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The occurrence of the rare species Circinella umbellata (Mucorales)

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Šimonovičová A. (1998): The occurrence of the rare species Circinella umbellata (Mucorales) – Czech Mycol. 50: 245–248

The rare species *Circinella umbellata* Tiegh. et Le Monn. was isolated from Eutric Fluvisol (Je) below an abandoned meadow, Gabčíkovo, Slovakia. In this paper we are giving a description and Scanning Electron Microscope photographs and light microscope photographs.

Key words: microfungi, Zygomycetes, Mucorales, *Circinella umbellata*.

Šimonovičová A. (1998): Výskyt vzácneho druhu Circinella umbellata (Mucorales) – Czech Mycol. 50: 245–248

Vzácny druh *Circinella umbellata* Tiegh. et Le Monn. bol izolovaný z fluvizeme typickej (Je) pod neobhospodarovaným lúčnym porastom v Gabčíkove. V práci uvádzame popis kultúry a fotodokumentáciu na SEM a vo svetelnom mikroskope.

The genus *Circinella* was erected by Tiegh. et Le Monn. in 1873 (Hesseltine and Fennell 1955). We isolated the species *Circinella umbellata* Tiegh. et Le Monn. from soil samples from the region of Gabčíkovo. The first detailed description of the species *Circinella umbellata* is given by Hesseltine and Fennell (1955). A brief description of the culture without pictures is presented by Milko (1967, 1974). The species is mentioned from soil in Slovakia by Bernát et al. (1984).

Material and Methods

We isolated the species *Circinella umbellata* from Eutric Fluvisol (Je) below an abandoned meadow. The monitoring locality is situated in the region of Bodíky on the right bank of the Danube river system near the water power plant Gabčíkovo. The species *Circinella umbellata* was isolated from a mixed culture on Sabouraud agar under dilution of 1.10–5 CFU from 10 g of fine soil. A pure culture was maintained on Sabouraud and Czapek-Dox agars in the dark at room temperature (Milko 1974).

Samples of the culture were observed and photographs (Figs 1–9) were taken on a Scanning Electron Microscope (SEM) model Tesla BS 301 by Mrs. J. Blahutiaková (Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, Ivanka pri Dunaji). Before that, the samples were dried under room temperature and coated with gold. Observations under the light microscope (Figs 10–12) were made too, in a drop of distilled water with Tween 80.

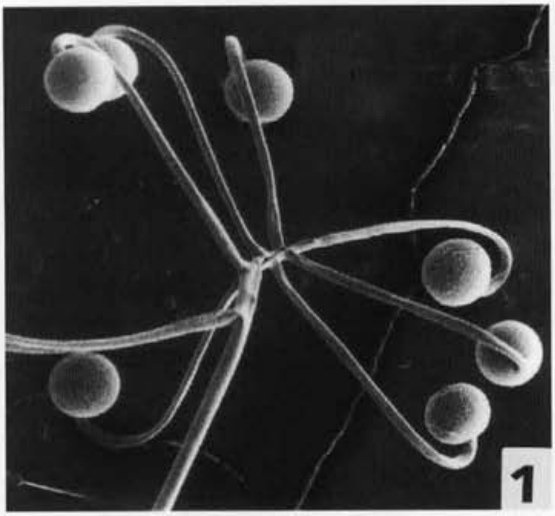
Our strain of *Circinella umbellata* is maintained in the Culture Collection of fungi, Department of Botany, Charles University, Prague (CCF 2971).

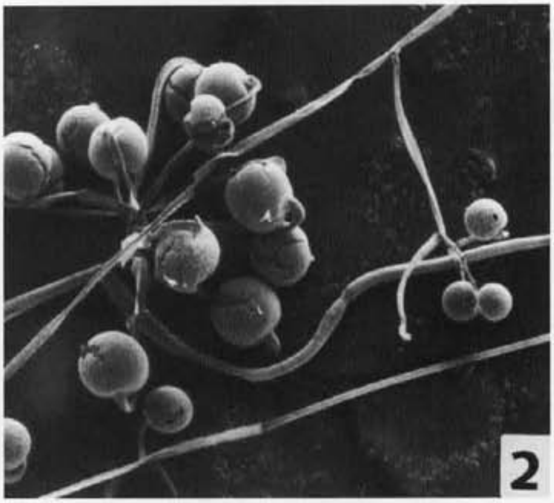
Circinella umbellata Tiegh. et Le Monn., 1873

Syn: *Helicostylum moreliae* Berk. et Broome, 1883
Mucor umbellatus (Tiegh. et Le Monn.) J. Schroet., 1886
Mucor umbellatus (Tiegh. et Le Monn.) J. Schroet. var. *asperior* J. Schroet., 1886
Circinella aspera Lendn., 1908
Circinella conica Moreau, 1913

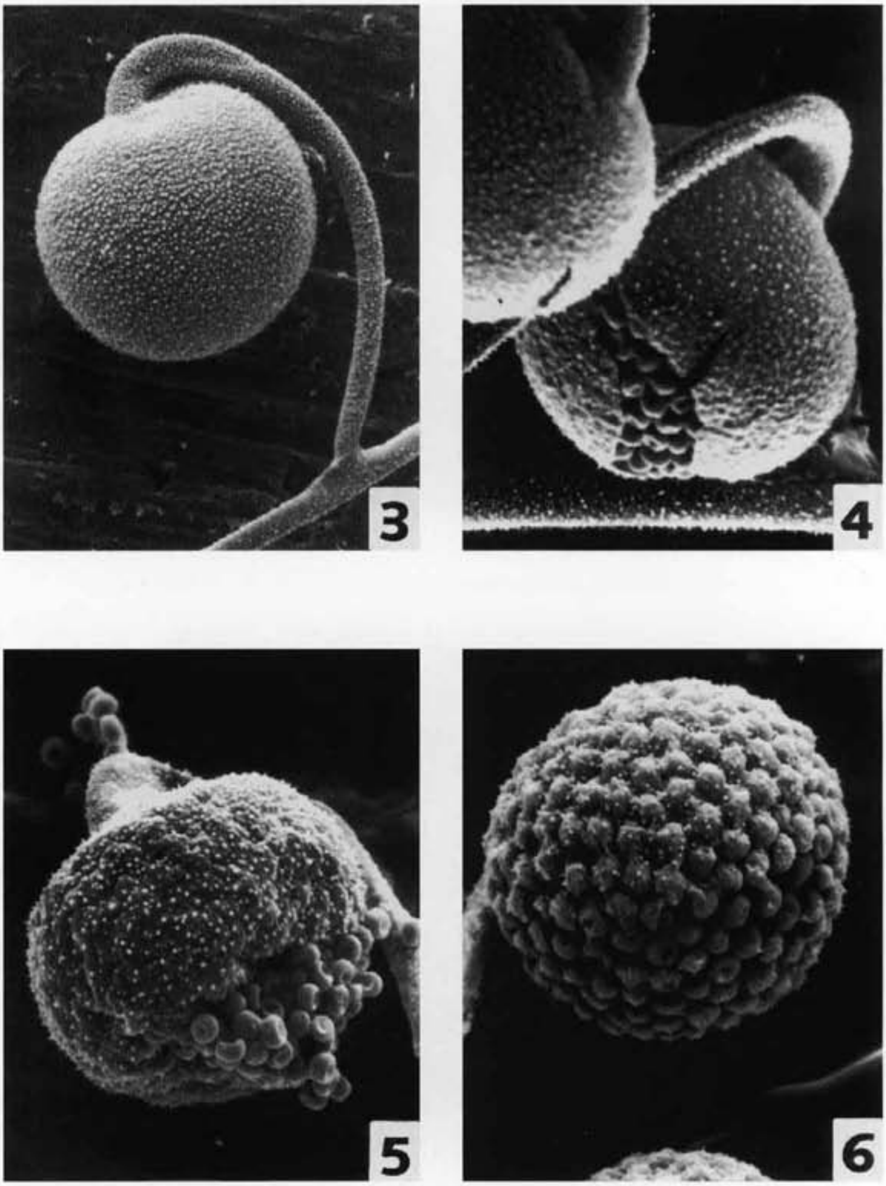
Systematic position: Zygomycota, Zygomycetes, Mucorales

Circinella umbellata forms fast growing colonies with rich air mycelium. At the beginning they are pubescent or slightly pubescent, light brown or ochre coloured, later their hight is 1.5 to 2.0 cm and the colonies are grey-brown or ochre-brown coloured. Sporangiophores grow from the mycelium to 2.0 cm high, and can be simple and straight with one globose circinate sporangium (Fig. 3), then the sporangiophore continues and is terminated by a curved sterile spine, or the sporangiophores are borne terminally on circinate branches. They are joined into umbels, 3 to 7 per umbel (Fig. 1), sometimes 9 or, 2 to 12 per umbel (Fig. 2), or up to 15. Beneath the umbel of sporangiophores a club-shaped sterile spine is formed (Fig. 2 on the right side). The stalks and side branches in young cultures are bound together, however later they are fan-shaped and are then opened. The circinate globose sporangium is located at the stalk terminals. The surface of the sporangium is rugged and wartlike and even the surface of the side branches is slightly rugged, evident below the basis of the sporangium (Figs 3–5). In young cultures the sporangium is light brown. During senescence of culture the sporangium ripens and becomes dark brown, its wall is fissured. The ripe sporangium cracks and opens by a fissure (Figs 4 and 5), which is prolonged and extended along the sporangium periphery to the basis of

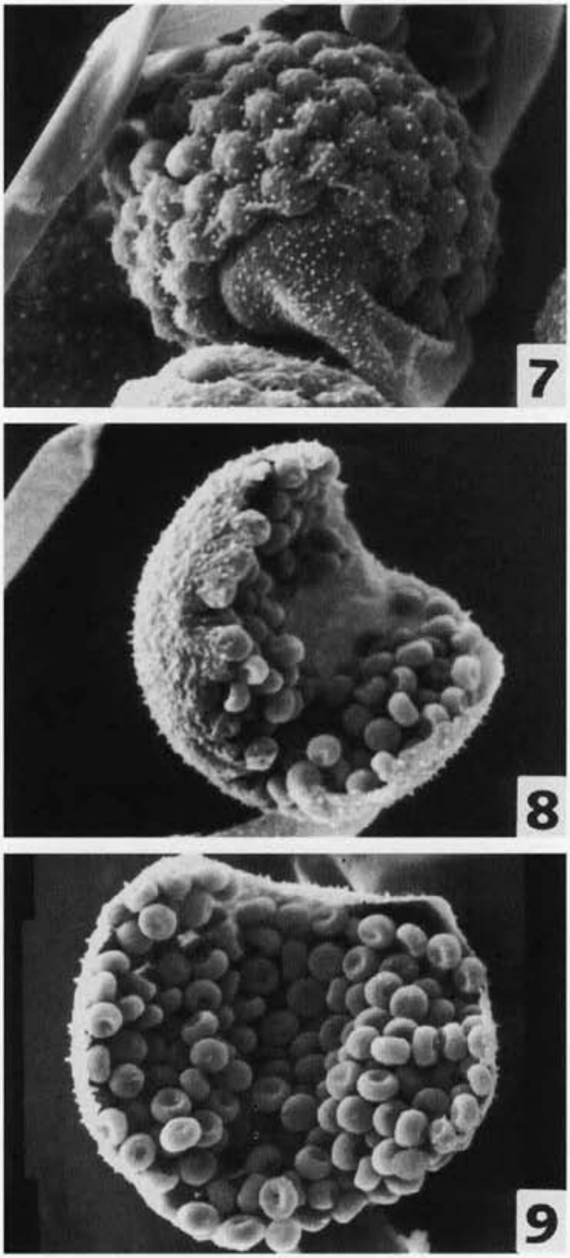




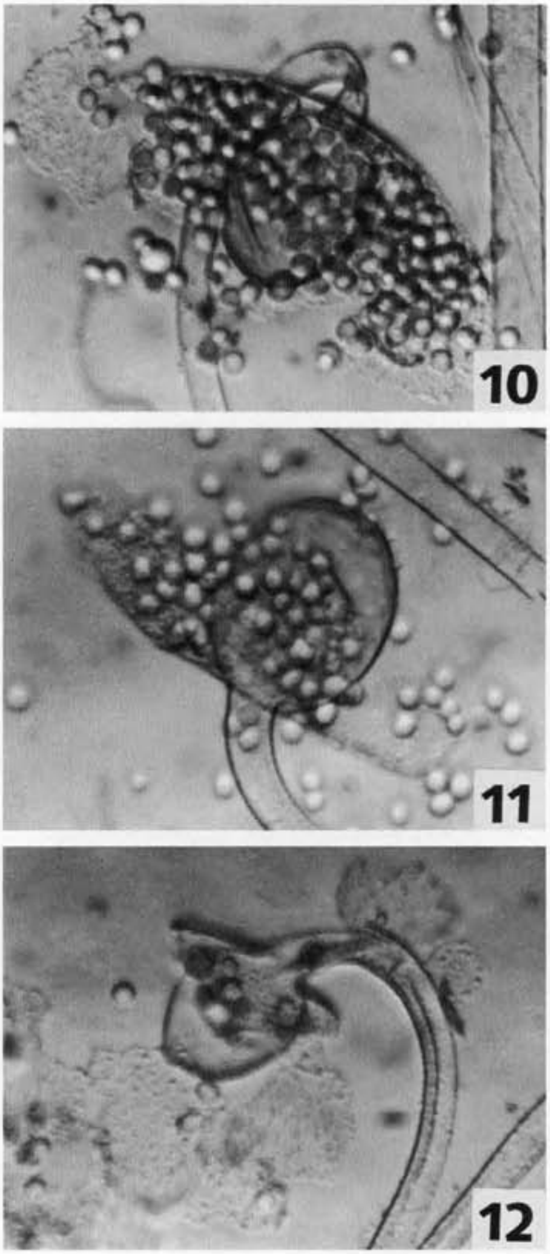
Figs 1–2. *Circinella umbellata* Tiegh. et Le Monn.
1. Sporangiophore with seven branches. Beneath the branching in the left part there is sterile spine. 150 ×.
2. Branched sporangiophore with twelve side stalks that carry ripened and cracked sporangia. At the right side of the picture the sporangiophore has only three side branches, below them is a club-shaped sterile fibre. 150 × [SEM photomicrographs].



Figs 3–6. *Circinella umbellata* Tiegh. et Le Monn.
3. Sporangiophore with one globose circinate sporangium with a rugged and wartlike surface. 750 ×.
4. Ripened sporangium forming a fissure. 800 ×.
5. Sporangiospores are released from the ripe sporangium through the fissure. 750 ×.
6. The surface membrane of the ripe sporangium at the fissure is getting thinner and this thinning leads to the basis of the columella. 850 × [SEM photomicrographs].



Figs 7–9. *Circinella umbellata* Tiegh. et Le Monn.
7. Basal part of the columella of a ripe sporangium. 1300 ×.
8. Halved sporangium with remarkable remain of columella. 1300 ×.
9. Halved sporangium with sporangiospores that are globose. Slightly flattened sporangiospores with depression on the side are caused by rehydratation (artifact on SEM). 1300 × [SEM photomicrographs].



Figs 10–12. *Circinella umbellata* Tiegh. et Le Monn.
10. The membrane of a ripped sporangium cracks and remains as a collar on the basis of columella. 150 ×.
11. Columella of globose shape with remains of the membrane of the ripe sporangium. 150 ×.
12. Conical columella slightly flattened in its apical part. The remains of the membrane from the ripe sporangium are minimal. 150 × [on a light microscope].

the columella. In the other case the surface membrane at the fissure is gradually getting thinner (Fig. 6) and its thinning also leads to the basis of columella (Fig. 7). The sporangiospores of *Circinella umbellata* are light green and globose. In Fig. 8 and 9 the sporangiospores are half-globose and slightly flattened with the whole depression on the side. This alternation (modification, artefact) is caused by scanning of dry (rehydratated) sporangiospores. The columella has a globose or half-globose, conical or cylindrical form, and is sometimes slightly flattened. On its basis part of the surface membrane of the ripped and cracked sporangium remains as a collar of various size (Figs 10–12). Zygospores were not observed. It is a heterothallic species.

Milko (1974) presented 11 species of the genus *Circinella,* which are characterized as physiologically non-specific saprophytes living on animal excrements, on decayed plant remains and even in soil. Milko (1974) made descriptions of the species *Circinella umbellata* based on his own isolate from excrements of a forest mouse.

In Slovakia the species was isolated from agricultural soils for the first time by Bernát et al. (1984). It was found in Haplic Phaeozem (Hh) with a frequency of occurrence of 0.01 and from (Vermi) Haplic Chernozem (Ch) with a frequency of occurrence of 0.05. Both soil types occur in the Danubian lowland (Žitný ostrov) or on alluvial soils along the river Váh. They are found in the warmest and the driest regions of Slovakia. We isolated the species *Circinella umbellata* from Eutric Fluvisol (Je). All soil types where *Circinella umbellata* has been found so far i. e. Haplic Phaeozem, (Vermi) Haplic Chernozem and Eutric Fluvisol, are soils with high contents of high quality humus and with a naturally high fertility (Sotáková 1982).

For Mucorales it is typical that they are found on substrates where a sufficient amount of easily attainable substances. Although the soil types mentioned above are sufficiently rich in organic substances, we consider the occurrence of the species *Circinella umbellata* in them as very seldom. Our identification of this species in Eutric Fluvisol will be a valuable contribution to its ecology and morphology, too.

