1	1
Penicillium thomii Maire			1	
Penicillium ser. *Minioluteum* Pitt			1	
Sesquicillium candelabrum (Bonorden) W. Gams	1	4	1	
Streptomyces sp.		2		

Table 2. (Cont.)

Species of fungi	*Quercus robur*		*Quercus petraea*	*Quercus rubra*
	Healthy (2 trees)	Diseased (12 trees)	Diseased (4 trees)	Healthy (1 tree)
Trichoderma atroviride P. Karsten sensu Bissett		7		
Trichoderma citrinoviride Bissett		1		
Trichoderma hamatum (Bonorden) Bainier	1	4	1	
Trichoderma harzianum Rifai		4	1	
Trichoderma koningii Oudemans	1	1	1	
Trichoderma minutisporum Bissett	1			1
Trichoderma viride Persoon: Fries agg.	1	3	1	
Zygorhynchus moelleri Vuillemin		1		
Sterile mycelia			1	
Number of species	17	47	22	17

* The isolated strains belong to the complex species *Penicillium aurantiogriseum*. Identification of these strains is possible by chemical analysis of secondary metabolites only.

Fusarium solani, Penicillium glandicola, P. glabrum, P. simplicissimum, Acremonium curvulum, Trichoderma atroviride, Penicillium spinulosum, P. daleae and *Gliocladium catenulatum* were found in at least 50 % of the studied diseased trees of *Q. robur* (Table 2).

Fusarium solani, Penicillium glabrum, P. glandicola, Acremonium curvulum and *Ophiostoma* sp. 1 were isolated from the roots of at least 50 % of diseased trees of *Q. petraea*.

The mycoflora of roots of *Q. rubra* was studied on one tree only. Therefore it is impossible to give the most frequently occurring species (Table 2).

During the study, 23 species of *Penicillium* were identified from the oak roots. Twenty species were found in *Quercus robur*, 14 species in *Quercus petraea* and ten species in *Quercus rubra*. Dominant species were *Penicillium glandicola, P. glabrum, P. simplicissimum, P. spinulosum, P. daleae* and *P. minioluteum*. The first four were isolated from all studied species of oak (Table 2).

The same dominating fungi were found in roots of all species of oak.

Two ophiostomatoid fungi (*Ophiostoma piceae* s.l. – probably *O. querci* and *Ophiostoma* sp.) were observed in the roots of diseased *Quercus robur* and *Q. petraea* (Table 1).

Forty-six species of fungi were isolated from the roots of oaks growing in the locality Bučina (No. 113C5). *Fusarium solani, Penicillium glandicola, P. glabrum, P. simplicissimum, P. spinulosum, P. daleae, Acremonium curvulum* and *Gliocladium catenulatum* were found most frequently in these roots (Table 3).

Table 3. Fungi isolated from roots of three different species of oak from two localities.

Species of fungi	Locality Bučina				Locality Na křivánkách		
	All trees (12 tr.)	*Quercus robur* (10 trees)	*Quercus petraea* (1 tree)	*Quercus rubra* (1 tree)	All trees (7 tr.)	*Quercus robur* (4 trees)	*Quercus petraea* (3 trees)
Absidia cylindrospora Hagem	1	1					
Acremonium butyri (van Beyma) W. Gams					1	1	
Acremonium curvulum W. Gams	7	7			4	2	2
Alternaria tenuissima (Kunze: Fries) Wiltshire					1	1	
Aspergillus fumigatus Fresenius	1	1					
Aspergillus versicolor (Vuillemin) Tiraboschi	2	2					
Beauveria brongniartii (Saccardo) Petch					1		1
Botrytis cinerea Persoon: Fries					1		1
Cladosporium cladosporoides (Fresenius) de Vries	1			1			
Cylindocarpon destructans (Zinssmeister) Scholten	7	6		1	1		1
Cylindocarpon sp.	1			1			
Doratomyces stemonitis (Persoon: Fries) Morton et G. Smith	1	1					
Engyodontium sp.	1	1					
Epicoccum nigrum Link	1	1					
Fusarium oxysporum Schlechtendahl: Fries	1		1		1	1	
Fusarium solani (Martius) Saccardo	12	10	1	1	4	2	2
Fusarium sp.	1			1			
Gliocladium catenulatum Gilman et Abbott	7	6		1	1	1	
Graphium sp.	2	2					
Mariannaea elegans (Corda) G. Arnaud ex Samson	1	1					
Mucor hiemalis Wehmer	1	1					
Oedocephalum glomerulosum (Bulliard: Fries)	1	1					
Ophiostoma piceae (Münch) H. et P. Sydow					2	1	1
Ophiostoma sp.	5	4	1		2	1	1
Penicillium arenicola Chalabuda	3	3					
Penicillium aurantiogriseum Dierckx *	2	1		1			
Penicillium canescens Sopp					1		1
Penicillium chrysogenum Thom	2	1		1			
Penicillium commune Thom	2	2					
Penicillium daleae Zalessky	7	6		1			
Penicillium expansum Link	2	2					
Penicillium funiculosum Thom	1	1					
Penicillium glabrum (Wehmer) Westling	10	9		1	4	1	3
Penicillium glandicola (Oudemans) Seifert et Samson	10	9		1	5	3	2

Table 3. (Cont.)

Species of fungi	Locality Bučina				Locality Na křivánkách		
	All trees (12 tr.)	*Quercus robur* (10 trees)	*Quercus petraea* (1 tree)	*Quercus rubra* (1 tree)	All trees (7 tr.)	*Quercus robur* (4 trees)	*Quercus petraea* (3 trees)
Penicillium griseofulvum Dierckx	1	1					
Penicillium hordei Stolk					1	1	
Penicillium implicatum Biourge					1	1	
Penicillium janczewski Zalessky	2	1		1			
Penicillium janthinellum Biourge	1	1			2	2	
Penicillium manginii Duché et R. Heim	1	1					
Penicillium minioluteum Dierckx	6	5		1			
Penicillium roquefortii Thom	1			1	3	2	1
Penicillium simplicissimum (Oudemans) Thom	8	7		1	4	3	1
Penicillium cf. *solitum* Westling	2	2					
Penicillium spinulosum Thom	7	6		1	3	2	1
Penicillium thomii Maire					1		1
Penicillium ser. *Minioluteum* Pitt					1		1
Sesquicillium candelabrum (Bonorden) W. Gams	6	4	1	1			
Streptomyces sp.	2	2					
Trichoderma atroviride P. Karsten sensu Bissett	6	6			1	1	
Trichoderma citrinoviride Bissett	1	1					
Trichoderma hamatum (Bonorden) Bainier	5	5			1		1
Trichoderma harzianum Rifai	3	3			2	1	1
Trichoderma koningii Oudemans	3	2	1				
Trichoderma minutisporum Bissett	1	1			1	1	
Trichoderma viride Persoon: Fries agg.	6	5	1				
Zygorhynchus moelleri Vuillemin					1	1	
Sterile mycelia					1		1
Number of species	46	41	6	17	28	20	18

* Isolated strains belong to the complex species *Penicillium aurantiogriseum*. Identification of these strains is possible by chemical analysis of secondary metabolites only.

Twenty-eight taxa were isolated from the roots of trees growing in the locality Na křivánkách (No. 104F4). *Penicillium glandicola, P. glabrum, P. simplicissimum, Fusarium solani* and *Acremonium curvulum* were found most frequently in these roots (Table 3).

Sixteen species of fungi were found in roots from both localities. Four species (*Penicillium daleae, P. minioluteum, Sesquicillium candelabrum* and *Trichoderma viride* agg.) were observed very frequently in roots from the locality Bučina

(No. 113C5), but were not isolated from roots from the locality Na křivánkách (No. 104F4) (Table 3).

Discussion

On the roots and stems of the studied trees not any dark necrotic patches in bark or phloem were observed. These features are described by many researchers as characteristic of oak decline in other countries (Kehr and Wulf 1993, Kowalski 1991, Przybyl 1995). The observed roots of dying trees were in better health state than the aboveground parts. This difference is probably caused by water deficiency. Branches and leaves dry sooner than roots, because water easier reaches to organs in lower positions than organs in higher position. Abiotic factors are probably the primary reason of dying in these localities.

The wet chamber method was used in this study. A different spectrum of fungi than recorded by Kowalski (1983) and Amos and True (1967) was found. This difference is probably caused by using different methods. These mycologists used methods based on strong surface sterilisation and incubation on agar media. Similar results as in the present study were obtained by Kubátová and Prášil (1995) and Fassatiová et al. (1995), who used the method of wet chambers, too.

Amos and True (1967) found most frequently *Trichoderma lignorum, Umbellopsis versiformis, Penicillium* spp., *Gliocladium roseum* and *Cephalosporium* spp. (= *Acremonium* spp.). Kowalski (1983) recorded most frequently *Trichoderma viride, Mycelium radicis atrovirens, Cylindrocarpon destructans* and *Coniothyrium fuckelii*. The dominant fungi isolated in the present work were *Fusarium solani, Penicillium* spp. and *Acremonium curvulum*. Six species of *Trichoderma* were isolated and members this genus occurred in roots of 73.7 % of oak trees. Fungi belonging to *Mucorales* and dematiaceous anamorphs were recorded very rarely. Kowalski (1983) did not observe Penicillia in roots.

Cylindrocarpon destructans was recorded in the oak roots using both different methods. Kowalski (1983) found it in 27.3 % of trees of *Q. robur* and in 38.5 % of trees of *Q. rubra*. During this study it was recorded in 50 % of trees of *Q. robur* and in 25 % of trees of *Q. petraea*. Amos and True (1967) did not isolated this species.

The Penicillia were found in stems, branches or roots of oaks by Amos and True (1967), Kowalski (1991), Halmschlager et al. (1993), Kehr and Wulf (1993) and Przybyl (1995), but they did not identify them to the species level. Species of Penicillia isolated from roots, stems and branches of oaks were identified by Kubátová (2000). She isolated 23 species from three species of oak (*Q. petraea, Q. robur* and *Q. pubescens*). Dominant species were *Penicillium glandicola, P. glabrum, P. minioluteum* and *P. simplicissimum*. The spectrum of Penicillia found in the present work is very similar.

In the present study *Fusarium solani* was isolated very frequently. This species was recorded by Fassatiová et al. (1995) from roots and by Kowalski (1991, 1996) and Przybyl (1995, 1996) from aboveground parts, too. They observed it not so often as in this study.