ACKNOWLEDGEMENT

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Loss of antifungal activity of selected fungicides in treated wood due to natural ageing
Part 1: Activity against moulds

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Reinprecht L. (1998): Loss of antifungal activity of selected fungicides in treated wood due to natural ageing. Part 1: Activity against moulds – Czech Mycol. 50: 249–258

The activity of 2-thiocyanomethylthiobenzothiazole (TCMTB) and selected organotin compounds (OTC = TBTO, TBTS, TBTCA and TBT-DEDTK) against moulds was evaluated by means of mycological tests in which treated and subsequently naturally aged beechwood samples were exposed to the effect of a mixture of six moulds under laboratory conditions. Natural ageing of the treated samples took place under an angle of 45°, and during periods of 0, 2 or 4 months. TCMTB was characterized as the relatively most stable fungicide with antimould activity.

Key words: 2-thiocyanomethylthiobenzothiazole, organotin fungicides, beechwood, natural ageing, moulds.

Reinprecht L. (1998): Strata protihubovej účinnosti vybratých fungicídov v impregnovanom dreve následkom starnutia. 1. časť: Účinnosť proti plesniam. – Czech Mycol. 50: 249–258

Účinnosť 2-thiocyanomethylthiobenzothiazolu (TCMTB) a vybratých organociničitých látok (OTC = TBTO, TBTS, TBTCA, TBT-DEDTK) proti plesniam sa hodnotila prostredníctvom mykologických skúšok, keď sa impregnované a následne prirodzene stárnuté vzorky buka vystavili aktivite zmesi šiestych plesní v laboratórnych podmienkach. Prirodzené stárnutie impregnovaných vzoriek sa vykonalo pod uhlom 45°, v trvaní 0, 2 alebo 4 mesiacov. TCMTB sa prejavil ako pomerne najstabilnejší fungicíd s protiplesňovou účinnosťou.

INTRODUCTION

Wood can be colonized by various microorganisms and fungi, including moulds. Moulds often grow on relatively moist wood surfaces and deteriorate mainly the aesthetical quality of wood due to pigmentation processes. However, the ability of moulds to damage the complicated lignin-saccharidic composition of wood cells and decrease the strength of wood, wood-based materials or wooden structures is obviously very small, in spite of the fact that moulds can also produce enzymes (cellulases, xylanases, lignin peroxidases, and also others) which catalyze depolymerization and decomposition reactions of individual wood components – cellulose, hemicelluloses, lignin, or extracts (Eriksson et al. 1990, Kirk and Cowling 1984).

Protection of wood against moulds and wood-destroying fungi, with the aim of raising its natural durability, is obtained by applying various types of inorganic and organic fungicides. The original antifungal efficacy of fungicides can be decreased due to evaporation and leaching processes, or due to chemical changes in the fungicide molecules.

Inorganic fungicides containing boron (H_3BO_3, $Na_2B_4O_7.10H_2O$), copper ($CuSO_4.5H_2O$, ...), zinc ($ZnCl_2$, ...), chromium ($Na_2Cr_2O_7$, $K_2Cr_2O_7$, ...), fluorine (NaF, $NaHF_2$, ...), or other bioactive atomes are normally water-soluble. Their chemical fixation to the wood substrate or their transformation to insoluble compounds directly in treated wood is important for such products, which must be resistant to leaching. Some of the above mentioned fungicides can be either fixed on the lignin-polysaccharidic complex individually (e.g. Cu^{2+} complexes with lignin and cellulose; ${C_2O_7}^{2+}$ complexes with guaiacyl lignin) and mutually (e.g. $CuCrO_4$-lignin complexes), or – in presence of wood substance – can be gradually transformed into water-insoluble complex compounds (reduction $Cr^{6+} \rightarrow Cr^{3+}$, etc.) (Nicholas and Preston 1984).

Organic compounds can be applied (Reinprecht 1994):

- as liquids (e.g. creosote);
- in organic solutions (e.g. organotin compounds [TBTO, TBTN], 1,2,4-triazole derivatives [Azaconazole, Tebuconazole, Propiconazole], carbamates:– 3-iodo-2-propanylbutyl carbamate [IPBC]);
- in water emulsions (e.g. 2-thiocyanomethyltiobenzothiazole [TCMTB]);
- in water solutions (e.g. alkyl ammonium salts, cyclohexyldiazeniumdioxy-copper [Cu-HDO] in the presence of special additives).

They usually resist better to water and to leaching processes, but some of them are more or less evaporative, or can be chemically changed into less effective compounds due to UV-radiation, oxygen, microorganisms, etc. (Plum 1982).

This work deals with the antimould resistance of wood treated with fungicides and concentrates on the stability of the tested fungicides in wood during its ageing under climatic conditions.

Material and Methods

Wood

Beechwood (*Fagus sylvatica* L.) samples with dimensions of 50 × 10 × 5 mm (longitudinal × radial × tangential), free from tyloses, without false red heart, knots, splits or biodefects, and with sanded surfaces.

Treatment of beechwood samples with fungicides

The beechwood samples were treated with:

a) water emulsions of the 2-thiocyanomethylthiobenzothiazole (TCMTB) fungicide in the following concentrations:

C_{TCMTB} = 0.45 %, 0.9 %, 1.8 %, and 3.6 %

[Note: In the experiments the commercial preservative product Busan 30 L – Buckman Laboratories, which contains 30 % of TCMTB, was used.]

b) ethanole solutions of selected organotin compounds (OTC), that is, with the bis-(tributyltin)oxide (TBTO), tributyltin sulfamate (TBTS), tributyltin chloroacetate (TBTCA) and tributyltin-N,N-diethyldithiocarbamate (TBT-DEDTK) fungicides, in the following concentrations:

C_{OTC} = 0.1 %, 0.33%, and 1 %

[Note: OTC were synthesized and submitted by Mr. Doc. Ing. Juraj Kizlink, CSc. – STU CHTF Bratislava. In this test only those OTC were tested, which in previous tests using the poisoned soil method (Kizlink, Fargašová and Reinprecht 1996, Reinprecht and Kizlink 1996) showed the relatively highest activity.]

Treatment of the beechwood specimens was carried out with the following two impregnation techniques:

dipping (time = 45 minutes; temperature = 20 °C; Note: only with TCMTB);

pressure impregnation (pressure = 0.6 MPa; time = 15 minutes; temperature = 20 °C)

Natural ageing of treated samples

The natural or accelerated assessment of the stability of woods, coatings and preservatives against external factors can be carried out by various methods (e.g.: Feist and Williams 1991, Hoey and Hipwood 1974, Palashev and Abrashev 1993, Podgorski et al. 1994, Reinprecht et al. 1989).

In this test the following natural ageing method of treated wood prior to mycological testing was applied:

Before natural ageing the treated beechwood samples were conditioned (4 weeks) on a moisture level of about 12 %.

Natural ageing of the samples was carried out without contact with the ground, in the industrial zone of the town of Zvolen, at an altitude of 320 m, the southern exposure, under an angle of 45 °, during periods of 0, 2 or 4 months, from 15th April to 15th August.

(Procedure: The wood samples treated with fungicides were attached onto supporting boards 300 × 100 × 20 mm large, and subsequently placed into special frames to undergo natural ageing.)

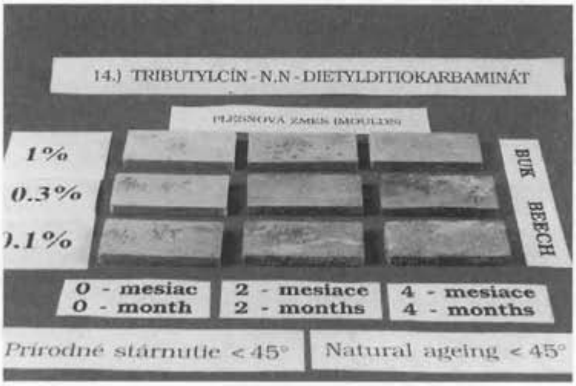

Fig. 1. The antimould resistance of treated beechwood against the tested mixture of moulds have been decreased due to ageing processes: growth of moulds on samples

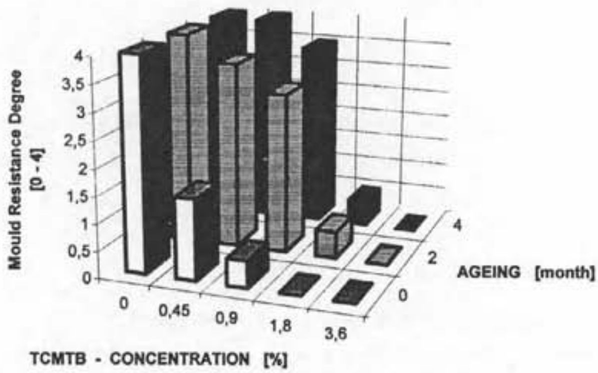

Fig. 2. Mould resistance degrees (MRD) of beechwood which was primarily treated with TCMTB using the dipping technique, and naturally aged 0, 2, or 4 months

Resistance of treated and (un)aged samples against moulds

Naturally aged surfaces 50 mm × 10 mm of the treated wood samples (50 × 10 × 5 mm), which had been aged under the influence of climatic and other open-air factors (rain, UV-radiation, emissions, etc.) during 0, 2, or 4 months, were vaccinated with a spore mixture of the following moulds:

Aspergillus amstelodami (Mangin) Thom et Church	No. 2437
Aspergillus niger van Tieghem	No. 1877

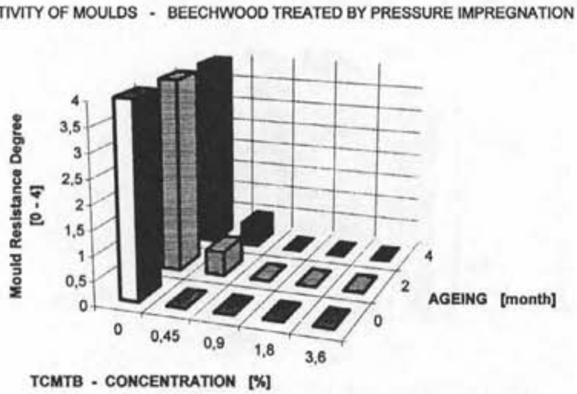

Fig. 3. Mould resistance degrees (MRD) of beechwood which was primarily treated with TCMTB by pressure impregnation, and naturally aged 0, 2, or 4 months

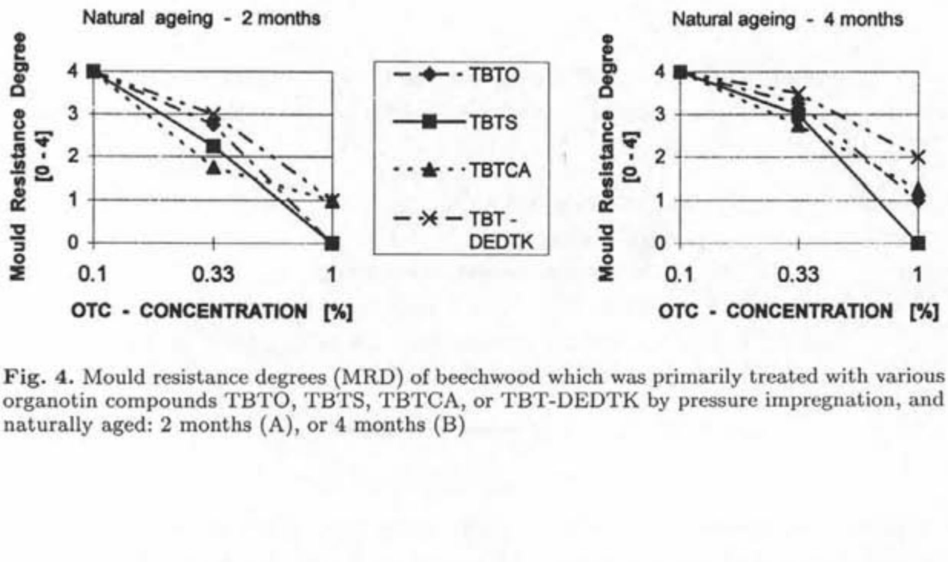

Fig. 4. Mould resistance degrees (MRD) of beechwood which was primarily treated with various organotin compounds TBTO, TBTS, TBTCA, or TBT-DEDTK by pressure impregnation, and naturally aged: 2 months (A), or 4 months (B)

Paecilomyces varioti Bainier	No. 2693
Penicillium cyclopium Westling	No. 2332
Chaetomium globosum Kunze	No. 358
Trichoderma viride Pers.: Fr.	No. 1403

The treated samples with aged and vaccinated surfaces were placed into Petri dishes on stiffened plaster and conditioned during 28 days at a temperature of $T = 29 \pm 1\,^{\circ}C$, and at a relative air humidity $RH = 95 \pm 3$ %.

ACTIVITY OF MOULDS - BEECHWOOD TREATED BY PRESSURE IMPREGNATION

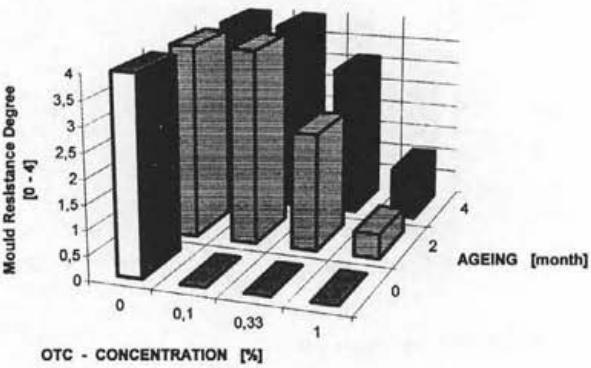

Fig. 5. Mould resistance degrees (MRD) of beechwood which was primarily treated with organotin compounds (OTC) by pressure impregnation, and naturally aged 0, 2, or 4 months (Note: MRD = mean value obtained from all tested OTC: TBTO, TBTS, TBTCA, and TBT-DEDTK)

The resistance of the treated and aged surfaces against moulds (MRD – mould resistance degree) was established after 28 days according to the following criteria (STN 49 0604 standard):

MRD	*MOULDS ON TESTED SURFACES*
0	No moulds (enlargement 50 ×)
1	< 10 % of surface covered with moulds
2	< 25 % of surface covered with moulds
3	< 50 % of surface covered with moulds
4	> 50 % of surface covered with moulds

RESULTS AND DISCUSSION

Results concerning losses of the antimould activity of the tested fungicides in treated beechwood due to its natural ageing are presented in Tables 1 and 2, Figures 1, 2, 3, 4 and 5.

For the TCMTB and organotin (OTC = TBTO, TBTS, TBTCA, TBT-DEDTK) fungicides the following critical toxic values, i.e. critical obligatory retentions of fungicide in kilograms per cubic meter of treated wood to guarantee MRD = 0, were determined:

a) apparently lower toxic values testing the antimould resistance of the treated samples which were not naturally aged:

TOXIC VALUES OF FUNGICIDES AGAINST MOULDS – WITHOUT AGEING

Fungicide	Toxic value [kg.m^{-3}]	Treatment
TCMTB	1.22–2.55	dipping
	< 2.05	pressure impregnation (p.i.)
TBTO	< 0.42	p.i.
TBTS	< 0.40	p.i.
TBTCA	< 0.40	p.i.
TBT-DEDTK	< 0.41	p.i.

b) apparently higher toxic values testing the antimould resistance of the treated samples which were naturally aged (2 or 4 months of ageing):

TOXIC VALUES OF FUNGICIDES AGAINST MOULDS – WITH AGEING

Ageing	Fungicide	Toxic value [kg.m^{-3}]	Treatment
2 months	TCMTB	2.55–4.60	dipping
		2.05–3.81	pressure impregnation (p.i)
	TBTO	1.38–4.15	p.i.
	TBTS	1.38–4.06	p.i.
	TBTCA	> 4.20	p.i.
	TBT-DEDTK	> 4.08	p.i.
4 months	TCMTB	2.55–4.60	dipping
		2.05–3.81	p.i.
	TBTO	> 4.15	p.i.
	TBTS	1.38–4.06	p.i.
	TBTCA	> 4.20	p.i.
	TBT-DEDTK	> 4.08	p.i.

In unaged samples the TCMTB fungicide had a lower antimould activity (higher toxic values) in comparison with the organotin fungicides TBTO, TBTS, TBTCA or TBT-DEDTK (Table 1 and 2).

On the other hand, the achieved results of the mycological tests with aged beechwood samples indicate indirectly, that the TCMTB fungicide (or wood treated with TCMTB) is relatively better resistant to external factors acting during natural ageing than organotin compounds – OTC (Table 1 and 2, Fig. 2, 3 and 5).