Ophiostomatoid fungi were observed on the roots of trees with any symptoms of oak decline only. They were not found on the roots of trees with healthy abovegrounds parts, but they were present in trees with healthy roots and drying aboveground parts. Kowalski and Bartnik (1990), who used the method based on strong surface sterilisation, isolated them from 53.3 % of dead or dying trees and from 33.3 % of diseased trees. In the present study, ophiostomatoid fungi were recorded in 81.8 % of diseased trees. Sieber et al. (1995) isolated *Ophiostoma querci* from lesions of *Quercus robur* twigs only once, but they observed it frequently in the sapwood of stumps of recently cut oaks.

In the present study two species of these fungi were isolated. *Ophiostoma piceae* s.l. (probably *O. querci*) was found twice (once in *Q. robur* and once in *Q. petraea*). The other species was isolated from the roots of five trees of *Q. robur* and two trees of *Q. petraea*. This species is similar to *Ophiostoma stenoceras* observed by Kowalski and Bartnik (1990) in oak roots.

Penicillium daleae, P. minioluteum and *Sesquicilium candelabrum* were found frequently in roots from the locality Bučina, but have not been isolated in the locality Na křivánkách. Fassatiová et al. (1995) isolated these species from trees in the locality Bučina, too. Occurrence of these fungi may depend on soil moisture. The stand of the locality Bučina was classified as a "fresh, oak-beech wood moderately rich in nutrients" and that of the locality Na křivánkách as a "water-deficient oak-beech wood". Also *Trichoderma viride* agg. was identified in roots from the locality Bučina only. The morphology of this species is very variable and it is similar to *Trichoderma atroviride*, which was found in both localities.

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Yeasts isolated from the lakes of Dhanmondi and Ramna, Bangladesh

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The occurrence of yeasts in the water of two lakes located in Dhaka City over a period from September to December 1999 was investigated. The number of yeasts of lake Dhanmondi and Ramna ranged from 9.5×10^4 to 35×10^4 and 2.3×10^4 to 11×10^4 CFU/l, respectively. The isolated yeast strains belonged to 5 species: *Saccharomyces cerevisiae, Rhodotorula glutinis, Rhodotorula mucilaginosa, Debaryomyces hansenii* var. *fabryi* and *Candida suecica*. The maximum number of yeasts was found to be 3 times higher in the water samples of Dhanmondi lake than that of Ramna lake. The higher number of yeasts was correlated with the temperature of the water and with pH values.

Key words: yeast community, occurrence, lake water, Bangladesh

Khan M. R., Saha M. L., Anisuzzaman M. a Sláviková E. (2001): Yeasts isolated from the lakes of Dhanmondi and Ramna, Bangladesh – Czech Mycol. 53: 223–228

Študoval sa výskyt kvasiniek vo vode dvoch jazier v meste Dhaka v období od septembra do decembra 1999. Počet kvasiniek v jazere Dhanmondi sa pohyboval v rozmedzí $9,5\times10^4$ až 35×10^4 CFU/l a v jazere Ramna $2,3\times10^4$ až 11×10^4 CFU/l. Izolované kmene kvasiniek boli zaradené do 5 druhov: *Saccharomyces cerevisiae, Rhodotorula glutinis, Rhodotorula mucilaginosa, Debaryomyces hansenii* var. *fabryi a Candida succica*. Zistilo sa, že maximálny počet kvasiniek bol 3-krát vyšší vo vzorkách vody z jazera Dhanmondi než z jazera Ramna. Vyšší počet kvasiniek koreloval s vyššou teplotou vody a s pH hodnotami.

INTRODUCTION

Water is a natural habitat for many micro-organisms but few surveys have been conducted to estimate the numbers of yeasts in waters. The relation between the extent of water pollution and the yeast flora are not known both regarding numbers and varieties of yeasts. Simard and Blackwood (1971) reported yeasts isolated from water samples taken from five widely separated collection points on the St. Lawrence River. Although yeasts have been shown to be common inhabitants of water, yet it is important to determine the yeast populations of both polluted and relatively unpolluted waters (Spencer et al. 1974). Their number and species composition depend on the type and purity of water (Hagler and Mendonca-Hagler 1981).

Yeasts can enter the water with swimmers, fish, animals, plants and also with sewage. Yeasts were found in the water of three artificial freshwater lakes. The lakes were used for recreational purposes and situaed in the area of Lowland Záhorie of Slovakia (Sláviková et al. 1992).

The numbers and species of yeast occurring in lakes and streams might serve as an index of the amount of pollution in such waterbodies. The presence of yeasts in any numbers in water could be taken as an indication of the presence of sewage as well. The need for detection and determination of yeasts which are able to biodegrade and/or accumulate organic and inorganic toxicants has become greater as water pollution has increased (Kwasniewska 1988).

In Bangladesh, limnological studies of lake water were made from time to time (Islam et al. 1979; Khondker et al. 1988; Khondker and Parveen 1992, 1993). It was reported that Lake Dhanmondi was highly eutrophic to hypertrophic and also contaminated by sewage, garbage from nearby residential colonies, markets, dopes used by anglers, direct input of waste products from slum areas situated on the bank, and had a high concentration of O_2, N_2 and P too. On the other hand, Lake Ramna was free from those contaminants. However, isolation of yeasts from lake water was not attempted. The present study represents an initial investigation on the occurrence of yeasts in water of the two artificial lakes Dhanmondi and Ramna.

MATERIAL AND METHODS

Studysites

The lakes of Dhanmondi and Ramna are artificial lakes in the city of Dhaka, Bangladesh. They were selected for the present study and sampled from September to December 1999. Samples were taken from five different sites (widely separated) at each lake. Water was collected in sterile plastic bottles and transported to the laboratory on ice. Samples were processed within 2 hours after collection.

Methods

Ten-millilitre aliquots of water were precipitated with a colloidal solution containing 0.5 ml 10 % sodium carbonate and 0.25 ml 10 % ferric sulphate and centrifuged for 2 minutes. Three drops of 20 % sodium-potassium tartarate were added and the mixture were streaked on malt agar plates containing 100 μg/ml chloramphenicol (Sláviková et al. 1992).

The isolation of yeast was carried out with the serial dilution technique. Malt agar plates containing chloramphenicol were all used throughout of the study. The pH of the medium was adjusted to 6.81 and the plates incubated from 3 to 7 days at 22 °C. Colonies of different appearance were counted. A digital colony counter

was used for the purpose of yeast count. The density of yeasts was estimated by calculating the number of colony forming unites (CFU) per litre.

Morphological and physiological characteristics of isolates were examined by the methods described by Yarrow (1998). Isolates were identified according to Kurtzman and Fell (1998).

RESULTS AND DISCUSSION

Temperature, pH and yeast densities (CFU/l) are shown in the Table 1. The temperature of the water of Lake Dhanmondi ranged from 19.9 °C to 22.4 °C, in Lake Ramna from 20.0 °C to 23.5 °C. The pH of the water differed in the two lakes. The pH of the water from Lake Dhanmondi ranged from 5.99 to 8.50, in Lake Ramna from 6.01 to 7.75. The results revealed that the pH, temperature and yeast densities differed between the lakes. Yeast densities in Lake Dhanmondi ranged from 9.5×10^4 to 35.0×10^4 CFU/l, in Lake Ramna from 2.3×10^4 to 11.0×10^4 CFU/l. The highest yeast population density was observed in the water of Lake Dhanmondi. Higher numbers of yeasts were found at a temperature of 22.4 °C both in Lake Ramna and Lake Dhanmondi (Table 1).

Table 1. Temperature, pH and yeast densities of the lakes of Dhanmondi and Ramna.

Lake	Temperature (°C)	pH	Yeast densities CFU/l
Dhanmondi	19.9	5.99	9.5×10^4
	20.0	6.00	15×10^4
	21.0	6.02	28×10^4
	22.2	7.04	22×10^4
	22.4	8.50	35×10^4
Ramna	20.0	6.01	2.3×10^4
	21.1	6.50	3.5×10^4
	22.4	6.65	5.0×10^4
	23.5	6.85	7.1×10^4
	22.4	7.75	11.0×10^4

Simard and Blackwood (1971) published yeast densities in the St. Lawrence river during summer varying from 0 to 9500 per 100 ml. The yeast densities in water samples of the river Danube ranged from 100 to 21,100 CFU/l (Sláviková and Vadkertiová 1997). Sláviková et al. (1992) reported densities of *Aureobasidium pullulans* (black yeast) reaching about 5×10^3–10×10^3 cells per litre in some regions of Lake Jakubov.

The isolated 18 yeast strains belonged to 5 species. Out of these strains 7 represented the species *Saccharomyces cerevisiae* Meyen ex E. C. Hansen,

Table 2. Main characteristics of the isolates

Identified species	Isolate No.	Source	Colony colour	Cell measurement (μm)	Pseudo-mycelium	Urea hydrolysis	Fermentation of saccharides	Assimilation of KNO_3	Growth at 37°C
Saccharomyces cerevisiae	Y1, Y2, Y3	Ramna	Cream	(6.6–9.9) × (9.9–13.2)	Rudimentary	Negative	Positive	Negative	Positive
	Y4, Y6, Y12, 14	Dhan-mondi							
Rhodotorula glutinis	Y15	Ramna	Red	(3.5–5.0) × (5.0–6.6)	Absent	Positive	Negative	Positive	Positive
	Y8, 10c	Dhan-mondi							
Rhodotorula mucilaginosa	Y7, Y11	Ramna	Pink	(3.3–8.3) × (3.3–8.3)	Absent	Positive	Negative	Negative	Positive
	Y10r, Y17	Dhan-mondi							
Debaryomyces hansenii var. *fabryi*	Y16	Ramna	White	(3.3–7.4) × (3.3–9.9)	Present	Negative	Negative	Negative	Positive
	Y5	Dhan-mondi							
Candida suecica	Y13	Ramna	White	(3.3–6.6) × (6.6–12.0)	Present	Negative	Negative	Negative	Positive
	Y13	Ramna							
	Y9	Dhan-mondi							

3 strains belonged to the species *Rhodotorula glutinis* (Fresenius) F. C. Harrison, 4 strains to *Rhodotorula mucilaginosa* (Jörgensen) F. C. Harrison, 2 strains to *Debaryomyces hansenii* var. *fabryi* (Ota) Nakase et M. Suzuki and the remaining 2 strains were identified as *Candida suecica* Rodrigues de Miranda & Norkrans. These five different species varied in colour, cell size, urease activity, fermentation of saccharides and assimilation of nitrate (Table 2). The occurrence of these species could also be influenced by their ability to grow at higher temperatures. All isolated strains grew well at 37 °C.

In aquatic environments, that is rivers, ponds and lakes, representatives of the genera *Candida, Trichosporon, Rhodotorula, Hansenula, Cystofilobasidium,*

Geotrichum and *Saccharomyces* were isolated most frequently (Spencer et al. 1970, 1974; Simard and Blackwood 1971; Sláviková et al. 1992; Sláviková and Vadkertiová 1997).