Table 1
Mould resistance degrees (MRD) of the aged surfaces of beech samples primarily treated with the 2-thiocyanomethylthiobenzothiazole (TCMTB) fungicide present in the commercial product Busan 30 L (Busan 30 L = 30 % TCMTB)
(C_{TCMTB} – concentration of TCMTB; n – number of samples; R_f – retention of TCMTB; MRD – mould resistance degree)

Moulds: *Mixture of microscopic fungi*							
C_{TCMTB}	Ageing	TCMTB application technique					
		DIPPING			PRESSURE IMPREGNATION		
[%]	[month]	n	R_f [kg.m^{-3}]	MRD [0–4]	n	R_f [kg.m^{-3}]	MRD [0–4]
0	0	6	—	4	6	—	4
	2	6	—	4	6	—	4
	4	6	—	4	6	—	4
0.45	0	6		1.5	6		0
	2	6	0.65	3.5	6	2.05	0.5
	4	6		4	6		0.5
0.9	0	6		0.5	6		0
	2	6	1.22	3	6	3.81	0
	4	6		3.5	6		0
1.8	0	6		0	6		0
	2	6	2.55	0.5	6	7.75	0
	4	6		0.5	6		0
3.6	0	6		0	6		0
	2	6	4.60	0	6	14.80	0
	4	6		0	6		0

Comparing antimould activity of the individual organotin compounds based on absolute MRD values (Table 2, Fig. 4), it is evident that, due to climatic factors, the tributyltin-N,N-diethyldithiocarbamate (TBT-DEDTK) lost antimould activity earlier in comparison with other organotin compounds (TBTO, TBTS, TBTCA).

Conclusions

Assessing the mycological tests carried out with treated and (un)aged beechwood samples, the following conclusions can be drawn:

Toxic values of both the 2-thiocyanomethylthiobenzothiazole (TCMTB) fungicide present in the commercial product Busan 30 L, and selected organotin compounds (OTCs = TBTO, TBTS, TBTCA, TBT-DEDTK) were significantly increased due to natural ageing processes in the intervals of 0 to 4 months.

The stability of TCMTB against natural ageing factors (rain, UV radiation, etc.) was slightly higher in comparison with the tributyltin compounds, in spite of the fact that TCMTB is less effective – having higher toxic values in unaged wood.

Tributyltin-N,N-diethyldithiocarbamate (TBT-DEDTK), which was considered as a prospective organotin compound from efficacy tests on poisoned soils (Kizlink et al. 1996), had a lower weather stability compared to other tributyltin compounds (TBTO, TBTS or TBTCA).

Table 2
Mould resistance degrees (MRD) of the aged surfaces of beech samples primarily treated with organotin (OTC = TBTO, TBTS, TBTCA, TBT-DEDTK) fungicides applying the pressure impregnation technique
(C_{OTC} – concentration of the used OTC-fungicide; n – number of samples in each series [n = 4]; R_f – retention of the fungicide; MRD – mould resistance degree)

Moulds: *Mixture of microscopic fungi*									
C_{OTC}	Ageing	OTC-fungicide application technique PRESSURE IMPREGNATION							
		TBTO		TBTS		TBTCA		TBT-DEDTK	
[%]	[month]	R_f [kg.m^{-3}]	MRD [0–4]	R_f [kg.m^{-3}]	MRD [0–4]	R_f [kg.m^{-3}]	MRD [0–4]	R_f [kg.m^{-3}]	MRD [0–4]
0.1	0		0		0		0		0
	2	0.42	4	0.40	4	0.40	4	0.41	4
	4		4		4		4		4
0.33	0		0		0		0		0
	2	1.38	2.75	1.38	2.25	1.32	1.75	1.35	3
	4		3.25		3		2.75		3.5
1.0	0		0		0		0		0
	2	4.15	0	4.06	0	4.20	1	4.08	1
	4		1		0		1.25		2

TBTO = tributyltin oxide;
TBTS = tributyltin sulfamate;
TBTCA = tributyltin chloroacetate;
TBT-DEDTK = tributyltin-N,N-diethyldithiocarbamate

Mean toxic values of fungicides against moulds [kg.m^{-3}]

"Pressure impregnation"	TCMTB	OTCs
unaged – 0 month	< 2.05	< 0.42
aged – 2 months	2.05–3.81	$1.38 \geq 4.20$
aged – 4 months	2.05–3.81	$1.38 \geq 4.20$

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Loss of antifungal activity of selected fungicides in treated wood due to natural ageing
Part 2: Activity against wood-destroying fungi

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Reinprecht L. (1998): Loss of antifungal activity of selected fungicides in treated wood due to natural ageing. Part 2: Activity against wood-destroying fungi – Czech Mycol. 50: 259–269

The activity of TCMTB and selected organotin TBTO, TBTS, TBTCA and TBT-DEDTK fungicides against the brown-rot fungus *Serpula lacrymans* and the white-rot fungus *Trametes versicolor* was evaluated by means of mycological tests in which treated and subsequently naturally aged beechwood samples were exposed to the effect of fungi in Kolle's flasks. In accordance with Part 1 of this work (activity against moulds), the TCMTB fungicide could again be characterized as more weather stable than organotin fungicides.

Key words: fungicides, beechwood, natural ageing, *Serpula lacrymans*, *Trametes versicolor*.

Reinprecht L. (1998): Strata protihubovej účinnosti vybratých fungicídov v impregnovanom dreve následkom starnutia. 2. časť: Účinnosť proti drevokazným hubám. – Czech Mycol. 50: 259–269

Účinnosť TCMTB a vybratých organociničitých TBTO, TBTS, TBTCA, TBT-DEDTK fungicídov proti celulózovornej hube *Serpula lacrymans* a ligninovornej hube *Trametes versicolor* sa hodnotila prostredníctvom mykologických skúšok, pri ktorých sa impregnované a následne prirodzene starnuté vzorky buka vystavili aktivite húb v Kolleho bankách. TCMTB fungicíd sa v zhode s 1. časťou tejto práce (účinnosť proti plesniam) prejavil poveternostne stabilnejším v porovnaní s organociničitými fungicídmi.

INTRODUCTION

Wood-destroying fungi are divided into three classes based on the type of their enzymatic system, i.e. white-rot, brown-rot and soft-rot fungi (Eriksson et al. 1990). Rot in wood can be started both under outdoor and indoor exposures, if its moisture is above 20 %. Wood adequately treated with suitable fungicide(s) is resistant to fungi. However, in situations when treated wood is exposed to aggressive weather conditions, with or without contact with the ground, the original activity of the fungicides is often decreased and can start up a rotting process in the previously chemically protected wooden products or building structures.

The present work deals with the reduced resistance against rot of wood treated with fungicides after its exposure to model natural ageing processes.

MATERIAL AND METHODS

Wood

Sound and quality beechwood (*Fagus sylvatica* L.) samples with dimensions of 120 × 8.5 × 8.5 mm (120 mm in the longitudinal direction). All tested samples were weighed in oven dry state, next treated with fungicides, subsequently naturally aged, and finally exposed to the brown-rot fungus *Serpula lacrymans* or the white-rot fungus *Trametes versicolor*.

Treatment of beechwood samples with fungicides and natural ageing of treated samples

Treatment and natural ageing of the beechwood samples was carried out in accordance with Part 1 of this work (Reinprecht 1998).[1)]

Resistance of treated and (un)aged samples against wood-destroying fungi

Treated and aged wood samples underwent to laboratory mycological tests with the following wood-destroying fungi:

Serpula lacrymans (Wulf.: Fr.) Schroet. – No. 6, brown-rot fungus;

Trametes versicolor (Fr.) Pil. [Stamm CTB 863 A – *Coriolus versicolor* (L.) Quél.], white-rot fungus.

Samples from the tested series[2)] were primarily conditioned to a moisture level of ≈ 18 % and then placed into 1/2 litre Kolle's flasks on a fungal mycelium grown

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[1)] Treatment of samples with two techniques:
dipping (45 minutes at 20 °C);
pressure impregnation (15 minutes at 20 °C and 0.6 MPa); using the following fungicides:
2-thiocyanomethylthiobenzothiazole (TCMTB) in water emulsions in the following concentrations:

C_{TCMTB} = 0.45 %, 0.9 %, 1.8 %, and 3.6 %.

Organotin compounds (OTC): –bis-(tributyltin)oxide (TBTO), tributyltin sulfamate (TBTS), tributyltin chloroacetate (TBTCA) and tributyltin-N,N-dietyldithiocarbamate (TBT-DEDTK), in ethanole solutions in the following concentrations:

C_{OTC} = 0.1 %, 0.33 %, and 1 %.

Natural ageing of treated and conditioned samples was carried out without having contact with the ground, in the industrial zone of the town of Zvolen, at an altitude of 320 m, the southern exposure, under an angle of 45 °, during periods of 0, 2 or 4 months.

[2)] Each series (totally 96 series → type of fungicide × concentration × treatment × ageing × fungus: see Tables 1, 2 and 3) contained 12 samples (8 tested samples = treated, aged and exposed to fungal activity; 4 comparative samples = treated and aged, but not exposed to fungal activity, i.e. used for the determination of the corrective coefficient k_f — relation 2).

grown on stiffened agar malt cultivating medium. Mycological tests lasted 16 weeks in a dark sterile room at a temperature of $T = 20 \pm 1\ ^{\circ}C$, and a relative air humidity $RH = 70 \pm 5\ \%$.

The resistance of treated and aged samples against fungi was assessed visually (e.g. Fig. 1) and also according to the quantitative criterion "loss of wood mass – δm_F" (relation 1).

$$\delta m_F = \frac{(m_o + m_{fungicide} \cdot k'_f) - m_F}{m_o} \cdot 100\ [\%] \quad (1)$$

where: δm_F – loss of wood mass in the tested sample due to a rot caused by the applied fungus

m_o – mass of the original tested sample in oven dry state

m_F – mass of the tested sample after treatment, ageing and fungus activity in oven dry state

$m_{fungicide}$ – mass retention of the applied fungicide in the tested sample

k'_f – mean corrective coefficient (arithmetic mean of 4 comparative samples in each series)

$$k_f = (m_{Ak} - m_{ok}) : m_{fungicide-k} \quad (2)$$

where: m_{ok} – mass of the original comparative sample in oven dry state

m_{Ak} – mass of the comparative sample after treatment and ageing in oven dry state

$m_{fungicide-k}$ – mass retention of the applied fungicide in the comparative sample

RESULTS AND DISCUSSION

The achieved results[3)] concerning the resistance of treated and aged beechwood samples against the wood-destroying fungi are presented in Tables 1, 2 and 3, Figures 1–5.

For the TCMTB and organotin (OTC = TBTO, TBTS, TBTCA, TBT-DEDTK) fungicides the following critical toxic values, i.e. critical obligatory retentions of fungicide in kilograms per cubic meter of treated wood to guarantee $\delta m_F < 3\ \%$ (by the EN 113 standard) have been determined:

[3)] Evaluating the mycological tests (relation 1), negative values of δm_F (from 0 % to -3.1 %) were rarely determined, probably as a consequence of particular methodical or experimental errors, e.g.: unequal absorption of emissions during ageing processes, possible malt diffusion in wood samples, etc. In the tables an figures these uniformly are marked as zero $\rightarrow \delta m_F = 0$.

Table 1
Rot characteristic (δm_F) determined for beechwood samples primarily treated with the TCMTB fungicide (present in Busan 30 L) and exposed to natural ageing, and subsequently tested against the brown-rot fungus *Serpula lacrymans*
(C_{TCMTB} — concentration of TCMTB fungicide; n — number of samples; R_f — retention of TCMTB; δm_F — loss of wood mass caused by fungus activity)

Fungus: *Serpula lacrymans*							
C_{TCMTB}	Ageing	Technique of the TCMTB fungicide application					
		DIPPING			PRESSURE IMPREGNATION		
[%]	[month]	n	R_f [kg.m^{-3}]	δm_F [%]	n	R_f [kg.m^{-3}]	δm_F [%]
0	0	16	—	22.8	16	—	22.8
	2	16	—	21.4	16	—	21.4
	4	16	—	24.5	16	—	24.5
0.45	0	8	0.57	6.3	8	2.13	1.8
	2	8	0.69	12.2	8	2.04	8.0
	4	8	0.63	16.1	8	1.95	11.0
0.9	0	8	1.21	3.7	8	3.87	0.2
	2	8	1.19	10.8	8	3.87	3.7
	4	8	1.16	15.2	8	3.84	6.7
1.8	0	8	2.39	0	8	7.17	0
	2	8	2.75	8.9	8	7.71	0
	4	8	2.81	14.9	8	7.53	0.7
3.6	0	8	4.67	0	8	14.31	0
	2	8	4.45	4.2	8	13.98	0
	4	8	4.72	13.3	8	14.61	0

Table 2
Rot characteristic (δm_F) determined for beechwood samples primarily treated with organotin (OTC = TBTO, TBTS, TBTCA, TBT-DEDTK) fungicides applying the pressure impregnation technique and exposed to natural ageing, subsequently tested against the brown-rot fungus *Serpula lacrymans*
(C_{OTC} — concentration of the used OTC-fungicide; n — number of samples; R_f — retention of the fungicide; δm_F — loss of wood mass caused by fungus activity)

Fungus: *Serpula lacrymans*							
C_{OTC}	Ageing	Fungicide application technique					
		PRESSURE IMPREGNATION					
	δm_F	n	R_f	$_F$	n	R_f	δm_F
[%]	[month]		[kg.m^{-3}]	[%]		[kg.m^{-3}]	[%]
		TBTO			**TBTCA**		
0.1	0	8	0.41	0	8	0.38	0
	2	8	0.41	7.2	8	0.39	9.7
	4	8	0.39	9.8	8	0.40	12.7
0.33	0	8	1.21	0	8	1.34	0
	2	8	1.35	0	8	1.34	0
	4	8	1.35	2.5	8	1.31	1.9
1.0	0	8	3.97	0	8	4.07	0
	2	8	3.90	0	8	4.00	0
	4	8	3.94	0	8	3.96	0
		TBTS			**TBT-DEDTK**		
0.1	0	8	0.40	0	8	0.40	0
	2	8	0.39	7.9	8	0.39	15.7
	4	8	0.39	14.4	8	0.39	20.2
0.33	0	8	1.35	0	8	1.32	0
	2	8	1.34	0	8	1.34	0
	4	8	1.34	0.2	8	1.34	3.6
1.0	0	8	3.76	0	8	4.05	0
	2	8	4.06	0	8	3.86	0
	4	8	4.08	0	8	3.91	0

TBTS = tributyltin sulfamate; TBTCA = tributyltin chloroacetate;
TBT-DEDTK = tributyltin-N,N-diethyldithiocarbamate;
TBTO = tributyltin oxide

Table 3
Rot characteristic (δm_F) determined for beechwood samples primarily treated with organotin (OTC = TBTO, TBTS, TBTCA, TBT-DEDTK) fungicides applying the pressure impregnation technique and exposed to natural ageing, subsequently tested against the white-rot fungus *Trametes versicolor*
(C_{OTC} — concentration of the used OTC-fungicide; n — number of samples; R_f — retention of the fungicide; δm_F — loss of wood mass caused by fungus activity)

Fungus: *Trametes versicolor*							
C_{OTC}	Ageing	Technique of fungicide application					
		PRESSURE IMPREGNATION					
[%]	[month]	n	R_f [kg.m^{-3}]	δm_F [%]	n	R_f [kg.m^{-3}]	δm_F [%]
		TBTO			**TBTCA**		
0.1	0	8	0.40	10.2	8	0.40	10.1
	2	8	0.40	31.6	8	0.40	26.0
	4	8	0.40	29.1	8	0.39	34.0
0.33	0	8	1.32	0	8	1.34	0
	2	8	1.32	5.9	8	1.38	4.0
	4	8	1.31	3.9	8	1.36	14.2
1.0	0	8	4.11	0	8	3.99	0
	2	8	3.60	2.1	8	3.98	2.3
	4	8	4.00	2.6	8	4.14	4.0
		TBTS			**TBT-DEDTK**		
0.1	0	8	0.37	10.7	8	0.40	12.6
	2	8	0.39	19.9	8	0.39	36.3
	4	8	0.41	24.7	8	0.38	39.0
0.33	0	8	1.31	0	8	1.30	0.1
	2	8	1.31	8.0	8	1.35	5.4
	4	8	1.33	13.3	8	1.31	13.5
1.0	0	8	3.98	0	8	3.94	0
	2	8	4.04	3.4	8	4.01	4.4
	4	8	3.96	3.2	8	4.06	13.2

TBTO = tributyltin oxide
TBTS = tributyltin sulfamate TBTCA = tributyltin chloroacetate
TBT-DEDTK = tributyltin-N,N-diethyldithiocarbamate

a) lower toxic values for unaged samples:

TOXIC VALUES OF FUNGICIDES AGAINST WOOD-DESTROYING FUNGI – WITHOUT AGEING

Fungicide	Toxic value [kg.m^{-3}]		Treatment
	Serpula lacrymans	*Trametes versicolor*	
TCMTB	1.21–2.39		dipping
	< 2.13		pressure impregnation (p.i.)
TBTO	< 0.41	0.40–1.32	p.i.
TBTS	< 0.40	0.37–1.31	p.i.
TBTCA	< 0.38	0.40–1.34	p.i.
TBT-DEDTK	< 0.40	0.40–1.30	p.i.

b) higher toxic values for aged samples (2 or 4 months of ageing):

TOXIC VALUES OF FUNGICIDES AGAINST WOOD DESTROYING FUNGI – WITH AGEING

Ageing	Fungicide	Toxic value [kg.m^{-3}]		Treatment
		Serpula lacrymans	*Trametes versicolor*	
2 months	TCMTB	> 4.45		dipping
		3.87–7.71		pressure impregnation (p.i.)
	TBTO	0.41–1.35	1.32–3.60	p.i.
	TBTS	0.39–1.34	> 4.04	p.i.
	TBTCA	0.39–1.34	1.38–3.98	p.i.
	TBT-DEDTK	0.39–1.34	> 4.01	p.i.
4 months	TCMTB	> 4.72		dipping
		3.84–7.53		p.i.
	TBTO	0.39–1.35	1.31–4.00	p.i.
	TBTS	0.39–1.34	> 3.96	p.i.
	TBTCA	0.40–1.30	> 4.14	p.i.
	TBT-DEDTK	1.34–3.91	> 4.06	p.i.