The ascosporogenous yeast species *Saccharomyces cerevisiae* was the predominant species isolated from samples taken from both studied lakes. It was reported that *S. cerevisiae* was a stable constituent of the activated sludge biocenosis of different industrial waste waters (Grabinska-Loniewska et al. 1993). Spencer et al. (1974) isolated this species together with *Trichosporon cutaneum* as a dominant species in a domestic sewage treatment plant; it was often found in river and fish pond water, too (Spencer et al. 1970; Sláviková and Vadkertiová 1995, 1997). *S. cerevisiae* is able to ferment saccharides. The incidence of fermentative yeasts in the environment are reported characteristic for sewage (Hagler et al. 1981).

Carotenoids producing red and pink yeasts were also isolated very frequently from both lakes. During periods of bright sunlight, carotenoids protect the vital structures and processes of yeast cells and this is a possible reason for the predominance of red yeasts in the upper layers of water (Kwasniewska 1988). All isolated strains belonged to the basidiomycetous species *Rhodotorula glutinis* and *R. mucilaginosa* (Table 2). Species of the genus *Rhodotorula* have also been observed in Lake St. Clair (Kwasniewska 1988) and pink yeast isolates were collected from the St. Lawrence River (Simard and Blackwood 1971). Higher densities of red yeasts were found in surface water layers, where phenolic contaminants and petrochemicals in higher concentration were discharged (Kwasniewska 1988).

The black yeast *Aureobasidium pullulans* was often isolated from artificial lake waters (Sláviková et al. 1992). No black yeasts were found in Dhanmondi and Rhamna lakes but the white yeasts *Debaryomyces hansenii* var. *fabryi* and *Candida suecica* were found during this study (Table 2).

In this study the main representatives of the yeast population were found to be similar in both two lakes. The highest densities were observed in Dhanmondi Lake into which organic waste, domestic, industrial, sewage and residential waste are discharged. In Lake Ramna only human activity takes place. Therefore the densities of yeast populations were relatively low in Lake Ramna.

The studied water environment probably positively affects the presence of yeast species by metabolic activities within compounds of natural and industrial origin and could be one of the reason for their dominance there. This suggests that they may play an important role in the aquatic environment of lakes. Much more study will be necessary to provide a reasonable explanation for the presence of yeast populations and their possible interaction with the environment.

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Fusarium moniliforme, F. subglutinans and Aspergillus flavus in maize products in Slovakia

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Piecková E. and Jesenská Z. (2001): Fusarium moniliforme, F. subglutinans and Aspergillus flavus in maize products in Slovakia – Czech Mycol. 53: 229–235

Ubiquitous microfungi *Fusarium moniliforme, F. subglutinans, F. proliferatum* represent frequent contaminants of maize products and can produce some mycotoxins: beauvericin, fusaproliferin and, the most important, fumonisins A_1, A_2, B_1–B_4, C_1 etc. Fumonisins are known to cause serious veterinary, and potentially human, mycotoxicosis. The aim of our study was to characterize the incidence of *F. moniliforme* and *F. subglutinans* in the presence of *Aspergillus flavus* in maize products produced in Slovakia during a four-years period. One hundred and forty samples of maize grain, groat, semolina, flour, and 28 samples of maize straw, cornhusk, corn silk and soil from the maize fields were mycologically evaluated for the named strains using potato-dextrose agar with 0.02 % chloramphenicol and 0.3 % of 0.2 % Botran and incubation in dark at 25 °C for 7–10 days. No *Fusarium* sp. and *A. flavus* strains were present in 40 % of the maize samples. The highest number of *F. moniliforme, F. subglutinans* and *A. flavus* isolates were encountered in flour samples in 1996 (4 264 cfu/g on average), in groat in 1998 (17 743.7 cfu/g on average), and in groat in 1996 (353 cfu/g on average). Twenty two per cent *A. flavus* isolates and 10 *F. moniliforme* strains were tested for their ability to produce aflatoxins, or fumonisin B_1, *in vitro*. No aflatoxin-producing *A. flavus* isolate was found, but all tested *F. moniliforme* strains produced fumonisin B_1 in amounts detectable by TLC. According to the results presented in this paper it is evident that strains of *F. moniliforme, F. subglutinans* and *A. flavus* were not very important contaminants of maize products from crops harvested in 1995–98 in Slovakia.

Key words: *Fusarium moniliforme, F. subglutinans, Aspergillus flavus*, mycotoxins, maize

Piecková E. a Jesenská Z. (2001): Fusarium moniliforme, F. subglutinans a Aspergillus flavus kukuričných produktoch na Slovensku – Czech Mycol. 53: 229–235

Celosvetovo rozšírené mikroskopické huby *Fusarium moniliforme, F. subglutinans, F. proliferatum*, často sa vyskytujúce v kukuričných produktoch, sú schopné produkovať viaceré mykotoxíny: beauvericín, fuzaproliferín, ako aj najviac sledované fumonizíny A_1, A_2, B_1–B_4, C_1 adť. Je známe, že fumonizíny spôsobujú vážne veterinárne, a pravdepodobne aj humánne, mykotoxikózy. Cieľom 4-ročnej štúdie bolo charakterizovať incidenciu *F. moniliforme* a *F. subglutinans*, spolu s *Aspergillus flavus*, v kukuričných produktoch slovenskej proveniencie. Po kultivácii na zemiakovo-dextrózovom agare s 0,02 % chloramfenikolu a 0,3 % z 0,2 % Botranu počas 7–10 dní pri 25 °C v tme sa mykologicky vyšetrilo 140 vzoriek kukuričného zrna, šrotu, krupice a múky a 28 vzoriek kukuričnej slamy, šúpolia, vlásia a pôdy z kukuričných polí. Zo 40 % kukuričných vzoriek sa neizoloval žiaden kmeň *Fusarium* sp. a *A. flavus*. Najviac izolátov *F. moniliforme, F. subglutinans* a *A. flavus* sa získalo zo vzoriek múky v r. 1996 (priemerne 4264 kolónie tvoriacich jednotiek – KTJ/g), šrotu v tom istom roku (priemerne 353 KTJ/g) a v r. 1998 (priemerne 17743 KTJ/g). Dvadsaťdva percent izolátov *A. flavus* a 10 kmeňov *F. moniliforme* sa testovalo na schopnosť produkovať aflatoxíny, resp. fumonizín B_1 *in vitro*. Nezistil sa ani jeden aflatoxín-produkčný kmeň *A. flavus*, ale všetky testované kmene *F. moniliforme* produkovali fumonizín B_1 v množtve detekovateľnom tenkovrstvovou chromatografiou. Na základe získaných výsledkov možno konstatovať, že kmene *F. moniliforme, F. subglutinans* a *A. flavus* významne nekontaminovali kukuričné produkty z úrody v r. 1995–98 na Slovensku.

INTRODUCTION

Ubiquitous worldwide-spread microfungi *Fusarium moniliforme, F. subglutinans* and *F. proliferatum* represent frequent contaminants of maize products. They are able to produce some mycotoxins: beauvericin, fusaproliferin and, the most important, fumonisins A_1, A_2, B_1–B_4, C_1 and their isoderivatives. *F. napiforme, F. nygamai* and *F. dlamini* which occurr more rarely in plants are also producers of fumonisins (Ritieni et al. 1997a, Sibanda et al. 1997, MacKenzie et al. 1998).

It is known that fumonisins cause serious veterinary mycotoxicosis, including fatal organ debilitites of laboratory animals. The consumption of contaminated maize grain and maize-based products has been statistically associated with an elevated incidence of human oesophageal cancer in some parts of the world (Nair 1998, Rosiles et al. 1998), and also has been reported to have caused accute toxicosis in consumers (Bhat et al. 1997). According to a study by Ueno et al. (1997) in some parts of China endemic for primary hepatocarcinoma, fumonisins B and deoxynivalenol represented risk factors for this disease while aflatoxin B_1 was postulated as the initiating factor for hepatocarcinogenesis. According to IARC fumonisins are potential human carcinogens (class 2B). Because of the ability of these highly heat- and fermentation-resistant mycotoxins presence in cereal products, usually contaminated with carcinogenic aflatoxins, the Joint FAO/WHO Expert Committee on Food Additives and Contaminants reccommends their study in any way (Shephard et al. 1996, Jackson et al. 1997, Torres et al. 1998). Their level should not exceed 100–200 μg/kg in contaminated maize. The maximum daily tolerated intake (MDTI) for humans is being concidered for 1 000 ng, and the maximum tolerated level of fumonisins A and B in foodstuffs, 1 mg/kg, is the current regulation in Switzerland (Diener 1997, de Nijs et al. 1998).

In contrast to neighbouring countries with similar climates (Austria, the Czech Republic, Hungary and Poland) there has been a lack of information on the incidence of *F. moniliforme* and *F. subglutinans* in maize products produced in Slovakia. The aim of our study was to characterize their colonisation by both fusaria strains in the presence of *A. flavus*.

MATERIAL AND METHODS

In 1995–98 one hundred and forty samples of maize products (43 samples of maize grain, 24 groat, 54 semolina and 19 flour, stored at a silo with relative air humidity 58–68 % and temperature approx. 2–3 °C less than outside ambient), 2 samples of maize straw, 5 samples of cornhusk, 1 sample of corn silk, and 20 samples of soil from maize fields, were taken in southern Slovakia where climatic conditions are suitable for corn cultivation. Climatological information on evaluated period are given in Table 1 (according to the Slovak Hydrometeorological Institute, Bratislava,

Slovakia). June–August are months with the highest average temperatures, and December – February the coldest ones. Average month's air relative humidity is usually the highest in December and January while the lowest in July.

Table 1. Climatological characterization of southern region of Slovakia (measuring station Gabčíkovo) in 1995–98

Year	average month's T /C/	average max. month's T /C/	Range average min. month's T /C/	average month's air relat. humid. /%/	average month's precipitation /mm/
1995	−0.3–23.1	1.8–29.8	−2.9–15.6	63.9–90.0	1.2–90.4
1996	−3.0–19.7	−0.3–25.7	−7.9–14.2	64.4–89.7	11.4–136.2
1997	−2.5–20.4	−0.3–27.7	−5.5–14.3	66.9–92.2	7.1–175.1
1998	−1.5–20.7	1.0–27.5	−3.8–15.4	62.1–86.0	2.4–124.6

Note: T – temperature

Every product sample was mycologically evaluated for *F. moniliforme, F. subglutinans* and *A. flavus* strains by a dilution method (max. 4-times) in sterile saline on 10 plates of potato-dextrose agar (Difco) with 0.02 % of chloramphenicol and 0.3 % of 0.2 % Botran (0.2 ml of suspension per plate) or 50 grains from every seed sample were put onto the 10 agar plates. After incubation in the dark at 25 °C for 7–10 days the number of colony forming units (cfu) per 1 g of sample or relative number [%] of contaminated seeds was quantified. Every isolated colony of both fusaria and *A. flavus* was identified according to their macro- and micromorphology.

One hundred and thirty six, i. e. 22 % of isolates of *A. flavus* were tested for their ability to produce aflatoxins *in vitro* using the method described by Abarca et al. (1988) in liquid medium with 20 % of sucrose and 2 % of yeast extract. The ability of 10 *F. moniliforme* strains to produce fumonisin B_1 *in vitro* was tested semi-quantitatively by TLC (silica gel GF 254, Merck; mobile phase acetonitrile: toluene: water – 95: 5: 3; v/v) after 20 d of cultivation on sterile corn with 40 % humidity (Pepeljnjak et al. 1998).

RESULTS

No strains of *A. flavus* were isolated from soil, straw, cornhusk and corn silk samples (all taken in 1998). No fusaria were present in 46 % of these samples while *F. moniliforme* was isolated from 50 % of the soil samples with 110.5 cfu/g on average, one straw and corn silk samples yielded 65 000, and 40 cfu/g, respectively, and 20 % of the soil samples and the other straw sample were contaminated with *F. subglutinans* yielding 22.3 cfu/g, and 205 000 cfu/g, respectively.

No searched moulds were present in 40 % of maize samples during our study. The only strains isolated from 6.4 % of the samples were *F. subglutinans,* while

no sample was contaminated just with *F. moniliforme* strains. Both these fusaria occurred in 27 % of the samples. *A. flavus* strains, as well as fusaria together with *A. flavus*, occurred in 12 %of the samples investigated (Table 2).

Table 2. Occurrence of *Fusarium moniliforme* (FM), *A. subglutinans* (FS) and *Aspergillus flavus* (AF) strains in maize products in 1995–98.

Crop year	1995		1996		1997		1998	
	abs.	[%]	abs.	[%]	abs.	[%]	abs.	[%]
No. of negative samples	11	52	12	36	20	37	3	9
No. of samples with only FM	0	0	0	0	0	0	0	0
No. of samples with only FS	0	0	1	3	1	2	7	22
No. of samples with FM+FS	4	19	2	7	19	35	13	41
No. of samples with only AF	2	10	3	9	8	15	4	12.5
No. of samples with AF+FM	1	5	5	15	4	7	0	0
No. of samples with AF+FS	0	0	1	3	1	2	1	3
No. of samples with AF+FM+FS	3	14	9	27	1	2	4	12.5
Total	21	100	33	100	54	100	32	100

The incidence of *F. moniliforme* in grain was 3, 1, 0.8 or 0.3 % and that of *F. subglutinans* was 22, 1, 0.5 or 0.4 % in 1995, 1996, 1997 and 1998, respectively. *A. flavus* contaminated 1, 0.7, 2 or 1.4 % of tested grains in these years (Table 3).

Table 3. Contamination of maize grain with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 43 samples).

Year Total No. of samples/No. of contaminated samples/average percentage of contaminated grains	1995	1996	1997	1998
FM	8/1/3 %	7/5/1 %	20/9/0.8 %	8/8/0.3 %
FS	8/1/22 %	7/5/1 %	20/9/0.5 %	8/8/0.4 %
AF	8/1/1 %	7/5/0.7 %	20/9/2 %	8/8/1.4 %

In 1995 *F. moniliforme* had a higher incidence than that of *F. subglutinans* in similar samples, while *A. flavus* had the least contamination levels. None of the 6 *A. flavus* isolates produced aflatoxin B_1 *in vitro*. In 1996 *F. subglutinans* had a higher incidence in groat samples, while *F. moniliforme* remained high in semolina and flour. *A. flavus* on the other hand had a high incidence in groat samples and of the 46 (28 %) isolates tested none produced aflatoxin B_1 *in vitro*. *F. subglutinans* had a higher frequency of occurrence in 1997 than *F. moniliforme* with incidences of 2 638.5 and 53.7 cfu/g compared to 145 and 6.7 cfu/g in groat

and semolina, respectively. *A. flavus* had consistently lower incidences, 103.5 and 0.4 cfu/g. None of 22 (63 %) evaluated *A. flavus* isolated strains produced aflatoxin B_1 *in vitro* under experimental conditions. In 1998 strains of *F. subglutinans* had a higher incidence in groat and flour than *F. moniliforme,* 17 743. 7, 1 195 and 948.7 compared to 185.9 and 220 cfu/g. However, incidences were comparable in semolina samples. *A. flavus* had an occurrence of 41.2, 30 and 177.7 cfu/g. None of the 62 (20 %) *A. flavus* isolates produced aflatoxin B_1 *in vitro* (Table 4–6).

Table 4. Contamination of maize groat with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 24 samples).

Year Total No. of samples/No. of contaminated samples/average No. of isolates [cfu/g]	1995	1996	1997	1998
FM	1/1/200	5/3/166	10/3/145	8/8/185.9
FS	1/1/0	5/3/1326	10/3/2638.5	8/8/17743.7
AF	1/1/0	5/3/353	10/3/103.5	8/8/41.2

Table 5. Contamination of maize semolina with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 54 samples).

Year Total No. of samples/No. of contaminated samples/average No. of isolates [cfu/g]	1995	1996	1997	1998
FM	9/6/61.1	13/8/416.9	24/22/6.7	8/8/1408.7
FS	9/6/15.5	13/8/3.1	24/22/53.7	8/8/1195
AF	9/6/4.4	13/8/4.8	24/22/0.4	8/8/30

Table 6. Contamination of maize flour with *Fusarium moniliforme* (FM), *F. subglutinans* (FS) and *Aspergillus flavus* (AF) (total 19).

Year Total No. of samples/No. of contaminated samples/average No. of isolates [cfu/g]	1995	1996	1997	1998
FM	3/2/1850	8/5/4264.1	0/0/0	8/5/220
FS	3/2/50	8/5/46.9	0/0/0	8/5/648.7
AF	3/2/1.7	13/5/12.5	0/0/0	8/5/177.7

Two (20 %) *F. moniliforme* strains isolated from grain in 1996 produced 125–185 mg/kg of biomass and 8 strains (80 %) with the same origin 232–417 mg/kg of fumonisin B_1 detected by TLC (Pepeljnjak et al. 1998).

DISCUSSION

According to Schlechter et al. (1998) American maize products in 1991–92 were contaminated with 10^5 cfu of *F. moniliforme* and max. 3 605 ng of total fumonisins per 1 g and products originated in South Africa 10^3 cfu of *F. moniliforme* and 465 ng of total fumonisins per g. *F. moniliforme* and *F. proliferatum* strains represented the most frequent contaminants of Italian maize in which also fumonisin B1, beauvericin and fusaproliferin, all together, were present (Ritieni et al. 1997b). Logrieco et al. (1998) regarded *F. subglutinans, F. proliferatum* and *F. semitectum* as natural contaminants of maize in Europe, South America and South Africa. They can produce beauvericin which has been already isolated from maize in named parts of the world as well as in North America (Logrieco et al. 1997). Czech cereal products from corn harvested in 1995 (71 samples) contained 278 ng of fumonisins B_1–B_3 and from 1996 harvest (76 samples) 131 ng of the same fumonisins per g (Ostrý and Ruprich 1997). The same authors evaluated also maize products by ELISA, from which 89 % were contaminated with fumonisins (180 ng/g on average), but 4 % with more than 1 000 ng/g (Ostrý and Ruprich 1998). Dutch authors (de Nijs et al. 1998) found that 93 % of tested samples of maize, imported to the Netherlands from 18 countries, were contaminated with fumonisin B_1 in average amount 1359 ng/g.

From our results of investigation of maize products from crop harvested in 1995–98 in Slovakia, it can be seen that more than one third of tested samples did not contain *F. moniliforme* or *F. subglutinans*. From the rest of the samples, both fusaria were hardly isolated in counts higher than 10^3 cfu/g, however, they were usually found in the presence of *A. flavus* strains. The occurrence of propagules of micromycetes in evaluated materials was not apparent in this study.

The *A. flavus* strains tested for *in vitro* production of aflatoxin B_1 were all negative, and *F. moniliforme* were able to produce only small quantities (10^2 mg/kg of biomass) of fumonisin B_1 *in vitro*.

CONCLUSION

Probably good conditions of farming technology during harvest and storage of raw maize and maize-based products in observed period were resulted in their low contamination with *F. moniliforme, F. subglutinans* and *A. flavus* moulds. Their ability to produce fumonisin B_1, or aflatoxin B_1 *in vitro* was not significant, too.

AKNOWLEDGMENT

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Book Review

D. PRUSKY, S. FREEMAN & B. DICKMAN (EDS.):

Colletotrichum. Host specifity, pathology, and host-pathogen interaction.
APS Press, The American Phytopathological Society,
St. Paul, Minnesota, USA, 2000, 393p.,
ISBN 0–89054–258–9.
(The book is deposited in the library of the Society)

Fungi from the genus *Colletotrichum* and its teleomorph *Glomerella* are considered very important plant pathogens. They cause economically significant damage to crops in tropical, subtropical and temperate regions. Cereals, grasses, legumes, ornamentals, vegetables and fruit may be attacked by this pathogen. The ability to give rise to latent or quiescent infections has caused *Colletotrichum* to be ranked among the most important post-harvest pathogens.

This book includes recent results of research on *Colletotrichum*. Lectures were performed at the International Workshop on Host Specifity, Pathology, and Host-Pathogen Interaction of *Colletotrichum*, supported by BARD (United States – Israel Binational Agricultural Research and Development Fund) in Jerusalem from 29, August. to 1. September, 1998. Problems in the areas of systematics and sexual stage, infection process, host specifity, population genetics, epidemiology, pathogenicity genes, regulation of pathogenicity and host-resistance, important *Colletotrichum* diseases, mycoherbicides and their use, new strategies to the study and the control of new epidemics (i.e. *C. acutatum* in fruit crops, *C. coccodes* in vegetables, and *C. lindemuthianum* in beans) were presented. Important contributions have been summarized and appear as 23 chapters in eight thematic parts of this book. The chapters are supplemented with tables, figures, photos and literature.

The first part "Systematics, Vegetative Compatibility and Sexual Stage" includes four chapters. The authors (from Spain, New Zealand, USA and Israel) present results of the research on systematics in the past, presence and future of systematics, describe the importance of phylogeny in understanding host-fungus relationships, genetic regulation of sexual compatibility in *Glomerella graminicola* and vegetative compatibility in *Colletotrichum*.