The TCMTB fungicide, compared to organotin (OTC = TBTO, TBTS, TBTCA or TBT-DEDTK) fungicides, was characterized with a several times lower efficacy, or with several times higher toxic values against the brown-rot fungus *Serpula lacrymans*, both for unaged and aged samples.

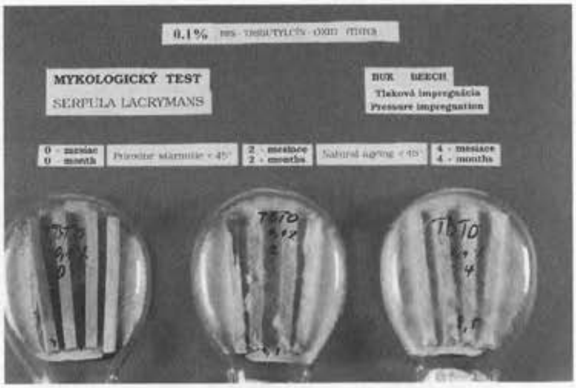

Fig. 1. The resistance of treated beechwood against the brown-rot fungus *Serpula lacrymans* decreased due to ageing processes: growth of its mycelia on samples

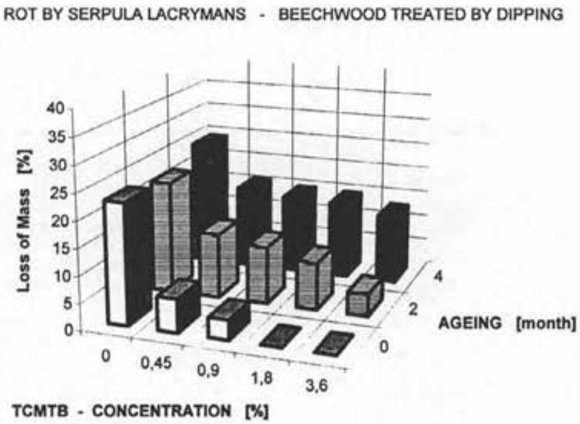

Fig. 2. Losses of mass (δm_F) of beechwood due to rot caused by the brown-rot fungus *Serpula lacrymans*. The wood was primarily treated with TCMTB by dipping, and naturally aged 0, 2, or 4 months.

The original resistance against rot of beechwood treated with TCMTB or OTCs decreased apparently due to natural ageing processes. This result is evident from the higher toxic values of those samples which were primarily aged (Table 1, 2 and 3, Fig. 2, 3, 4 and 5). The external factors had on the whole a comparable negative effect on both groups of tested fungicides (TCMTB and OTCs), assessing their antifungal efficacy against the brown-rot fungus *Serpula lacrymans*.

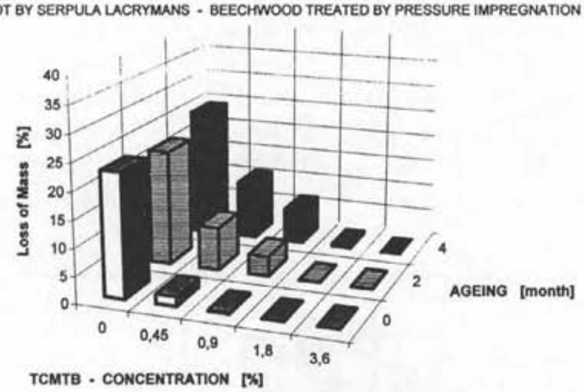

Fig. 3. Losses of mass (δm_F) of beechwood due to rot caused by the brown-rot fungus *Serpula lacrymans*. The wood was primarily treated with TCMTB by pressure impregnation, and naturally aged 0, 2, or 4 months.

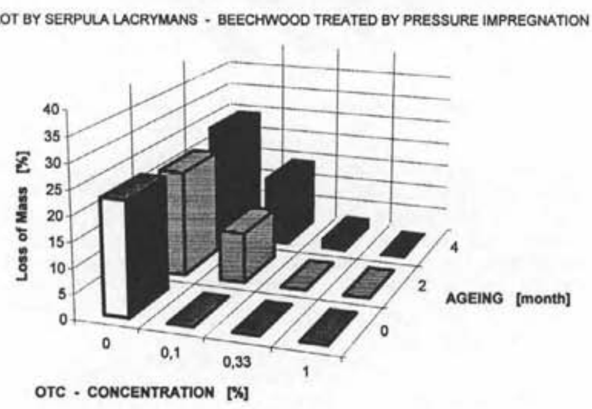

Fig. 4. Losses of mass (δm_F) of beechwood due to rot caused by the brown-rot fungus *Serpula lacrymans*. The wood was primarily treated with organotin compounds (OTC) by pressure impregnation, and naturally aged 0, 2, or 4 months. (Note: δm_F = mean value determined from all tested OTC: TBTO, TBTS, TBTCA, and TBT-DEDTK)

The toxic values of OTCs were apparently higher against the white-rot fungus *Trametes versicolor* compared to those determined against the brown-rot fungus *Serpula lacrymans*. This result corresponds with other authors referred to by Schwienfurth et al. (1991), who found that organotin compounds are more active against brown-rot and less active against white-rot and soft-rot fungi.

Comparing the absolute losses of wood mass (δm_F) as well as the toxic values (i.e. critical fungicide retentions to guarantee $\delta m_F < 3$ %), it is evident that tributyltin-N,N-diethyldithiocarbamate (TBT-DEDTK) lost antifungal activity earlier due to ageing processes in comparison with other organotin compounds (TBTO, TBTS, TBTCA). This result corresponds with Part 1 in which TBT-DEDTK demonstrated the lowest antimould activity after ageing processes.

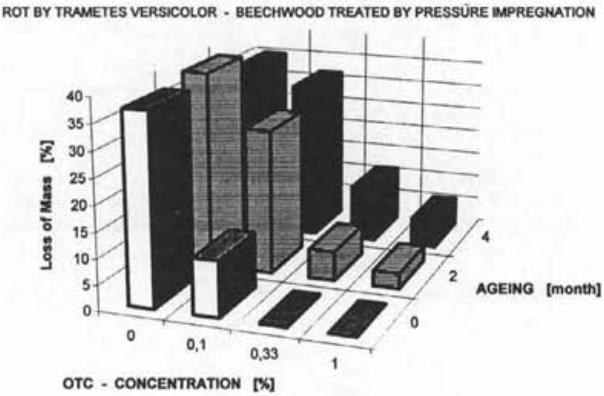

Fig. 5. Losses of mass (δm_F) of beechwood due to rot caused by the white-rot fungus *Trametes versicolor*. The wood was primarily treated with organotin compounds (OTC) by pressure impregnation, and naturally aged 0,2, or 4 months. (Note: δm_F = mean value determined from all tested OTC: TBTO, TBTS, TBTCA, and TBT-DEDTK)

CONCLUSIONS

Based on mycological tests with wood-destroying fungi carried out with treated and (un)aged beechwood samples, the following conclusions can be drawn:

The toxic values of all tested fungicides (TCMTB and OTCs = TBTO, TBTS, TBTCA, TBT-DEDTK) significantly increased due to natural ageing processes in intervals of 0 to 4 months.

Mean toxic values of fungicides against wood-destroying fungi [$kg.m^{-3}$]

"Pressure impregnation"	TCMTB	OTCs
Serpula lacrymans		
unaged – 0 month	< 2.13	< 0.41
aged – 2 months	3.87–7.71	0.41–1.35
aged – 4 months	3.84–7.53	0.40–3.91
Trametes versicolor		
unaged – 0 month	—	0.40–1.34
aged – 2 months	—	$1.32 \geq 4.04$
aged – 4 months	—	$1.31 \geq 4.14$

The TCMTB fungicide was characterized as slightly more stable under natural exposures in comparison with the organotin compounds (OTCs) TBTO, TBTS and TBTCA.

On the other hand, the tributyltin-N,N-diethyldithiocarbamate (TBT-DEDTK) was the most weather unstable fungicide.

The OTCs were less effective against the white-rot fungus *Trametes versicolor*, and also against moulds (Part 1), comparing their efficacy against the brown-rot fungus *Serpula lacrymans*.

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Distribution and ecology of the genus Thelocarpon (Lecanorales, Thelocarpaceae) in the Czech Republic

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Kocourková-Horáková J. (1998): Distribution and ecology of the genus Thelocarpon Nyl. (Lecanorales, Thelocarpaceae) in the Czech Republic. – Czech Mycol. 50: 271–302

Of the 20 currently accepted species of the genus *Thelocarpon* Nyl. 8 species are reported from the Czech Republic: *T. epibolum, T. impressellum, T. intermediellum, T. laureri, T. lichenicola, T. olivaceum, T. pallidum* and *T. superellum*. 5 species, *T. epibolum, T. intermediellum, T. olivaceum, T. pallidum* and *T. superellum*, are new for the Czech Republic. *T. laureri* is found to be a common species. *T. epibolum* and *T. lichenicola* are here reported from a number of localities, *T. superellum* is reported from 3 localities, *T. olivaceum* from 2 localities and both *T. impressellum* and *T. pallidum* only from 1 locality. A key to these species, distribution maps, localities as well as data on the ecology of all included species are provided. *T. impressellum* and *T. lichenicola* are reported for the first time from the Slovak Republic. Also, several additional records of *T. epibolum* and *T. laureri* are given from this country. Pycnidia of *T. epibolum, T. intermediellum* and *T. lichenicola* have been discovered and are described for the first time. Drawings of all these are added.

Key words: *Thelocarpon*, pycnidia, Czech Republic, Slovak Republic, distribution maps, ecology.

Kocourková-Horáková J. (1998): Rozšíření a ekologie rodu Thelocarpon Nyl. (Lecanorales, Thelocarpaceae) v České republice.– Czech Mycol. 50: 271–302

Z 20 současně uznávaných druhů rodu *Thelocarpon* Nyl. je nyní známo v České republice 8 druhů: *T. epibolum, T. impressellum, T. intermediellum, T. laureri, T. lichenicola, T. olivaceum, T. pallidum* a *T. superellum*. Poprvé jsou pro území České republiky uváděny: *T. epibolum, T. intermediellum, T. olivaceum, T. pallidum* a *T. superellum*. *T. laureri* je běžně se vyskytující druh, *T. epibolum* a *T. lichenicola* jsou známy z poměrně značného počtu lokalit. *T. superellum* je nyní dokumentován ze tří lokalit, *T. olivaceum* ze 2 lokalit, *T. impressellum* a *T. pallidum* jsou známy každý z 1 lokality. Ke všem druhům jsou podány mapy rozšíření, kompletní výčet známých lokalit a ekologie druhů, rovněž je zahrnut klíč k určování druhů vyskytujících se v České republice. Ze Slovenské republiky je poprvé publikován *T. impressellum* a *T. lichenicola* a uvedeny jsou nové lokality *T. epibolum* a *T. laureri*. U *T. epibolum, T. intermediellum* a *T. lichenicola* byl zjištěn nepohlavní způsob rozmnožování, jsou popsány pyknidy a připojeny perokresby.

INTRODUCTION

The species of the genus *Thelocarpon* Nyl. have already monographically been treated by Magnusson (1935) and Salisbury (1953, 1966, 1974). A diagnosis of the genus, a key to the species as well as references are given by Poelt and Vězda (1977). The lichen flora of Great Britain and Ireland recently published by Purvis et al. (1992) includes also summarized diagnoses of this genus and the species found in these countries. A key to the species is also provided. Kiszka and Nowak (1966) reported about the rather frequent occurrence of representatives of this genus in Poland and expressed the opinion, that *Thelocarpon* species are much more overlooked than rare. Poelt and Hafellner (1975) found a certain connection between the change in function of the ascocarps from apothecioid to perithecioid and a series of reductions of the ascoapical apparatus. Ahti (1973) recognized two varieties of *Thelocarpon epibolum* during his work on North American *Peltigera* collection. He called the variety distinguished by long spores *Thelocarpon epibolum* var. *epithallinum*, a taxon that was already mentioned by Magnusson under the name f. *longisporum*. Ahti has found most of them to occur only on *Peltigera aphtosa* and *Peltigera leucophlebia*.

Several new species were recently described, such as *Thelocarpon cyaneum* on *Polyblastia* cf. *gothica* from Antarctica by Olech and Alstrup (1990), *Thelocarpon macchiae*, the species with the lowest number of spores in the genus on bare soil in garrigue vegetation with evergreen low bush formation from Italy by Nimis, Poelt and Puntillo (1994). Finally, *Thelocarpon opertum* was described by David and Coppins (1997). This species is characterized by sphaerical spores and immersed growth in cyanobacterial colonies on mossy turf together with calciphilous lichens on calcareous coastal sands.

Thelocarpon laureri was the first species of the genus *Thelocarpon* Nyl. reported from the Czech Republic, distributed as Lojka's exsiccate collection Lichenotheca Universalis no. 197 in 1886 sub *T. epilithellum*. The species was collected for the exsiccate collection by Plosel jr. in 1886 in the same locality where two years before this collector had made the first find of this species, which was so far not published. All the other finds of *Thelocarpon* have been made as late as in 20th century. *T. laureri* was reported by Suza, Anders, Poelt and Vězda and by Vězda (Suza 1919, 1929 sub *T. prasinellum*; Suza 1925, 1931, 1947 sub *T. epilithellum*; Anders 1922 and 1936, both sub *T. epilithellum*; Vězda 1957, 1972 and 1979, Poelt and Vězda 1990). Several of these collections were later mentioned by Migula (1929), Šmarda (1931), Magnusson (1935) and Poelt and Hafellner (1975).

Thelocarpon intermediellum, T. impressellum and *T. lichenicola* are the other species published from the Czech Republic. *T. intermediellum* was recorded by Servít (1930: 31, sub *T. intermixtulum*), *T. impressellum* collected by A. Vězda was mentioned by Poelt and Hafellner (1975) and *T. lichenicola* was published and distributed in Lichenes Selecti Exsiccati no. 1400 by Vězda (1976 sub *Ahlesia strasseri*).

Although *Thelocarpon epibolum* was so far not reported from the Czech Republic, it was collected by Suza in 1919, but left as an unidentified specimen in his collection in the PRM herbarium.

Map 1. Location of Šumava Mts. and Protected Landscape Area Křivoklátsko, the areas which were most frequently visited.

A find of *Thelocarpon lichenicola*, a rich collection with many fruitbodies made in the Šumava Mts. in 1989, evoked my interest in this genus. Also some other Czech lichenologists are presently interested in searching for *Thelocarpon* species. Currently 8 species from the genus *Thelocarpon* are recognized in the Czech Republic: *T. epibolum, T. impressellum, T. intermediellum, T. laureri, T. lichenicola, T. olivaceum*, *T. pallidum* and *T. superellum*. The largest number of collections of the last years were made in the Šumava Mts. and in the Protected Landscape Area Křivoklátsko (see Map 1).

The relatively high number of listed localities of recently collected *Thelocarpon* species shows that these lichens are much more overlooked for their minute size than rare and by purposeful exploration they can be found very frequently.

The species *Thelocarpon impressellum* and *T. lichenicola* recently collected in the Slovak Republic are new to this country and belong together with *T. epibolum, T. depressulum, T. intermediellum* and *T. laureri* to the currently known species of the genus *Thelocarpon* there.

MATERIAL AND METHODS

This study is based mostly on material recently collected by Czech lichenologists including the author's own finds made between 1989–1998. I have attempted to observe as many of the accepted species in their natural habitats as possible because such experience is invaluable for the study of their ecological requirements. The revision of earlier collections is based on all the specimens referring to the area of the Czech Republic we could find. The studied specimens originate from the herbaria PRM, PRC and from the private herbaria of Š. Bayerová, R. Dětinský, J. Halda, Z. Palice and Dr. A. Vězda. The revision of several additional collections is based on material sent on loan from the herbarium in Munich (M).

Observations of external features were made with a MST 131 stereomicroscope. Photographs were made with an Olympus PM 10. Macrophotographs were taken on a stereomicroscope Olympus SZH 10 and photomicrographs on an Olympus BX-50 microscope with a Nomarski Differential Interference Contrast on a Fuji 200 ASA film. For microscopic examination squash preparations and hand cut sections were made in water, 10 % KOH or Lugol's iodine.

ABBREVIATIONS AND SYMBOLS

The four-digit numbers preceding the dates indicate coordinate squares of 10 by 6 minutes (MTB grid) of the listed localities. If several collections were made in the same coordinate square, only the most recent record is mentioned. Symbols used in distribution maps: empty circles and other symbols = specimens collected until 1949; nearly empty circles and other symbols = specimens collected between 1950 and 1974 and full circles and other symbols = specimens collected since 1975. Species marked * are reported from the Czech or Slovak Republic for the first time. All specimens have been revised except those designated "non vidimus"! The following abbreviations are used for the collectors: J. K. or J. H. = Jana Kocourková-Horáková, P. K. = Pavel Kocourek.