The results of the research to the cell biology of the *Colletotrichum* infection process, early molecular communication between *C. gleosporoides* and the host and regulation of melanin biosynthesis genes during appressorium formation by *C. lagenarium* are presented in the second part "Infection Process" (three chapters, by the authors from UK, USA and Japan).

Very interesting are the three following parts which deal with host specifity and genetic diversity, pathogenicity genes and regulation of pathogenicity and host resistance. Besides its considerable economic impact, *Colletotrichum* has become a valuable experimental pathogen to study genetic, molecular and biochemical aspects of plant – fungus associations. This part contains seven chapters (authors from USA, Israel, Australia, France).

The two following parts present results of the research on preparation and utilization of mycoherbicides and problems of control (two chapters, authors from UK and Izrael).

The part "Major *Colletotrichum* Hosts" contain 7 chapters (authors from USA, UK, Israel). In these chapters results of studies of *Colletotrichum* diseases on strawberry, citrus, almond, coffee, bean, potato and maize are included.

The book contributes to the understanding of problems dealing with *Colletotrichum* and future development in the the areas of biology, pathology and control.

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Jiřina Krátká

CZECH MYCOL. 53 (3), 2001

New records of micromycetes from the Czech Republic. IV. Acrodontium salmoneum, Chaunopycnis alba and Cylindrocarpostylus gregarius, and notes on Dactylaria lanosa and Trichoderma saturnisporum

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Kubátová A., Černý M. and Nováková A. (2001): New records of micromycetes from the Czech Republic. IV. Acrodontium salmoneum, Chaunopycnis alba and Cylindrocarpostylus gregarius, and notes on Dactylaria lanosa and Trichoderma saturnisporum. – Czech Mycol. 53: 237–255

Five saprotrophic mitotic microfungi, *Acrodontium salmoneum, Chaunopycnis alba, Cylindrocarpostylus gregarius, Dactylaria lanosa,* and *Trichoderma saturnisporum,* were found during studies of micromycete diversity in the Šumava Mts., Krkonoše Mts., Krušné hory Mts., Prague and Jevany near Prague in the Czech Republic. The microfungi were isolated from soil, except *Cylindrocarpostylus gregarius,* which was found on bark, and *Acrodontium salmoneum* isolated from air, seeds and mites. Three of them had not yet been reported from the Czech Republic and are considered to be the first records for this country. Descriptions and illustrations are given. All the fungi are maintained in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague.

Key words: conidial fungi, saprotrophs, biologically active metabolites, Bohemia

Kubátová A., Černý M. a Nováková A. (2001): Nové nálezy mikromycetů pro Českou republiku. IV. Acrodontium salmoneum, Chaunopycnis alba a Cylindrocarpostylus gregarius, a poznámky k Dactylaria lanosa a Trichoderma saturnisporum. – Czech Mycol. 53: 237–255

Během studia biodiverzity saprotrofních mikromycetů na Šumavě, v Krkonoších, Krušných horách, v Praze a Jevanech byly v posledních letech nalezeny tyto vzácnější houby: *Acrodontium salmoneum, Chaunopycnis alba, Cylindrocarpostylus gregarius, Dactylaria lanosa a Trichoderma saturnisporum.* Většinou byly izolovány z půdy, pouze *Cylindrocarpostylus gregarius* byl izolován z borky a *Acrodontium salmoneum* z ovzduší, semen a roztočů. Tři z uvedených hub jsou prvními nálezy těchto druhů z území České republiky. Jsou uvedeny popisy a vyobrazení. Kultury všech mikromycetů jsou uchovávány ve Sbírce kultur hub (CCF) katedry botaniky na Přírodovědecké fakultě UK v Praze.

INTRODUCTION

The rare microfungi treated below were recovered during several different surveys of the diversity of saprotrophic micromycetes in the Czech Republic carried out by the authors in the past years. These micromycetes are neither mentioned in Řepová (1989a, 1989b, 1990a, 1990b), who compiled a list of soil micromycetes known from the formerly Czechoslovakia, nor in other papers by our mycologists (with the exception of *Dactylaria lanosa* and *Trichoderma saturnisporum*). They appear to be new records for the Czech Republic.

MATERIALS AND METHODS

Surveys of micromycete diversity were made in the following areas:

— soil microfungi: Šumava Mts. (National Park and Protected Landscape Area) in the south of the Czech Republic (1993–96, see Kubátová et al. 1998), locality Jevany near Prague (1993–95), and Krkonoše Mts. (National Park) in the northern part of the Czech Republic (2000);
— microfungi in mycorhizosphere: Krušné hory Mts. in the north-western part of the Czech Republic (1984–1991, see Černý and Cudlín 1989), and Krkonoše Mts. (National Park) (1991–93)
— air-borne microfungi: outdoor air of Prague (1995–97);
— seed-borne microfungi: Prague (2000–01).

The strains were isolated using a soil dilution method, serial washing of roots or by a Harvard impactor. Isolation media used were soil extract agar with glucose, Rose Bengal and streptomycin (SEGA), malt-extract agar (MEA), wort-beer agar (WBA), or Sabouraud agar (SAB). For identification, cornmeal agar (CMA), Czapek yeast extract agar (CYA), malt-extract agar (MEA), potato-carrot agar (PCA), and potato glucose agar (PGA) were used.

Majority of the strains were lyophilised in skim milk. They are maintained in the Culture Collection of Fungi (CCF), Department of Botany, Faculty of Science, Charles University, Prague. Brief descriptions of morphological features and additional information follow.

Descriptions of the fungi based on our isolates and other notes.

Acrodontium salmoneum de Hoog 1972 (Fig. 1)

Systematic position: Mitotic fungus, teleomorph not known.

Examined strains

CCF 3106: outdoor air, Prague, Czech Republic, isol. A. Kubátová on WBA by Harvard impactor, IX.1996, as No.160/96, LYO.

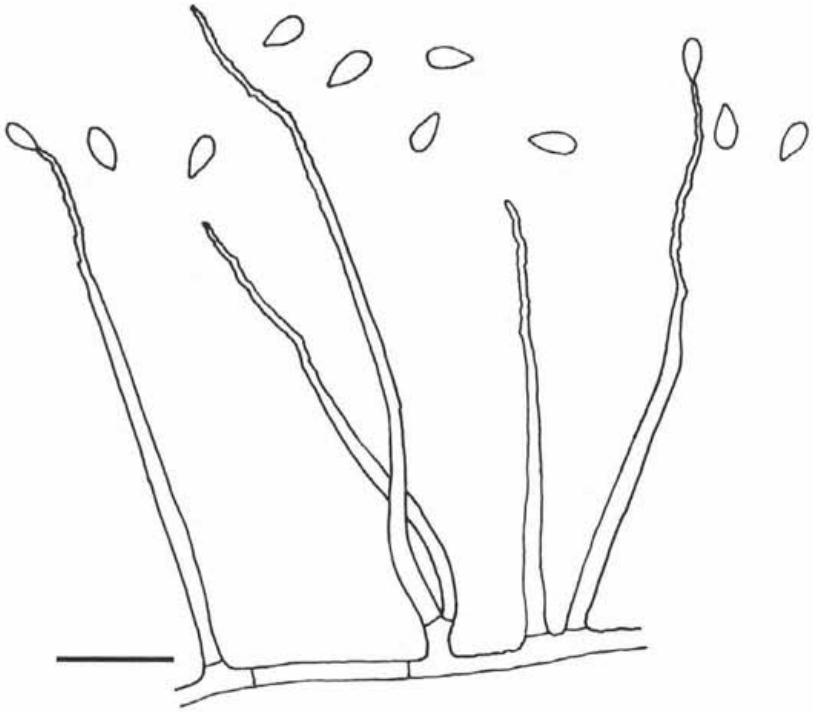

Fig. 1. *Acrodontium salmoneum*, strain CCF 3106, conidiophores with conidia (bar = 10μm). A. Kubátová del.

CCF 3174: digestion tract of mite *Acarus siro*, seed storehouse, Research Institute of Crop Production, Prague, Czech Republic, isol. J. Hubert on MEA, X.2000, as No. 40, det. A. Kubátová, LYO.

CCF 3175: seeds of *Lactuca sativa*, seed storehouse, Research Institute of Crop Production, Prague, Czech Republic, isol. J. Hubert on MEA, X.2000, as No. 8, det. A. Kubátová, LYO.

CCF 3220: digestion tract of mite *Caloglyphus* sp., mouldy wheat, Buštěhrad, Czech Republic, isol. J. Hubert on MEA, VI.2001, as No. 241, det. A. Kubátová, LYO.

Description

Colonies on CMA powdery, peach coloured, growing rather slowly, reaching 13–15 mm, 20–24 mm and 28–33 mm diam. after 7, 10 and 14 days at 25 °C, respectively. Growth on MEA and CYA is similar in growth rate, however sporulation on MEA is somewhat poor and the colonies on CYA are yellowish

peach and funiculose. Colonies at 30 °C after 7 days on CYA reaching 7–8 mm. Growth at 35 °C is nil.

Conidiogenous cells (see Fig. 1) long, growing from undifferentiated hyphae, sometimes in groups of 2–3, slightly tapering to a well-developed rachis, somewhat flexuous, c. 35–95 μm long (including rachis), 1.5–2 μm wide in the lower part and c. 1.2 μm wide in the rachis.

Conidia hyaline, smooth-walled, obovoid with apiculate base, 3.4–5.6 × 2.2–2.8 μm diam.

Our observations are generally in accordance with de Hoog (1972). However, no chlamydospores were observed and growth is somewhat faster in our strains.

Similar species

After de Hoog (1972) this species resembles pink-coloured species of *Nodulisporium* or *Geniculosporium*. They differ by either truncate conidia or short conidiogenous cells.

Habitats and distribution

The fungus was isolated from human sputum (The Netherlands), found as a culture contaminant (U. S. A.), isolated from soil of a beech forest in West Germany, and one strain is from an unknown source (de Hoog 1972). Two other strains were found in the soil of grotto in France (Seigle-Murandi et al. 1980). Steiman et al. (1995) have mentioned a strain isolated from decayed wood and from crab shell. These data do not allow to estimate the typical habitat of this fungus.

Notes: The strain of *Acrodontium salmoneum* found in grotto soil in France is known to produce acrodontiolamide, a secondary metabolite with antifungal properties (Buarque de Gusmão et al. 1993). After Steiman et al. (1995) the acrodontiolamide of this strain inhibits especially phytopathogenic and entomopathogenic fungi.

Chaunopycnis alba W. Gams 1980 (Figs. 2 and 3)

Systematic position: Mitotic fungus, teleomorph not known. The genus includes two species only (Möller and Gams 1993).

Examined strains

CCF 3119: soil in beech forest (*Fagus sylvatica*) near lake Čertovo jezero, Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1996, as No. 94/96.

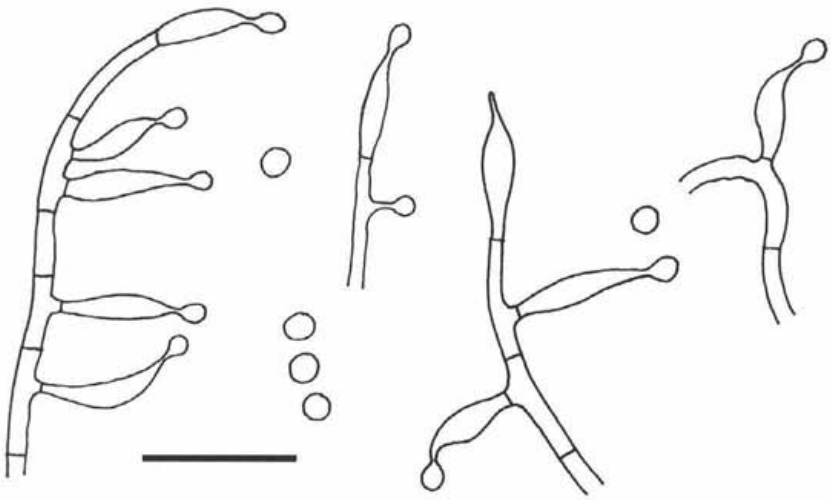

Fig. 2. *Chaunopycnis alba*, strain CCF 3119, conidiogenous cells with conidia (bar = 20μm). A. Kubátová del.

CCF 3176: soil in beech forest *(Fagus sylvatica)*, near Jevany, central Bohemia, Czech Republic, isol. A. Nováková on SEGA, I.1994, det. A. Kubátová, LYO.

CCF 3185: soil of the peat-bog Úpské rašeliniště, Krkonoše Mts., northern Bohemia, Czech Republic, isol. A. Kubátová on SEGA with LiCl, XI.2000, as No. 25/01, LYO.

Description

Colonies on PCA and CMA white, floccose, with yellow tint in reverse, growing rather slowly, reaching 15–22 mm, 25–28 mm and 31–35 mm diam. after 7, 10 and 14 days at 25 °C, respectively. Growth on WBA somewhat faster, colonies reaching ca 19–23 mm, 29–33 mm and 43–45 mm diam. after 7, 10 and 14 days, respectively. Growth at 37 °C is nil.

Conidiomata (Fig. 3c) were observed after 10 days or more on PCA and CMA (sometimes however even after 3 or 4 weeks), forming small globose granules on medium. They were 80–380 μm diam., with little-differentiated filamentous walls, and filled with a dense mass of small conidia. Sporulation on MEA was very poor. The sporulation of the strains CCF 3176 and CCF 3185 is much better than of strain CCF 3119.

Conidiogenous cells (phialides according to Gams 1980) (Figs. 2 and 3) were visible after disruption of the conidiomata. They were better observable in Melzer's reagent (potassium iodide, iodine and chloralhydrate) than in lactic acid stained by methylene blue. Phialides are solitary or irregularly arranged, grow from

hyaline, septate, smooth-walled hyphae c. 0.7–1.5 μm wide. They are cylindrical or inflated, straight or slightly curved, with narrow neck, 6–11 × 1.6–1.9 μm (terminal phialides sometimes longer) and are remarkably similar to those of *Tolypocladium geodes*. Below the terminal phialide, a short lateral neck is sometimes formed like in *Sesquicillium*.

Conidia small, hyaline, globose, smooth-walled, 1.6–1.9 μm diam.

Our observations of microscopic and macroscopic features are in accordance with Gams (1980).

Similar species

The other species of this genus, *Chaunopycnis ovalispora*, isolated from an antarctic lichen, differs from *C. alba* in size and shape of the conidia and phialides (see Möller and Gams 1993). Besides, *C. ovalispora* is not able to grow at temperatures above 20 °C. *Tolypocladium geodes* resembles *Chaunopycnis alba* in its conidiogenous cells and conidia, but it does not form individual conidiomata. *Sesquicillium microsporum,* another similar species, has irregularly branched conidiophores and has affinity to myxomycetes.

Habitats and distribution

Gams (1980) studied many strains from different types of soil (e.g. forest soil, greenhouse soil, páramo soil) and plant material (flower buds, needles etc.) from different parts of the world (Colombia, France, Scotland, Sri Lanka, Sweden, The Netherlands). Nilsson et al. (1992) found *C. alba* to be very frequent in a mire in Sweden. Other large collections of isolates are from lichens and mosses of Antarctica, Spitsbergen and the Swiss Alps (Möller et al. 1995). These authors also studied strains from Brazil, Germany, Great Britain, Madagascar, Malaysia, Singapore, U. S. A. etc. The fungus is considered to be cosmopolitan. In our region it was probably neglected due to its minute conidiogenous structures.

Notes: *Chaunopycnis alba* is interesting by its unusual conidioma type enclosed in a wall of loosely intertwined hyphae, somewhat resembling pycnidia. Thus, it is intermediate between Hyphomycetes and Coelomycetes.

Screenings of many strains made in the 1990s revealed high genetic, metabolic and physiological diversity within this species (Möller et al. 1995, 1996). Some isolates are interesting by its production of cyclosporin A, an immunosuppressive compound (Möller et al. 1995), and of pyridoxatin, which has a high cytotoxicity against human cancer cell lines and inhibits gelatinase A, playing an important role in cancer invasion (Lee et al. 1996).

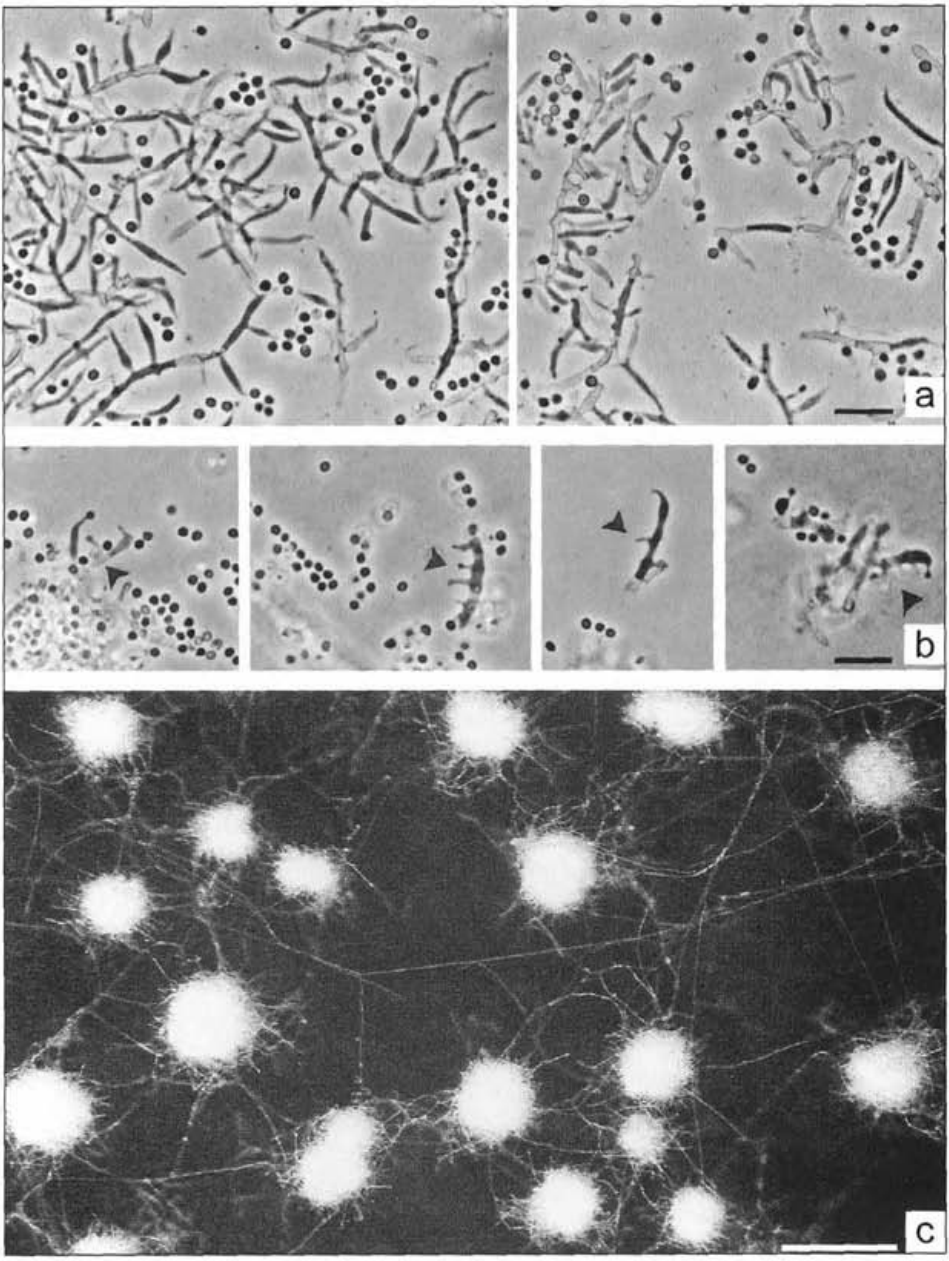

Fig. 3. *Chaunopycnis alba*: **a** – conidiogenous cells and conidia, strain CCF 3185 (CMA, 1 month), phase contrast (bar = 10μm); **b** – conidiogenous cells with terminal and lateral necks (arrows) and conidia, strain CCF 3176 (PCA, 10 days), phase contrast (bar = 10μm); **c** – conidiomata, strain CCF 3185 (PCA, 1 month) (bar = 250μm). Photo A. Kubátová

Cylindrocarpostylus gregarius (Bres.) Kirschner et Oberw. 1999 (Figs. 4 and 5)

Bas. *Diplocladium gregarium* Bres. 1903
Syn. *Cylindrocladium gregarium* (Bres.) de Hoog 1978

Systematic position: Anamorphic *Hypocreales* (Hawksworth et al. 1995). Teleomorph not known. The genus is monospecific.

Examined strain

CCF 2751: ex bark of stump of *Picea abies*, locality Kalek, Krušné hory Mts., Czech Republic, isol. M. Černý on WBA, X.1991.

Description

Colonies on MEA with sparse, whitish mycelium, reaching 15–18 mm, 23–26 mm and 32–37 mm diam. at 25 °C after 7, 10 and 14 days, respectively. Growth on CMA is somewhat faster. Colonies after 10 days have a light brown tint. Sporulation is better after 10–14 days on both media.