RESULTS AND DISCUSSION

Life cycle

Species of the genus *Thelocarpon* Nyl. have been regarded as shortliving pioneer lichens. This opinion was already reported by Zahlbruckner on the label of *Thelocarpon laureri*, the specimen collected by him for the exsiccate Kryptogamae exs. Vindobonensi no. 373 (as syn. *T. prasinellum*) in Slovakia in 1897. Zahlbruckner observed the development of this species during a one-year period. Also Vězda observed the life cycle of this species for a two-year period on the top of a wooden post of his garden fence in Brno (south Moravia) and published his observation with Poelt (Poelt and Vězda 1990: 387). They indicated that fruitbodies are probably occurring only for several months during the year and that new ones can arise either from ascospores or from existing thallus. In autumn he found fruitbodies with asci in unmature state. In May of the following year the asci examined with a microscope were mature. Vězda also repeatedly observed the disappearing of fruitbodies during dry sunny days during summer.

The development of fruitbodies and the ripening of asci of *Thelocarpon laureri* were found by him dependent on the seasons of the year (Poelt and Vězda 1990). This result seems to be clear, but we found the process to be more complicated. Asci of several species were found mature in various seasons of the year including winter and summer. We observed that groups of fruitbodies, forming some sorts of colonies, appear any time of the year, but the most frequently in spring and autumn under the most favourable moisture conditions. By microscopical examination we found various colonies of fruitbodies of *T. epibolum* and *T. lichenicola* on the same lying decaying trunk in different stages of development several times during year. The same observation was made in *T. laureri*, observed on several stumps in our garden. However, in one colony we always found asci in almost the same stage of development. In *T. lichenicola* a tendency was observed to form pyčnidia (see below for description) before the ascomata started to arise or ripen. Therefore, we would rather hold the opinion, that both the development of fruitbodies and the maturing of asci depend more on the duration of wet periods than on the changing of the seasons during the year.

According to our observation, *Thelocarpon* species are living short in less favourable conditions, but mostly they are living longer than the few months indicated by Poelt and Vězda (1990). They are not tolerant to excessive desiccation for a long period, which damages them up to destruction of the fruitbodies built of tender structures and they are neither able to survive for a long time (several months) when covered by snow. But as the data of finds listed below indicate, it is possible to collect them practically during the entire year. The frequency of finds of individual species during the year mostly depends on the frequency of field studies by diligent collectors in each season of the year. However there is probably a lower number of collections during winter and during summer holidays the Czech Republic.

Five of the currently known species (*T. epibolum, T. intermediellum, T. laureri, T. lichenicola* and *T. olivaceum*) in the Czech Republic were found to live in the winter season, several collection of *T. intermediellum* were even made after a period of very cold and frosty conditions in mountain altitudes. Fruitbodies of *T. epibolum, T. intermediellum, T. laureri* and *T. lichenicola* were observed to survive extremely cold weather even if the temperature staged deeply below freezing-point for a longer time. The fruitbodies of *T. laureri* were found living on cutting flat of a stump in our garden even if after being covered with snow for almost one month. But we were not able to make a more detailed study of life cycle and how these species survive the winter season in areas with an extremely long winter period in high mountain and alpine situations and (for example) snow cover.

Three species, *Thelocarpon epibolum, T. laureri* and *T. lichenicola* were confirmed to survive hot days during summer months. *T. epibolum* and *T. lichenicola* were observed by us several times on lying decaying trunks in two wet valleys in the Protected Landscape Area Křivoklátsko in Central Bohemia from the spring till the end of the summer of the year 1997. The same fruitbodies were found living at each observation.

In favourable habitats under wet conditions *Thelocarpon* species can be observed in the same habitat for several years up to destruction of the organic substratum, unless they are overgrown by other competitively stronger or more expansive lichens or bryophytes, because of weakness in competition.

For two years *Thelocarpon laureri* was observed in the village of Přílepy in Central Bohemia on old wooden roof beams lying in a place exposed to the sun from the eastern for two years. *Thelocarpon laureri* was collected accompanied only by *Absconditella delutula*. After one and half year *Absconditella delutula* vanished, but due to proceeding succession a more extensive covering of the beams by *Placynthiella icmalea* and *Trapeliopsis flexuosa* was observed and only several fruitbodies of *Thelocarpon laureri* were seen. These after *Thelocarpon epibolum* appeared on the decaying wood between *Placynthiella icmalea* and *Trapeliopsis flexuosa*, but only in small quantity (see also Kocourková-Horáková 1998).

Distribution and substrata

Thelocarpon species are distributed from the lowlands to the mountains. They mostly prefer shaded and wet habitats. They occur on a wide range of substrata which are mentioned below in detail under each species. It seems, that they mostly avoid substrata with a high pH. This phenomenon is also obvious from the tables of accompanying species added to each of species. These accompanying species usually also occur on acidic substrata. However, there are species of the genus which were found directly on a calcareous substratum such as *Thelocarpon albidum* or *Thelocarpon opertum*, the last recently described by David and Coppins (1997). *Thelocarpon laureri* is also known to grow rarely on calcareous or alcaline stones. *Thelocarpon* species, being relatively living short, are little affected by air pollution. In the Czech Republic they are for this reason distributed even in suburban areas heavily polluted by sulphur dioxide and its derivatives.

Thelocarpon epibolum, T. lichenicola and *T. superellum* can be found in similar types of habitats which are shaded and wet for a long time. The organic substrata which they colonize, can be found mostly in a more advanced state of

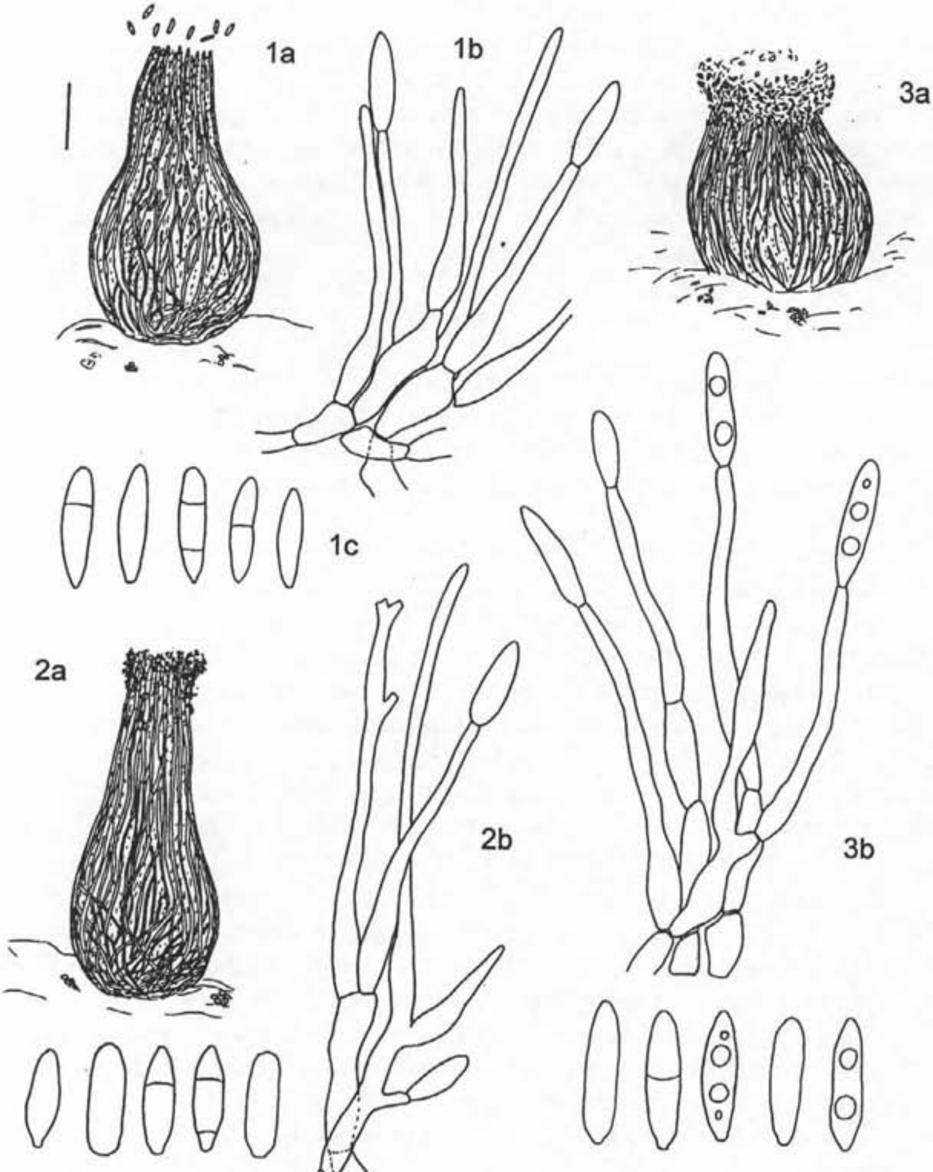


Fig. 1. *Thelocarpon. epibolum* var. *epibolum*. – **Fig. 2.** *Thelocarpon intermediellum*. – **Fig. 3.** *Thelocarpon lichenicola*. **Figs 1–3.** (a) pycnidium. Scale = 20 μm. (b) conidiophores and (c) conidia. Scale = 5 μm.

destruction, such as rotten or decaying wood. *T. laureri* rather occurs in more open habitats and on dry substrata and when on wood, than in an initial stage of destruction. *T. intermediellum* has intermediate requirements. *T. impressellum* and *T. olivaceum* were not collected by us and for that reason we hesitate to estimate their ecological requirements without personal observations. A rather more frequent occurrence of *T. epibolum* and *T. lichenicola* on some lichen hosts shows a certain ability to take up additional nutrients from a host thallus. *Thelocarpon epibolum* var. *epibolum* was found parasitic on thallus squamules of *Omphalina hudsoniana*.

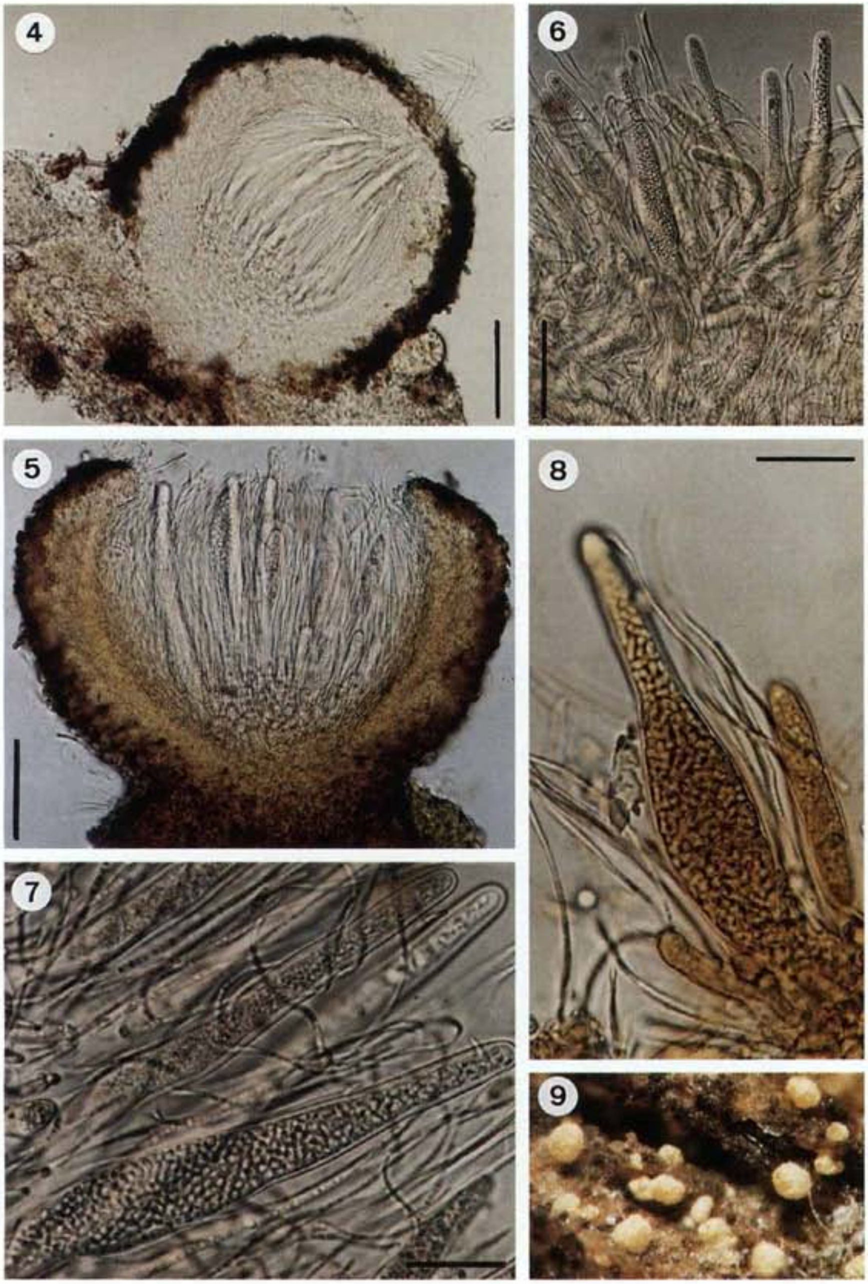
Pycnidia

Because earlier diagnoses of this genus and of all its species discussed here were already provided in detail several times before (Magnusson 1935, Salisbury 1966, 1974, Purvis and al. 1992), we insert only the description of newly discovered pycnidia and a key for easily orientation. Characteristic features of several species are shown in the Figs. 1–22.

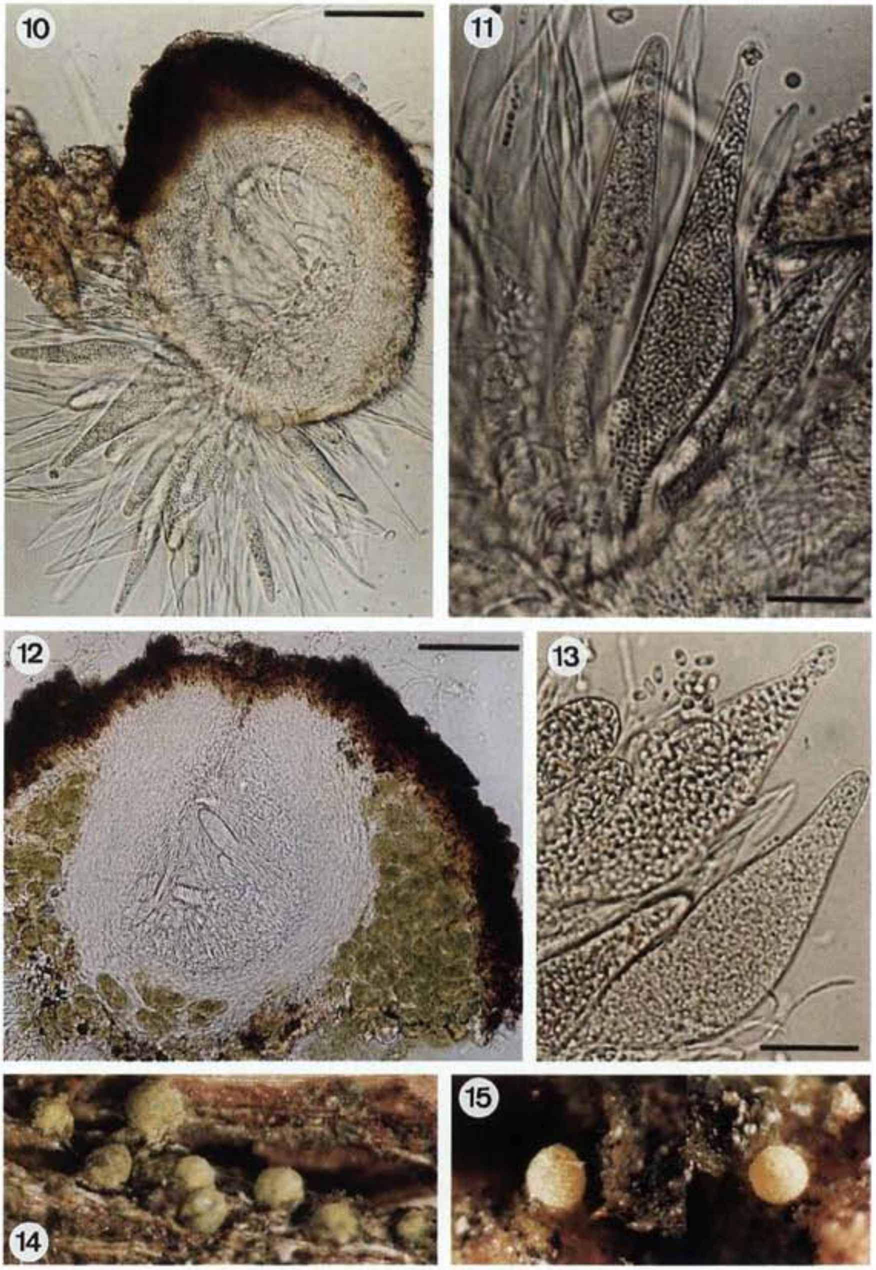
The occurrence of pycnidia within the genus *Thelocarpon* is very rare. Until now they have been known to occur only in *T. albidum, T. olivaceum* and *T. robustum*. The first note about pycnidia was provided for *T. olivaceum* by Magnusson (1936: 296 under *T. intermediellum*), according to a single observation of them on Phillips's collection made on old leather in Salop near Shrewsbury in 1873. This specimen should be deposited in Nylander's herbarium under No. 4127b. Salisbury (1966: 186) later revised probably another part of this collection possibly kept as a separated specimen, marked in Nylander's herbarium No. 4127e. He identified the species as *T. olivaceum*. Because he did not observe any pycnidia in this nor in anyother specimens, he provided only the description given already by Magnusson. A more detailed description of pycnidia is provided by Salisbury for *Thelocarpon albidum*. The origin of observed microconidia in *Thelocarpon robustum* was not traced either by Magnusson nor by Salisbury. A diagnosis of pycnidia for *Thelocarpon olivaceum* is also given by Purvis and al. (1992).