Conidiophores (Fig. 4a, b and 5a, c) mononematous, erect, c. 160–560 μm long, c. 15–30 μm wide in the basal part, penicillately branched at the top. Young stipes smooth, hyaline, mature ones warted and brownish, especially at the base. Conidiophores three to five-stage branched, metulae with warted walls (Fig. 5b).

Conidiogenous cells (phialides) cylindrical, hyaline, smooth, c. 25–33 × 2.5–3.5 μm.

Conidia (Fig. 4c and 5d) hyaline, smooth, slightly curved, 2–4-celled, c. 14–40 × × 4.2–5.2 μm, forming a drop at the tip of the conidiophore. Anastomoses were frequently observed between hyphae and even between conidia.

The above mentioned data correspond with measurements by Kirschner and Oberwinkler (1999). Conidiophore branching of *C. gregarium* resemble that of *Leptographium*, however *Leptographium* has one-celled conidia only. Morphology and habitat of *C. gregarium* are also very close to *Stilbella fusca*. However, the latter species forms predominantly synnemata (Seifert 1985).

Habitat and distribution

The species was first described (as *Diplocladium gregarium*) from bark of *Pinus sylvestris* in Poland (Bresadola 1903). Other known records are from Germany, where the fungus was found in bark beetle galleries in *Pinus sylvestris* and *Picea abies* and was also isolated from the beetles themselves (Kirschner and Oberwinkler 1999). Our find supports the probable affinity of this fungus for bark of conifers.

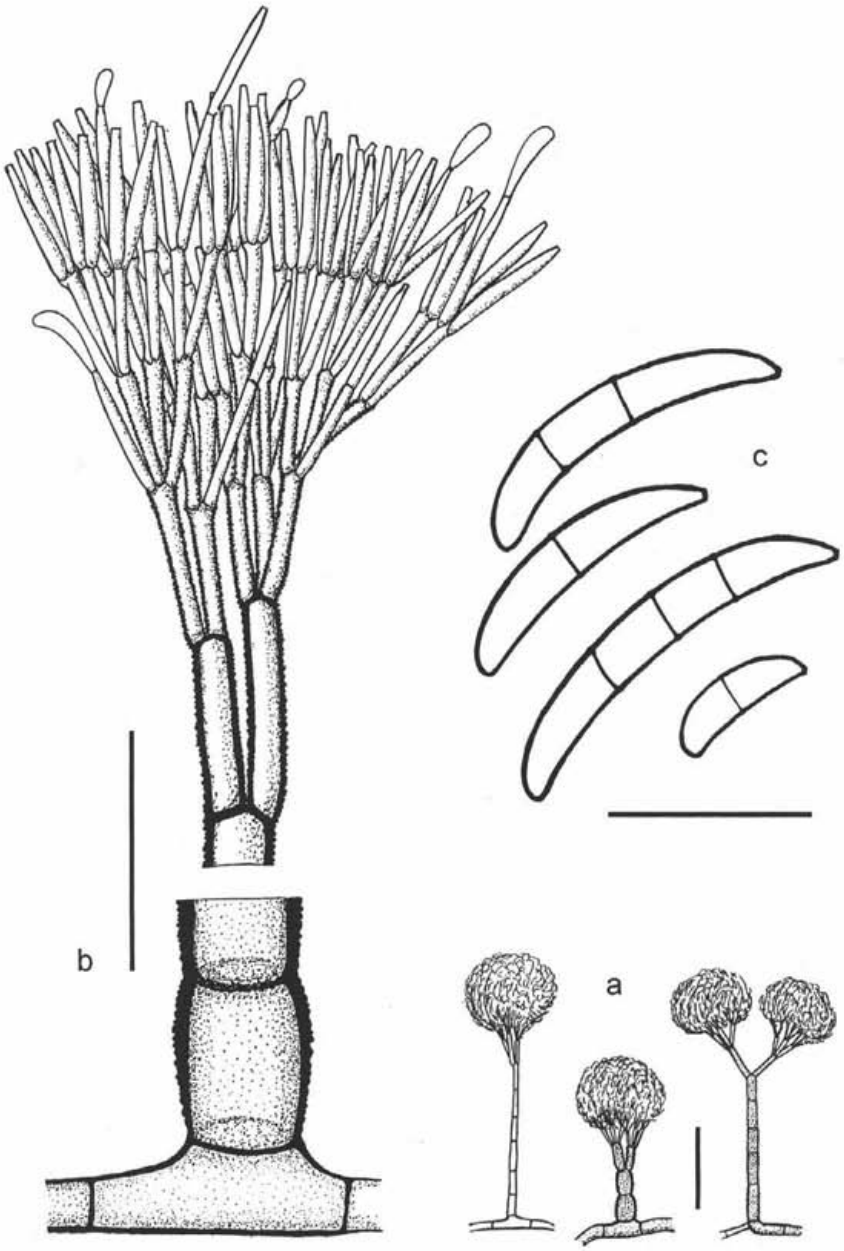

Fig. 4. *Cylindrocarpostylus gregarius*, strain CCF 2751: **a** – habitus of conidiophores (bar = 100μm); **b** – base of conidiophore and conidiophore branching (bar = 50μm); **c** – conidia (bar = 20μm). M. Černý del.

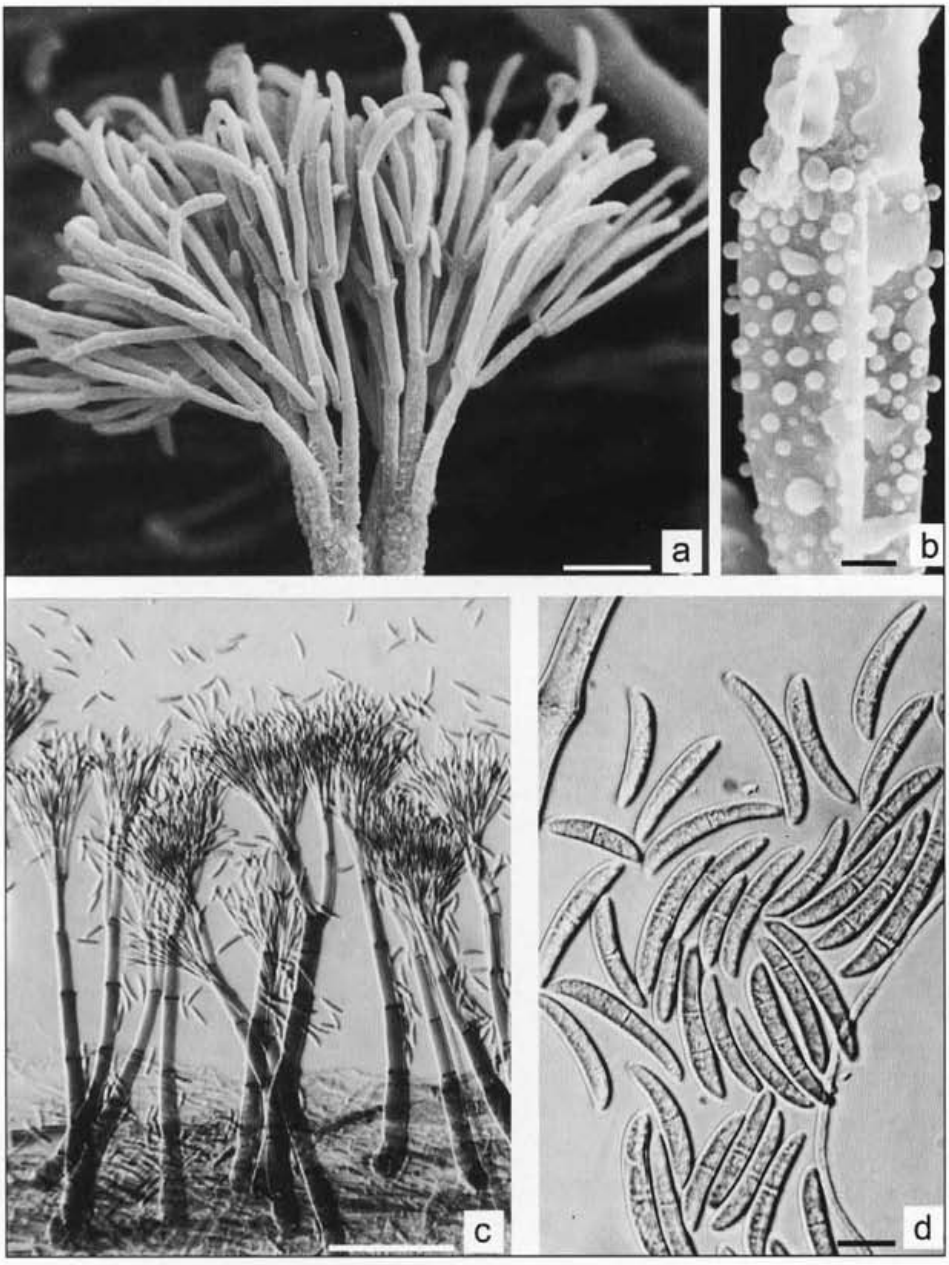

Fig. 5. *Cylindrocarpostylus gregarius*, strain CCF 2751: **a** – penicillately branched conidiophore tip, SEM (bar = 20μm); **b** – part of metula with warted walls, SEM (bar = 5μm); **c** – habitus of conidiophores (bar = 100μm); **d** – conidia (bar = 10μm).

Photos J. Nebesařová (a, b) and M. Černý (c, d)

Notes: The generic classification of this species has changed during the last decades. Hughes (1958) reduced *Diplocladium* Bonord. to a synonym of *Cladobotryum* Nees. De Hoog (1978) revised some fungicolous Hyphomycetes including *Cladobotryum*. He studied the type specimen of *Diplocladium gregarium* Bres. and due to its hyaline conidiophores, elongated 1–2-celled conidia and resemblance to *Cylindrocladium* he recombined it as *Cylindrocladium gregarium* (Bres.) de Hoog. Contrary to this, Crous and Wingfield (1994), who revised *Cylindrocladium*, excluded *C. gregarium* from this genus, emphasizing the resemblance of the conidiophore branching pattern to *Leptographium* Lagerb. et Melin and *Phialocephala* W. B. Kendr. Kirschner et Oberwinkler (1999) rediscovered fresh material of a fungus almost matching the description of *Diplocladium gregarium*. The only difference was the 0–1–septate conidia mentioned in Bresadola's description *versus* 0–3-septate conidia seen in their own material. Therefore they re-examined the type material of *D. gregarium*, found a few conidia with two septa and concluded that their fungus was conspecific with *Diplocladium gregarium*. As there was no appropriate genus for that species, they established the new genus *Cylindrocarpostylus* Kirschner et Oberwinkler with the single species *Cylindrocarpostylus gregarius* (Bres.) Kirschner et Oberwinkler. It is of interest to note, that M. Černý, who collected this fungus in Czechoslovakia in 1991, also observed bi- to tri-septate conidia in his material, which otherwise corresponded to the description of *Cylindrocladium gregarium*. Kirschner and Oberwinkler (1999) explain this phenomenon by the age of the colony: young colonies produce more conidia with three septa, whereas in old cultures single-septate conidia predominate.

Dactylaria lanosa Malla et W. Gams 1971 (Fig. 6)

Systematic position: Mitotic fungus, teleomorph not known (Hawksworth et al. 1995). In the genus *Dactylaria*, it is classified under the section *Dactylaria* (de Hoog 1985). The genus includes 82 species (Goh and Hyde 1997).

Examined strains

CCF 2739: ex rhizosphere of *Picea abies*, Kamenný vrch hill, Krušné hory Mts., Czech Republic, isol. M. Černý on WBA by serial washing of roots, XI.1990, as 15aPi-K, LYO.

CCF 2850: ex rhizosphere of *Picea abies*, Modrý důl valley, Krkonoše Mts., Czech Republic, isol. M. Černý on WBA by serial washing of roots, 1993, as No. 219b.

CCF 2982: ex forest soil under *Picea abies*, Svaroh Mt., Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1995, as No. 82/95, LYO.

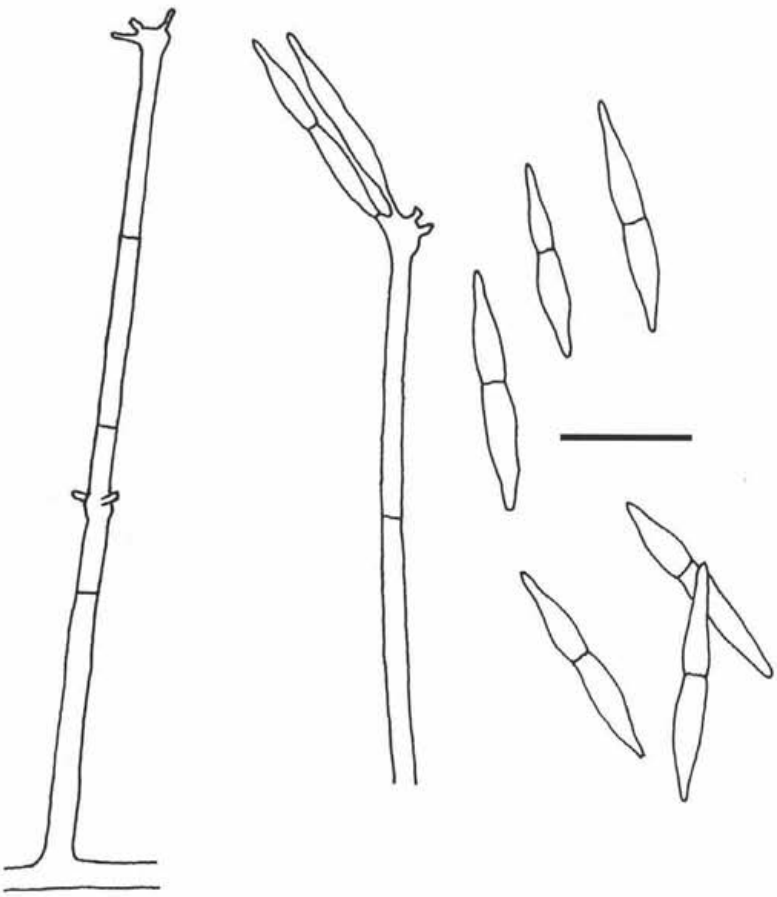

Fig. 6. *Dactylaria lanosa*, strain CCF 2982, conidiophores with conidia (bar = 10μm).
A. Kubátová del.

Other finds:

MC 99U: ex rhizosphere of *Picea abies*, Sluneční údolí valley, Krkonoše Mts., Czech Republic, isol. M. Černý on WBA by serial washing of roots, IX.1991.

GEF 20/95: ex forest soil under *Picea abies*, Mt. Jezerní hora, Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1995.

Description

Colonies on MEA whitish to yellow, lanose, reaching 14–17 mm, 21–24 mm and 28–35 mm diam. after 7, 10 and 14 days at 25 °C, respectively. Reverse brown-orange to brown. Old cultures are deep yellow and produce yellow diffused pigment into the agar. Growth at 30 °C is nil.

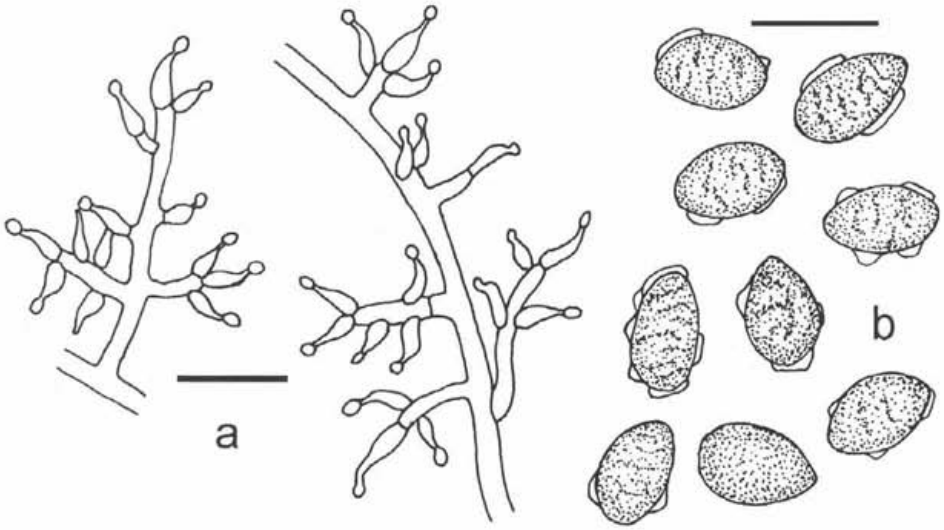

Fig. 7. *Trichoderma saturnisporum*, strain CCF 2983: a – conidiophores (bar = 20μm); b – conidia (bar = 5μm). A. Kubátová del.

Conidiophores (Fig. 6) mononematose, erect, long, simple or rarely with a branch, 1.8–2.3 μm wide, similar to vegetative hyphae, bearing 1–4 μm long conidiiferous denticles, which are often scattered at the terminal part of the conidiophores or arise below the apex. Mycelial hyphae sometimes with golden-yellow encrustations.

Conidia hyaline, smooth, narrow, fusiform, predominantly two-celled, c. 14–20.2 × 1.8–2.3 μm.

Macroscopic and microscopic features correspond to those reported by Malla and Gams (1971) and de Hoog (1985).

Habitats and distribution

The type strain was isolated from roots of *Picea abies* in Denmark (Malla and Gams 1971), other specimens were found in forest soil in Sweden, on a decaying *Picea* trunk in Germany, and on *Pseudohydnum gelatinosum* in The Netherlands (de Hoog 1985). These data and our own finds suggest a certain affinity of *Dactylaria lanosa* to *Picea* itself as a substrate or to habitats with *Picea* trees. A nematophagous character, observed in several *Dactylaria* species, was not recorded in this species.

The first three above cited strains were published by Kubátová et al. (1997) in the new catalogue of filamentous fungi and two of them also by Kubátová et al. (1998) in an article on soil microfungi of the Šumava Mts.

Trichoderma saturnisporum Hammill 1970 (Fig. 7)

Systematic position: Anamorphic *Hypocreales* (Hawksworth et al. 1995). Teleomorph is not known. In the genus *Trichoderma* it is classified in the section *Longibrachiatum* (Samuels et al. 1998).

Examined strain

CCF 2983: ex soil in beech forest (*Fagus sylvatica*), Mt. Ždanidla, Šumava Mts., southern Bohemia, Czech Republic, isol. A. Kubátová on SAB, VI.1996, as No. 106/96, LYO.

Description

Colonies on PGA, MEA and CMA fast growing, covering a 9 cm Petri dish in 3–4 days at 25 °C. Mycelium sparse, inconspicuous, whitish. On CMA yellow pigment diffusing into agar was observed. Fruiting areas dark olive green. At 37 °C, colonies on MEA cover the Petri dish in 7 days, mycelium inconspicuous. At present, our strain shows poor sporulation.

Conidiophores (Fig. 7a) are aggregated into tufts, irregularly branched.

Phialides solitary, paired or in verticils of 3, ampulliform or lageniform, often curved, ca 10–12 μm long.

Conidia (Fig. 7b) green, ovoid to ellipsoidal, 4.4–5.4 × 3.1–3.8 μm, mostly with distinct hyaline wing-like outgrowths c. 0.7 μm high, sometimes without wings.

Our observations are in accordance with data of Hammill (1970), Doi et al. (1987), and Gams and Bissett (1998). In contrast to Doi et al. (1987), no chlamydospores were detected.

A similar species is *Trichoderma ghanense* (Doi et al. 1987). Its conidia have irregular extensions on the surface, but are longer (up 8.2 μm).

Habitat and distribution

Trichoderma saturnisporum was first isolated from forest soil in Georgia, United States (Hammill 1970). After Samuels et al. (1998) it is known from Australia, Italy, South Africa, Turkey and the United States. The species was isolated predominantly from soil, in some cases from the rhizosphere of *Triticum* sp., straw of *Triticum* sp. and roots of *Pseudotsuga menziesii*, Alexandrova and Velikanov (1999) isolated an other strain from steppe soil in Kalmykia, southwestern Russia.

The first report of the above cited strain (CCF 2983) in the Czech Republic was published by Kubátová et al. (1997). The strain is mentioned by Kubátová et al. (1998), too. Noteworthy, Marvanová (1999) isolated this fungus at the same

time (June 1996) from alluvial meadow soil in southern Moravia, Czech Republic. She isolated it on soil agar at 42 C and characterised it therefore as thermotolerant.

Notes: Doi et al. (1987) placed *T. saturnisporum* in a new section, *Trichoderma* sect. *Saturnisporum*. However, molecular studies of Kuhls et al. (1997) showed a close relationship to section *Longibrachiatum*, therefore Gams and Bisset (1998) merged the section *Saturnisporum* with the section *Longibrachiatum*.

T. saturnisporum is known to produce some biologically active compounds. Rebuffat et al. (1993) isolated the peptides saturnisporins, peptaibols with antibiotic activity against *Staphylococcus aureus*. Ritieni et al. (1995) reported production of the antibiotics paracelsin A, B, C, D and E.

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