During the revision of several specimens of *Thelocarpon olivaceum* made by collectors in the Czech Republic and material from the Munich herbarium (M), we did not found any pycnidia in this species. However, we discovered pycnidia in *T. epibolum, T. intermediellum* and *T. lichenicola*. Since both *T. albidum* and *T. olivaceum* seem to form conidiomata and conidia of somewhat different form than in our species, a detailed description and drawings of our finds are provided.

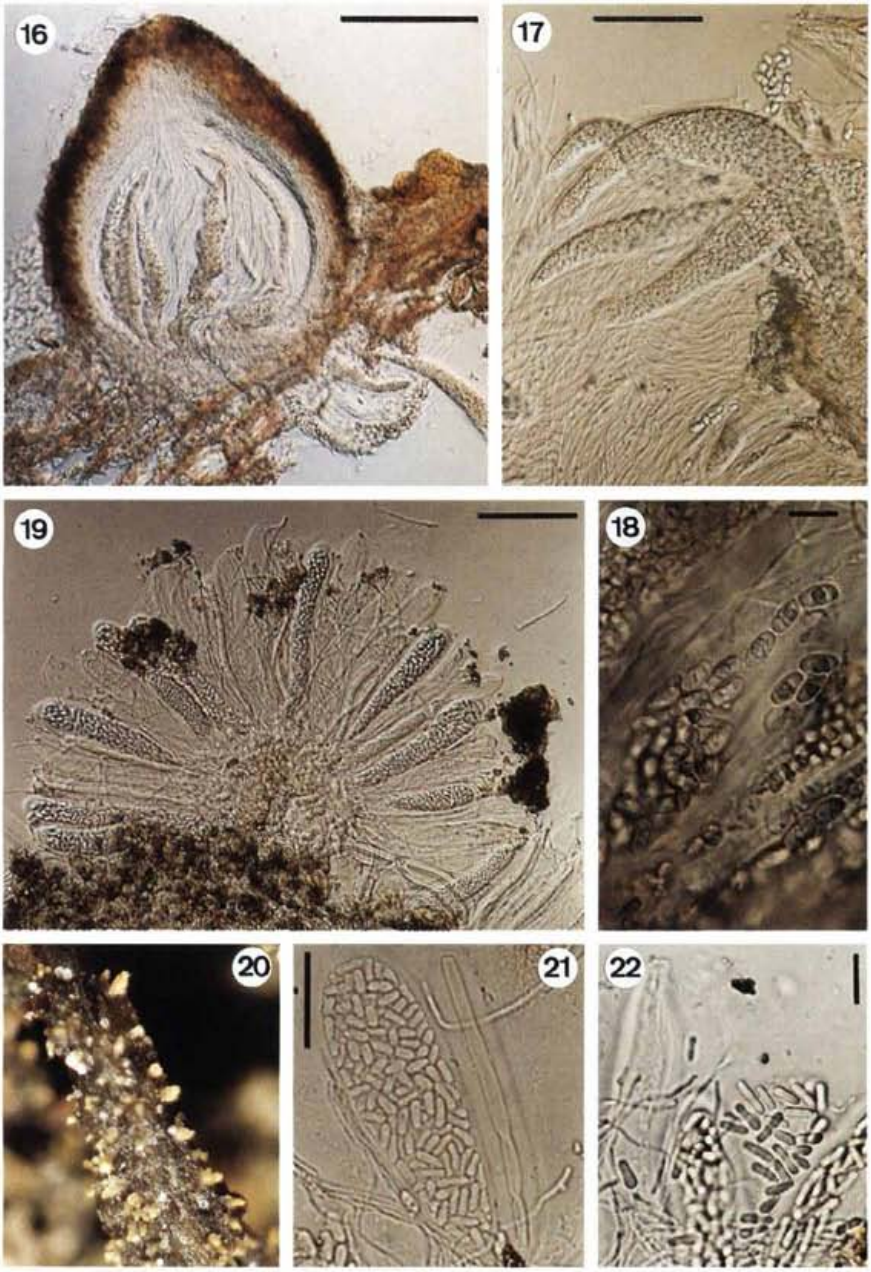
The pycnidia of *Thelocarpon albidum* are described by Salisbury as immersed in the thallus verrucae, irregularly shaped, up to 100 μm across, with conidiophores 25 μm long, up to 2 μm thick at base, tapering towards apex, simple; microconidia are produced apically, oblong, 3–3.5 $\times$ 1 μm, colourless, simple.



Figs 4–9. *Thelocarpon epibolum* var. *epibolum*. – **4.** Longitudinal section through immature ascoma. – **5.** Slightly squashed longitudinal section through mature ascoma showing also associated algae at the base. – **6–7.** Mature asci with ascospores and simple paraphyses as long as asci or longer. – **8.** Asci in Lugol's solution showing a typical red reaction of gel matrix. – **9.** Group of ascomata on rotten wood (× 48). (Figs 4–7. Scale = 50 μm; Figs 7–8. Scale = 20 μm; Figs 4–7. Water preparations.).



Figs 10–11. *Thelocarpon intermediellum*. – **10.** Vertical section of ascoma without paraphyses showing prolapsed asci. – **11.** Asci and ascospores. **Figs 12–14.** *Thelocarpon laureri*. **12.** Vertical section of ascoma with algal sheath present. – **13.** Flask-shaped asci and branched paraphyses shorter than asci. **14.** Group of ascomata on withered rests of grass (× 48). **15.** *Thelocarpon superellum*. Ascomata on soil (× 48,5). (Figs 10, 12. Scale = 50 μm; Figs 11, 13. Scale = 20 μm. All preparations in water.)



Figs 16–18. *Thelocarpon superellum*. – **16.** Longitudinal section through ascoma. – **17.** Asci and simple paraphyses. – **18.** Ascospores with occasionally developed pseudoseptum. Scale = 10 μm. – **Figs 19–22.** *Thelocarpon lichenicola*. – **19.** Squash preparation of ascoma. – **20.** Pycnidia in algal layer covering dead plant of *Sphagnum* (× 35) – **21.** Mature ascus with ascospores in squash preparation. Scale = 20 μm. – **22.** Ascospores. Scale = 10 μm. (Figs 16. Scale = 100 μm; Figs 17, 19. Scale = 50 μm. All preparatons in water.)

Thelocarpon olivaceum should form pycnidia sited in the algal sheath of the perithecial wall, and are globose, only 35 μm in diam., wall colourless; microconidia 4.5–5 × 1 μm. No conidiophores were described.

While the pycnidia of *T. lichenicola* were observed rather frequently (see the list of localities), pycnidia of *T. epibolum* and *T. intermediellum* were seen only in single collections.

Conidiomata of *Thelocarpon lichenicola* (Fig. 3a-c):

Conidiomata pycnidia, standing solitarily, dispersed to more or less close together, intermixed with ascomata, sessile, only with their bases slightly immersed in the algal film, sometimes rising from the base of apothecia, obpyriform or lageniform (flask-shaped), with a clearly narrower part below rounded ostiolum, hyaline to pellucid yellow in the lower part and clearly yellow pruinose towards the top, most intensively around the ostiolum; 75–120 μm tall, 50–85 μm wide, in the narrowest part below the ostiolum 0.2–0.4 mm wide. Wall composed of a textura intricata. Conidiophores simply or rarely branched, erect, in the lower part widened and ampuliform up to 2 μm, about 1.5 μm wide in upper part, 22–40 μm long. Conidia holoblastic, solitary, acrogenous, aseptate, rarely 1-septate, hyaline; amerospores oblong to fusiform, with narrower, slightly truncate base, guttulate, 6.5–8 × 1.8–2.1 μm in diam.

Apothecia and pycnidia occur very frequently in the below mentioned specimen of *T. lichenicola* from Hůrecká slať in the Šumava Mts. While the pycnidia are mature, only unmature asci were observed in apothecia.

Pycnidia of *Thelocarpon epibolum* (Fig. 1a-c) differ in having a less narrowed part below the ostiolum, hyaline walls, size 75–105 × 40–60 μm; conidiophores formed only simply, 15–25 μm; conidia aseptate to 2-septate, fusiform, with very slightly indicated truncate base, aguttulate, 5–7 × 1.2–1.6 μm. The pycnidia of *Thelocarpon intermediellum* (Fig. 2a-c) are hyaline or pellucid yellow, with a much less distinct pigmentation of the upper part than in *Thelocarpon lichenicola*, 105–165 × 45–80 μm; conidiophores branched, 18–45 μm; conidia 0–2-septate, oblong to fusiform, aguttulate, conidia fusiform, 4.5–6.5 × 1.1–1.8 (-2.1) μm.

Key to the Czech species:

Paraphyses absent 2
Paraphyses present 4
2 Algal sheath absent or rudimentary, spores oblong, slightly constricted ....
.... *T. intermediellum*
Algal sheath present, spores oblong-ellipsoid 3
3 Spores (2-)2.5–3.5(-4) × 1.5–2 μm .... *T. olivaceum*
Spores (6-)7–9(-10) × 3–3.5 μm .... *T. pallidum*
4 Paraphyses branched 5

Paraphyses simple 6
5 Algal sheath present, one globose apothecium developed in the thallus verruca .... *T. laureri*
Algal sheath absent, apothecia cylindrical or widened at apex .. *T. lichenicola*
6 Asci I– spores oblong .... *T. epibolum*
Asci I+ blue 7
7 Asci I+ dark blue, hymenial jelly I+ red, apothecia obpyriform, spores (6-)8–13 × 2.5–6 µm, pseudosepta usually present .... *T. superellum*
Asci I+ pale blue, hymenial jelly I+ red, apothecia depressed above, spores 6–8 (-11) × 4–4.5 µm, pseudosepta absent .... *T. impressellum*

Thelocarpon epibolum Nyl. var. **epibolum** (Figs 1, 4–9, 23)

Thelocarpon epibolum var. *epibolum* occurs in rather moist and shaded habitats for example in stream valleys, in peat bogs or near springs. Although the species is considered rather mountainous (Poelt and Vězda 1977), recent collections in the Czech Republic equally cover all altitudes between 190–1360 m above sea level, from the lowlands to the mountains. It was found on a wide range of substrata including soil, more or less rotten wood mostly of broad leaved trees, plant debris, peat (*Sphagnum girgensohnii*), basidiocarps (*Fomes fomentarius*) and thalli of lichens (*Baeomyces rufus, Peltigera* spec., *Omphalina hudsoniana* and *Verrucaria*). The most common substratum seems to be rotten wood. No collection was made directly on stone. Only a single collection was made on an immersed pebble, although on the thallus of an unidentified *Verrucaria* species.

In all Czech specimens *Thelocarpon epibolum* collected on thallus squamules of *Baeomyces rufus* was found to occur together with *Arthrorhaphis grisea*. *Arthrorhaphis grisea* is a parasitic species, which kills the fungus in *Baeomyces rufus*, takes over its alga and forms eventually its own thallus. This observation was recently published by Obermayer in his monography of the genus *Arthrorhaphis* (1994) and it also confirmed the observation that we made while searching for *Thelocarpon* species of *Baeomyces* thalli. Because *Thelocarpon epibolum* was also found to strongly damage host thalli of *Baeomyces rufus* in other revised specimens where *Arthrorhaphis grisea* was not present, the relation between both parasites when occurring together is not clear to us. Squamules of *Omphalina hudsoniana* in both Czech and Slovak specimens were also heavily affected. Therefore we consider *Thelocarpon epibolum* a parasite when lichenicolous. Additionally, the same observations were made when it was overgrowing algae of the genus *Coccomyxa*.

No occurrence of *T. epibolum* var. *epithallinum* (Leight. ex Nyl.) G. Salisb. distinguished by longer spores was established in the Czech Republic, although it was found in the Slovak Republic.

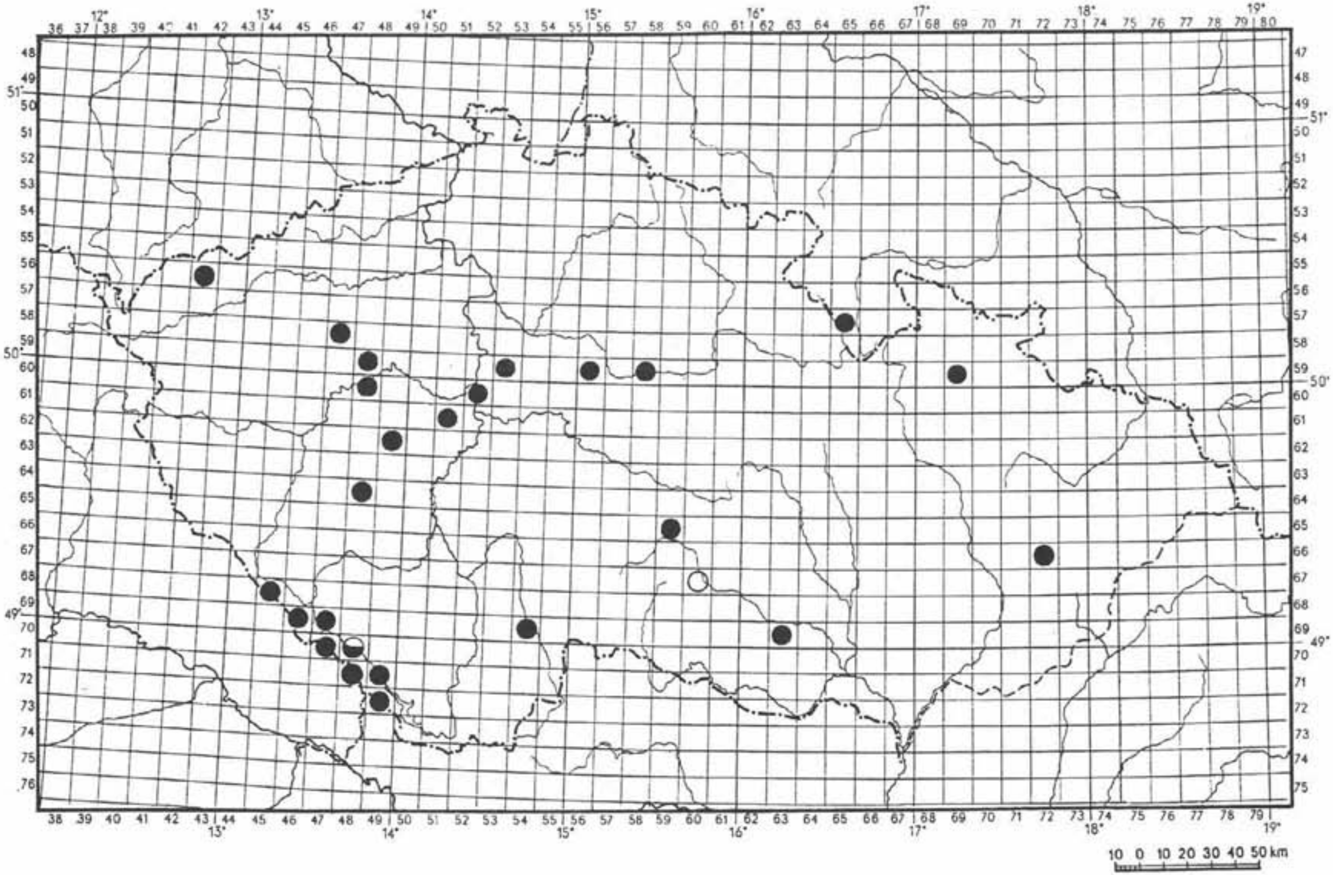


Fig. 23. Distribution of *Thelocarpon epibolum* Nyl. var. *epibolum* in the Czech Republic.

Table 1
Species of lichens and bryophytes accompanying *Thelocarpon epibolum* according to substrata.

substrata	rotten wood	soil	peat	Baeomyces rufus	saxicolous	Fomes fomentarius
lichens	*Absconditella* sp. *Cladonia* sp. *Lepraria* sp. *Micarea prasina* *Placynthiella icmalea* *Steinia geophana* *Thelocarpon lichenicola* *Vezdaea* sp.		*Omphalina hudsoniana* *Thelocarpon lichenicola*	*Arthrorhaphis grisea* *Belonia incarnata* *Bryophagus gloeocapsa*		*Lecania cyrtella*
bryophytes	*Cephalozia bicuspidata* *Cephalozia lunulifolia* *Chiloscyphus profundus* *Hypnum cupressiforme* *Tetraphis pellucida*	*Cephalozia bicuspidata* *Pogonatum aloides* *Pohlia nutans*	*Sphagnum girgensohnii*	*Dicranella heteromalla* *Nardia scalaris*	*Bryum caespiticium* *Ceratodon purpureus*	
algi	*Coccomyxa* sp.		*Coccomyxa* sp.			

Accompanied lichens and bryophytes observed in the Czech localities are the following species of cryptogams growing in the close vicinity of our species. The most frequently observed were *Micarea prasina*, *Placynthiella icmalea, Chiloscyphus profundus, Hypnum cupressiforme* and *Coccomyxa* sp. All species are listed in the Table 1 according to substrata.

Thelocarpon epibolum is reported for the first time from the Czech Republic, but it has been collected in many localities. All the collections concern recent finds with the exception of two earlier finds made by Suza in 1919 and Svrček in 1970.

Specimens examined:

CZECH REPUBLIC: Western Bohemia, Krušné hory Mts., Distr. Karlovy Vary, Nejdek, on slope of the hill Blatenský vrch, near Vlčí jámy ice pits, on stump of *Picea abies*, 1000 m, 5642; 24.6.1993, coll. J. H. (PRM 890489). – Šumava Mts., Distr. Klatovy, Železná Ruda, in glacier cirque of lake Černé jezero – central part, moist siliceous pebbles on the ground, on thallus of *Baeomyces rufus*, about 1200 m, 6845; 11.10.1995, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Klatovy, Modrava, forest track leading through the peat bog Mlynářská slať, on thallus of *Baeomyces rufus,* 1050 m, 6946; 28.6.1995, coll. Z. Palice (hb. Palice). – Brdy Mts., Distr. Plzeň-South, Míšov, near nature reserve Míšovské buky, by forest track, on decaying wood of *Picea abies*, 750 m, 6448; 12.12.1997, coll. J. Kocourková, Z. Pouzar, Š. Bayerová (PRM 891906). – Central Bohemia, Distr. Rakovník, Přílepy, at a house on old wooden beams, 340 m, 5847; 7.9.1997, coll. J. K. (PRM 891409). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, about 3.5 km SE of Rakovník, near the village of Dolní Chlum, by railway, on a moist granite stone, 5948; 300 m, 3.1.1998, coll. J. K. and P. K. (PRM 891900). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, Skryje, close by the confluence of the brooks Ostrovecký potok and Houpačkový potok, on a fallen decaying trunk of *Picea abies*, together with *T. lichenicola* and *Vezdaea* sp., 449 m, 6048; 21.6.1997, coll. J. K. (PRM 890834). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, nature reserve Týřov, valley of the Úpořský stream, near the confluence of the brooks Úpořský potok and Prostřední potok, on decaying wood, 285 m, 6048; 25.5.1997, coll. J. K. (PRM 890833). – Brdy Mts., Distr. Příbram, Jince, near Ohrazenice, on rotten stump, 550–600 m, 6249; 21.2.1998, coll. Š. Bayerová (hb. Bayerová). – Hřebeny Mts., Distr. Prague-West, Řevnice, on slope of Hvíždinec hill, in forest, on stump, about 400 m, 6151; 3.11.1996, coll. Š. Bayerová and O. Lopata (hb. Bayerová). – Prague, Komořany, right bank of the Vltava river, near railway station, rotten fallen trunk of *Populus nigra*, 190 m, 6052; 16.11.1996, coll. J. Horáková (PRM 890446). – Prague, Uhříněves, in the reserve near a brook, on top of stump, 280 m, 5953; 4.10.1995, coll. J. H. (PRM 887013). – Distr. Kolín, Veltruby, valley of the Labe river, in the floodplain forest "Veltrubský luh", on decaying wood, 190 m, 5956; 26.5.1995, coll. Z. Palice, det. J. H. (hb. Z. Palice.). – Southern Bohemia, Šumava Mts., Distr. Prachatice, Kvilda, in peat bog between the nature reserve Jezerní slať and the stream Hamerský potok, on *Baeomyces rufus*, together with *Arthrorhaphis grisea,* 990 m, 6947; 2.10.1990, coll. J. H. (PRM 887014). – Šumava Mts., Distr. Prachatice, Kvilda, 1 km SE of the village Kvilda, right bank of the Vltava river, on mosses on rocks, 1000 m, 6947; 13.6.1997, coll. Š. Bayerová and Z. Palice, det. Z. Palice (hb. Palice). – Ibid.: on *Sphagnum magellanicum* on squamules of *Omphalina hudsoniana*, 6947; 13.6.1997, coll. Š. Bayerová (hb. Bayerová, together with *T. lichenicola*). – Šumava Mts., Distr. Prachatice, near of the source of the Vltava river, on overhangs by a forest track, on thallus of *Baeomyces rufus*, 1170 m, 7047; 5.5.1993, coll. J. H. (PRM 887015). – Šumava Mts., Distr. Prachatice, in the peat bog "Velká Niva" near Lenora, on trunk of *Betula*, on upper side of basidiocarp of *Fomes fomentarius*, together with *Lecania cyrtella* (Ach.) Th. Fr., 750 m, 7048; 6.9.1970, coll. M. Svrček, det. J. H. (PRM 716208). – Šumava Mts., Distr. Prachatice, Mt. Stožec, nature reserve "Medvědice", rotten fallen trunk of?*Fagus*, about 900 m, 7148; 5.7.1994, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, Nové Údolí, valley of the Světlá brook, about 2 km ENE of Mt. Kamenná, on bark of

Sambucus racemosa, 850 m, 7148; 3.6.1995, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, in the village Černý Kříž, yard of gameskeeper's lodge, on wood, 745 m, 7149; 10.1996, coll. and det. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, Nová Pec, in glacier cirque of lake Plešné jezero, rock wall in central part, on debris of *Sphagnum girgensohnii*, on algal crust of *Coccomyxa*, about 1300 m, 7249; 15.8.1995, coll. Z. Palice (hb. Palice). – Ibid.: Plešné jezero lake, near bank, on semi-immersed wood, 7249; 1090 m, 11.7.1997, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, Nová Pec, Mt. Plechý, climax spruce forest, on dry stump of *Picea*, 1360 m, 7249; 1.10.1995, coll. Z. Palice (PRM 891903, hb. Palice). – Distr. Jindřichův Hradec, Třeboň, between Lomnice nad Lužnicí and Lužnice, near research station of Botanical Institute of Academy of Science, on rotten wood, 420 m, 6954; 22.5.1997, coll. Z. Palice (hb. Palice). – Eastern Bohemia, Distr. Pardubice, Chvaletice, sedimentation basin near power station c. 1 km E of the village, on immersed pebble, on thallus of *Verrucaria* sp., 220 m, 5958; 14.2.1997, coll. Z. Palice (hb. Palice). – Orlické hory Mts., Distr. Rychnov nad Kněžnou, valley of the Divoká Orlice river, Podlesí, at a forest margin, in denudated roadside verges, on soil, on *Baeomyces rufus* together with *Arthrorhaphis grisea*, 550 m, 5765; 19.4.1996, coll. Z. Palice (hb. Palice). – Western Moravia, Distr. Jihlava, below summit of Vysoký Kámen hill, on upper surface of rotten stump, 640 m, 6559; 14.10.1996, coll. Š. Bayerová (hb. Bayerová). – Distr. Třebíč, between the villages Heraltice and Opatov, in ditch by roadside, on soil, about 600 m, 6760; 2.8.1919, coll. J. Suza, det. J. H. (PRM 587610). – Northern Moravia, Hrubý Jeseník Mts., Distr. Bruntál, Malá Kotlina valley, by the brook Kotelný potok, on an old rotten stump, about 900 m, 5969; 12.7.1989, coll. J. H. (PRM 887016). – Eastern Moravia, Hostýnské vrchy Mts., Distr. Zlín, Košovy, Mt. Sochová, below the top, on a rotten fallen trunk, c. 700 m, 6672; 14.5.1995, coll. J. H. (PRM 891899). – Southern Moravia, Distr. Znojmo, Moravský Krumlov, Tábor hill, above the left bank of the Rokytná river, xerothermic slope, on dying lichens of *Peltigera* sp., 350 m, 6963; 19.5.1996, coll. Z. Palice (hb. Palice).

Slovak Republic: Northern Slovakia, High Tatra Mts., valley Temnosmrečinová dolina, near the lake Vyšné Temnosmrečinovské pleso, on *Omphalina hudsoniana*, 1720 m, 22.9.1993, coll. J. Horáková and V. Alstrup (PRM 889695). – Central Slovakia, Carpathians, Muránska planina plateau, Hrdzavá valley, peat bog "V machoch", on upper surface of rotting stumps, 760–800 m, 22.9.1995, coll. Z. Palice and Š. Bayerová, det. Z. Palice (hb. Palice).

Thelocarpon epibolum var. **epithallinum** (Leight. ex Nyl.) G. Salisb.

The collection was already mentioned by Alstrup (1996) together with other lichenicolous fungi collected during field studies of the Bryological-Lichenological

Section of the Czech Botanical Society in the High Tatra Mts. in 1993. If any specimen of this large-spored variety was earlier collected in the Slovak Republic, all lichenicolous finds of *Thelocarpon epibolum* would have to be revised. The occurrence of this taxon in relation to its substrate was more extensively discussed by Ahti (1973). The infected thalli of *Peltigera aphtosa* were not found damaged.

SLOVAK REPUBLIC: Northern Slovakia: High Tatra Mts., valley Temnosmrečinovská dolina, near the lake Vyšné Temnosmrečinovské pleso, on thalli of *Peltigera aphtosa*, 1720 m, 22.9.1993, coll. J. Horáková and V. Alstrup (PRM 889693).

***Thelocarpon impressellum** Nyl. (Fig. 27)

The only specimen known in the Czech Republic is the one collected by A. Vězda and revised by Poelt and Hafellner (1975). No find was made by A. Vězda and me during our visit of this only Czech locality in autumn 1997. Recently the species was found for the first time in the Slovak Republic, in the West Tatra Mountains. The find was made under very humid conditions on wooden trunks in the brook. The finds were made in quite different habitats. No accompanied species were found in neither of specimens.

Specimens examined:

CZECH REPUBLIC: Western Moravia, Distr. Blansko, Tišnov, near Deblín, at margin of forest by a road, on soil, 400 m, 6664; 1974, coll. A. Vězda, (GZU).

SLOVAK REPUBLIC: Western Slovakia, the West Tatra Mts., Oravice, valley Juráňova dolina, on a rotten trunk lying in a tributary of the brook Juráňový potok, 960 m, 6784; 1.6.1990, coll. J. Horáková (PRM 887017).

Thelocarpon intermediellum Nyl. (Figs 2, 10–11, 24)

This species appears to be quite common, but is probably much overlooked. There is one old published record of this species collected on sandstone at Vidoule in Prague (Servít 1930: 31 as *T. intermixtulum*). Unfortunately, the specimen is probably lost, although it should be deposited in Servít's collection in the PRM herbarium. It was neither found in the PRC herbarium, where some of Servít's earlier collections are situated. All the other Czech and Moravian recent collections were made between 1994–1997. The species is at present known from 16 localities in the Czech Republic.

The species occurs at altitudes from the lowlands to the mountains. It has often been collected at the margin of forests or in light deciduous forests. Rotten wood of trunks or stumps of broad-leaved trees was found to be the most common substratum of this species, found in association with *Micarea* species, *Placynthiella icmalea* and algae. A pine stump, peat, siliceous stones and the basidiocarp of

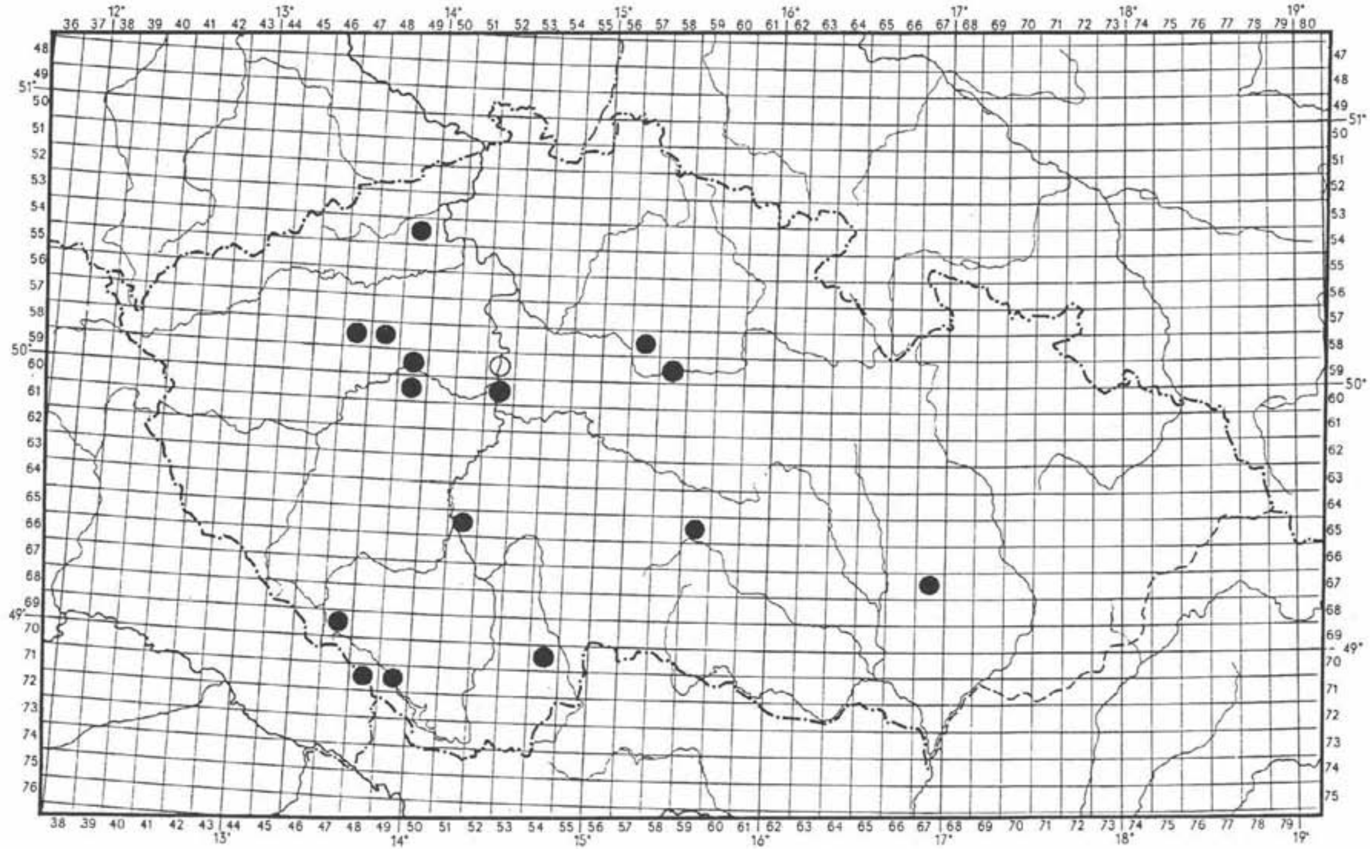


Fig. 24. Distribution of *Thelocarpon intermediellum* Nyl. in the Czech Republic.

Table 2
Species of lichens and bryophytes acompanying *Thelocarpon intermediellum* according to substrata.

substrata	rotten wood	soil	peat	saxicolous	Fomes fomentarius
lichens	*Chaenotheca sp.* *Cladonia sp.* *Hypogymnia physodes* *Lepraria sp.* *Lecanora conizaeoides* *Micarea denigrata* *Micarea prasina* *Placynthiella icmalea* *Placynthiella uliginosa*	*Micarea melaena* *Placynthiella icmalea*	*Cladonia digitata* *Placynthiella icmalea*		
bryophytes		*Ceratodon purpureus* *Tetraphis pellucida*		*Hypnum cupressiforme*	*Hypnum cupressiforme*

Fomes fomentarius were recorded as less common other substrata. One collections was made on a corroded plate of iron, on an algal layer. Accompanied species growing in the close vicinity of *Thelocarpon intermediellum* are given in Table 2.

Pycnidia of *Thelocarpon intermediellum* were discovered at the revision of specimens collected by Z. Palice in the Šumava Mts.

Specimens examined:

CZECH REPUBLIC: Western Bohemia, Šumava Mts., Distr. Klatovy, Zhůří, valley of the brook Pěnivý potok, on vertical side of old stump, growing together with *Chaenotheca* sp., about 750 m, 6947; 4.5.1995, coll. Z. Palice (hb. Palice). – Northern Bohemia, Distr. Litoměřice, České středohoří Mts., Mt. Milešovka, on lying trunk, on wood, 5449; 22.4.1995, coll. Z. Palice (hb. Palice). – Central Bohemia, Distr. Rakovník, Bedlno, 1 km SE of the village near quarry, on a fallen decaying trunk, 485 m, 5847; 4.3.1997, coll. J. H. (PRM 890484). – Distr. Rakovník, Olešná, in peat bog near the village, on peat among roots of fallen trunk, 350 m, 5848; 17.2.1996, coll. J. H. (PRM 887748). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, between Roztoky and Karlova Ves, valley of Klucná brook, on fallen decaying trunk, 310 m, 5949; 3.10. 1996, coll. J. H. (PRM 891902). – Distr. Beroun, 2 km SW of the village Nový Jáchymov, near brook Habrový potok, in glade close to a road, on lignum of stump of *Quercus*, 420 m, 6049; 23.3.1997, coll. J. H. (PRM 890491). – Distr. Prague, Vidoule, on knoll Vidoule, on decalcified clay slate, 5952; 1920–1926, coll. Servít (PRM?, non vidimus!). – Distr. Prague, Radotín, near the busstop "U cementárny", on piece of wood on bare soil in a quarry, 230 m, 6052; coll. Z. Palice (hb. Palice). – Distr. Nymburk, Libice nad Cidlinou, flood plain forest "Libický luh", NE border of nature reserve, together with *Placynthiella icmalea*, about 190 m, 5857; 18.3.1995, coll. Z. Palice (PRM 887114, hb. Palice). – Southern Bohemia, Šumava Mts., Distr. Prachatice, on S slope of Mt. Stožec near the nature reserve Medvědice, on a rotting trunk (cf. *Fagus sylvatica*), about 800 m, 7148; 22.10.1994, coll. Z. Palice (hb. Palice). – Ibid.: on top of stump (*Picea abies*), about 880 m, 7148; 22.10.1994, coll. Z. Palice (hb. Palice). – Ibid.: near Stožecká kaple chapel, on rotting wood, 930 m, 7148; 26.1.1997, coll. B. Buryová and Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, Černý Kříž, ca 200 m from the railway station in direction to České Budějovice, in pine forest by railway, on top of a rotten pine stump, about 745 m, 7149; 3.12.1994, coll. Z. Palice (hb. Palice, with pycnidia!). – Šumava Mts., Distr. Prachatice, 3 km S of the village Černý Kříž, Mt. Srnčí vrch, in the nature reserve Jelení vrch, on wood of a fallen decaying trunk of *Fagus sylvatica*, about 850 m, 7149; 29.12.1994, coll. Z. Palice (hb. Palice). – Šumava Mts., Černý Kříž, in pine forest by the railway station, on peat, 740 m, 7149; 16.4.1995, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, Černý Kříž, near the forest track Tovární cesta, c. 100 m from the forest track Hučicská cesta, siliceous stone overhang, 760–800 m, 7149; 16.12.1995, coll. Z. Palice (hb. Palice). – Distr. Písek, Zvíkovské Podhradí, above the right bank of the Vltava river, near the bridge, on slope in deciduous forest, on stump, about 400 m, 6551; 10.1996, coll. Z. Palice (hb. Z. Palice). – Distr. Jindřichův Hradec, Třeboň, nature reserve Stará řeka, on wood of *Quercus*, 435 m, 7054; 1.4.1997, coll. Z. Palice (hb. Palice). – Eastern Bohemia, Distr. Pardubice, Chvaletice, sedimentation basin near the power station c. 1 km E of the village, on bare soil, 220 m, 5958; 5.11.1995, coll. Z. Palice (hb. Palice). – Ibid.: on plate of corroded iron, 220 m, 5958; 14.4.1997, coll. Z. Palice (hb. Palice). – Western Moravia, Distr. Jihlava, on slope of the hill Vysoký Kámen, in pores of basidiocarp of *Fomes fomentarius*, 640 m, 6559; 14.10.1996, coll. J. H. (PRM 890493). – Southern Moravia, Distr. Vyškov, Olšany, in forest, on rotten stump, 500 m, 6767; 11.5.1996, coll. J. H. (PRM 887018).

Thelocarpon laureri (Flot.) Nyl. (Figs 12–14, 25)

Until the early seventies of this century, this species had been the only one known of the genus in the Czech Republic, except for an unverified specimen of *Thelocarpon intermediellum* collected by M. Servít and one unidentified specimen of *Thelocarpon epibolum* collected by Suza. This is the most common species of the genus.

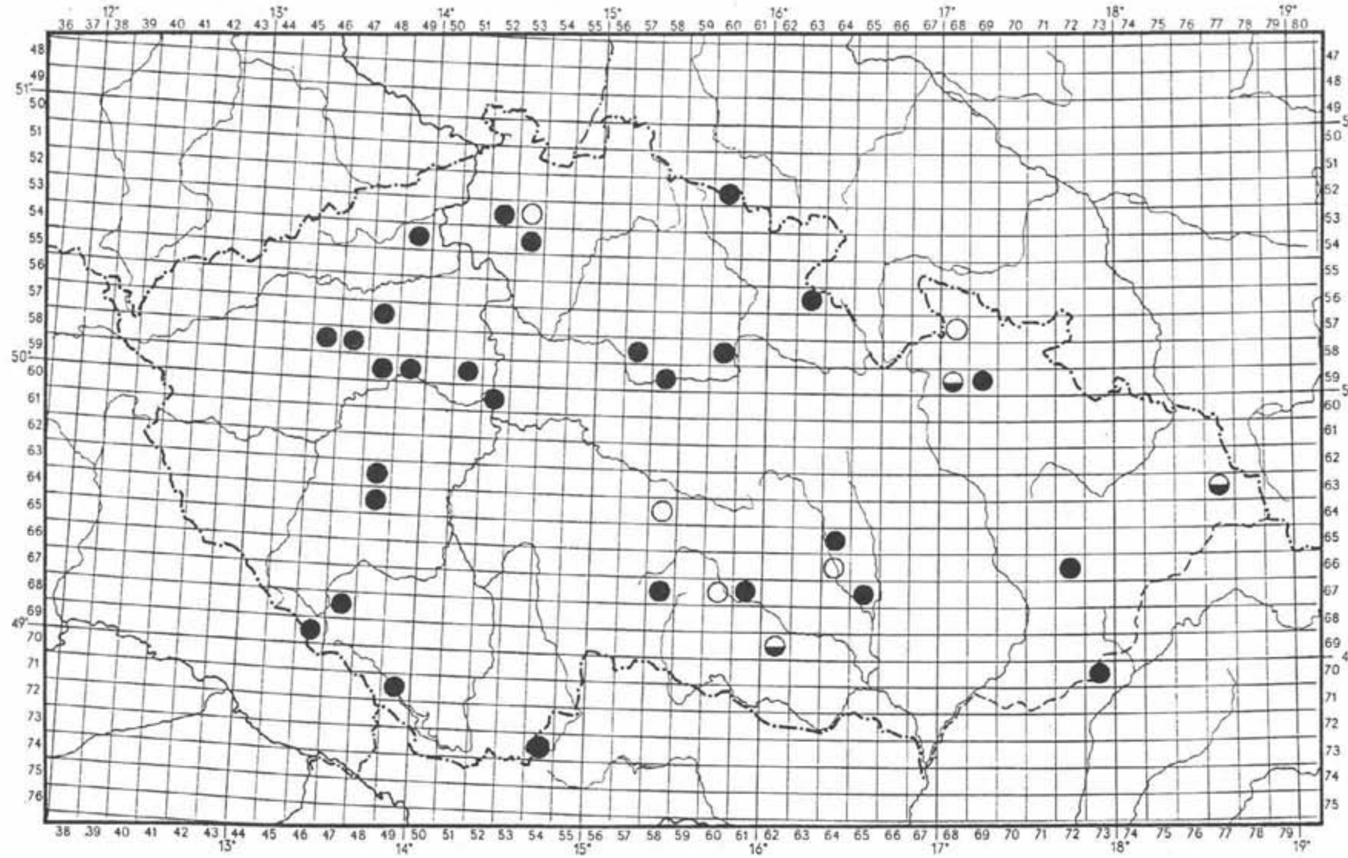


Fig. 25. Distribution of *Thelocarpon laureri* (Flot.) Nyl. in the Czech Republic.

Table 3.
Species of lichens and bryophytes acompanying *Thelocarpon laureri* according to substrata.

substrata	wood	soil	stones	plant debris
lichens	*Absconditella delutula* *Amandinea punctata* *Candelariella vitellina* *Candelaria concolor* *Hypogymnia physodes* *Lecanora conizaeoides* *Lecanora saligna* *Lepraria* sp. *Micarea denigrata* *Parmelia sulcata* *Placynthiella icmalea* *Trapeliopsis flexuosa*	*Steinia geophana*	*Acarospora* sp. *Candelariella vitellina* *Lecidea variegatula* *Micarea lithinella* *Trapelia involuta* *Trapelia* sp.	*Placynthiella icmalea* *Trapeliopsis flexuosa* *Trapeliopsis granulosa*
bryophytes	*Ceratodon purpureus*		*Cephaloziella divaricata*	

Unlike other species of the genus this one occurs rather in open sunny habitats. This phenomenon is probably caused by the photobiont sheath in its ascomawall as an adaptation to excessive exposition and following desiccation. *Thelocarpon laureri* grows on a wide range of substrata in initial states of succession as an rapid coloniser of substrata made or influenced by man, on tops of stumps, worked timber, on wooden fencing and also burnt wood, bricks, or on exposed bark of stumps and lying corticate trunks, often in dusty habitats, and in natural habitats on plant debris, pebbles, loose stones and boulders. The species was found in about 40 localities in different areas of the Czech Republic from the lowlands to mountainous situations.

Species of cryptogams found in the close vicinity of *T. laureri* are given below in the Table 3.

Exsiccata examined:

CZECH REPUBLIC: A. Vězda: Lichenes selecti exsiccati no. 1028 (PRM 721825). A. Vězda: Lichenes selecti exsiccati no. 1640 (PRM 820786, M 10631). J. Suza: Lichenes Bohemoslovakiae, no.164 (sub *T. prasinellum*; hb. Vězda, M 10608, PRC). H. Lojka: Lichenotheca Universalis, fasc. IV., no. 197 sub *T. epilithellum*, PRM 587592, 587594, 587596).

SLOVAK REPUBLIC: J. Suza: Lich. Bohemoslovakiae no. 44. – Southern Slovakia, Kremnica, on old roof, on shingles, about 500 m, 1927, coll. J. Suza (sub *T. prasinellum*; M 10609).

Kryptogamae exs. Vindobonenses no. 373. - Distr. Bratislava (Com. Posoniensis): ad ligna abietina, Sv. Jur (in St. Georgio), 5.1897, coll. Zahlbruckner. (sub *T. prasinellum* Nyl.; M 10666).

Additional specimens examined:

Czech Republic: Western Bohemia, Šumava Mts., Srní, S of the village along the yellow marked tourist track, on a siliceous boulder, about 850 m, 6946; 26.6.1995, coll. Z. Palice (hb. Palice). – Šumava Mts., Rejštejn, on stump of *Populus tremula*, 600 m, 6847; 29.5.1989, coll. J. H. (PRM 887021). – Brdy Mts., Distr. Plzeň, near the village Nové Mitrovice, in Chynínské polesí forest, on pebbles on the ground among roots of a fallen tree, with *Steinia geophana*, c. 660 m, 6448, 15.10.1997, coll. Š. Bayerová, det. J. K. (hb. Bayerová). – Ibid.: in forest on the N slope of the hill Nad Maráskem, 675 m, 6448, 16.10.1997, coll. and det. Š. Bayerová. (hb. Bayerová). – Northern Bohemia, České Středohoří Mts., between the village Velemín and Mt. Milešovka, by path, on pebbles and wood, 5449; 22.4.1995, coll. J. Liška and Z. Palice (hb. Palice). – Distr. Česká Lípa, Kravaře, on stone in a wall, 280 m, 5352; 26.9.1995, coll. J. Horáková, A. Vězda and Z. Palice (PRM). – Distr. Česká Lípa, Zahrádky, on sandstone pebbles among *Calluna vulgaris* shrubs, 5353; 2.1921, coll. J. Anders (PRM?, non vidimus!). – Distr. Česká Lípa, near Jestřebí, in peat bog, on wood, 270 m, 5453; 28.9.1995, coll. J. H. (PRM 890492). – Central Bohemia, Distr. Rakovník, Bedlno, on S slope of the hill Tobiášův vrch, on pebbles on the ground among roots of a fallen tree, 485 m s.m., 5846; 19.3.1997, coll. J. H. (PRM 890486). – Distr. Rakovník, Bedlno, on top of Tobiášův vrch hill, on a fallen trunk, 500 m, 5846; 19.3.1997, coll. J. H. (PRM 890487). – Distr. Rakovník, Bukov, foot of the hill Liščí vrch, on a gneissaceous boulder, 390 m, 5847; 1.5.1996, coll. J. H. (PRM 890488). – Distr. Rakovník, Přílepy, at a house, on old wooden beams, together with *Absconditella delutula*, 340 m, 5847; 3.9.1995, coll. J. H. and P. K. (PRM 887746, 887750). – Ibid.: 31.3.1997, coll. J. H. and P. K. (PRM 890824). – Distr. Rakovník, Kněževes, in garden, on stump of *Tilia* sp., 375 m, 5847; 8.4.1996, coll. J. H. and P. K. (PRM 891901). – Distr. Rakovník, near the railway station Mutějovice – Černá hora, on charred stump of *Fagus sylvatica* and on a dead basidiocarp of *Trametes versicolor*, ca 500 m, 5748; 27.1.1995, coll. J. H. (PRM 887751, 887024). – Distr. Rakovník, Kozojedy, Pochválovská stráň, on slope of the hill Dřevíč, on soil by forest track, on withered plant debris, 360 m, 5748; 17.3.1996, coll. J. H. and P. K. (PRM 887749). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, 1.5 km NE of the village Hracholusky, on bark of a stump of *Pinus sylvestris*, 370 m, 5748; 2.8.1997, coll. J. K. and P. K. (PRM 891898). – Distr. Příbram, Brdy Mts., near the village Teslíny, in cut down forest, on bark of fallen tree, 680–690 m, 6348; 15.10.1997, coll. and det. Š. Bayerová (hb. Bayerová). – Distr. Příbram, Brdy Mts., Radošice, on loose siliceous stone in meadow, 570 m, 6448; 15.2.1997, coll. Š. Bayerová and Z. Palice (hb. Palice). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, 1.5 km NE of the village Nezabudice, Nezabudické skály rocks, on stump of *Carpinus betulus*, 370 m, 5949; 13.4.1996, coll. J. H. (PRM 887767). – Prague, Šárka valley, Kozákova skála rock, on withered plant debris, 350 m, 5951; 28.9.1993, coll.

J. H. (PRM 887020, 890485). – Prague, Komořany, on the right bank of the Vltava river, near railway station, on bark of a fallen trunk of *Robinia pseudoacacia*, 190 m, 6052; 16.11.1996, coll. J. H. (PRM 890490). – Distr. Nymburk, Libice nad Cidlinou, flood plain forest Libický luh, at the NE border of the nature reserve, together with *Placynthiella icmalea,* about 190 m, 5857; 18.3.1995, coll. Z. Palice (hb. Palice). – Southern Bohemia, Šumava Mts., Distr. Prachatice, Volary, in the village Černý Kříž, at gamekeeper's lodge on top of wooden post of garden fence, 745 m, 7149; 25.9.1994, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, Volary, on the forest track Hučicská cesta, gravel pit, on "fresh" siliceous pebbles and stones, together with *Trapelia* sp., about 770 m, 7149; 29.12.1994, coll. Z. Palice (hb. Palice). – Novohradské hory Mts., Distr. Český Krumlov, 8 km SE of Benešov nad Černou, Žofín, on wooden fence, 750 m, 7354; 3.6.1997, coll. R. Dětinský, det. J. K. (hb. Dětinský). – Distr. Pelhřimov, above Krasoňov, on gneissaceous boulder in a meadow, 620 m, 6458; 9.5.1968, coll. A. Vězda (hb. Vězda). – Eastern Bohemia, Distr. Pardubice, Chvaletice, sedimentation basin near the power station, on a sandstone-shale boulder, 220 m, 5958; 14.10.1994, coll. Z. Soldán (PRC). – Krkonoše Mts., Pec pod Sněžkou, in the valley Obří důl, on stump of *Picea abies*, about 1000 m, 5260; 9.1991, coll. J. Horáková and J. Hubáčková (PRM). – Distr. Pardubice, Opatovice nad Labem, Bukovina nad Labem, sedimentation basin near the power station, on wood and on pebbles, 230 m, 5860; 10.3.1997, coll. and det. Z. Palice (hb. Palice).– Orlické hory Mts., Sedloňov, on wood, about 600 m, 5663; 5.1996, coll. J. Halda (hb. Halda). – Western Moravia, Distr. Jihlava, between Třešť and Horní Cerekev, near Rácov, on a gneissaceous boulder, c. 630 m, 6758; 15.10.1996, coll. J. Halda and B. Gruna (PRM 891437). – Ibid.: sub *Lecidea variegatula* Nyl., matrix: *Thelocarpon laureri*, 6758; 15.10.1996, coll. J. H., det. J. Hafellner (PRM 891436). – Distr. Třebíč, near Heraltice, on a gneissaceous boulder, about 600 m, 6760; 26.3.1929, coll. J. Suza (PRM 587598, 587601, 587602, 587603). – Distr. Třebíč, Vladislav, in forest, on stump of *Robinia pseudoacacia*, about 420 m, 6761; 6.6.1990, coll. E. Lisická (PRM 887025). – Distr. Třebíč, Rouchovany, between Boříkovský dvůr and the brook Mlýnský potok, at forest margin, on gneissaceous pebbles, 380 m, 6962; 14.9.1971, coll. A. Vězda (A. Vězda: Lichenes selecti exsiccati no. 1028; PRM 721825). – Distr. Blansko, Doubravník, in valley of the Svratka river near the village Prudká, about 300 m, 6564; 30.5.1975, coll. A. Vězda (hb. Vězda). – Distr. Žďár nad Sázavou, near Tišnov, between the villages Křížovice and Skorotice, on fence in a meadow, on wood, 500 m, 6564; 18.9.1977, coll. A. Vězda (A. Vězda: Lichenes selecti exsiccati no. 1640; PRM 820786, M 10631). – Distr. Blansko, Tišnov, in the village Borač, on fir wood of a fence at house no. 43, 400 m, 6564; 8.1928, coll. J. Suza (sub *T. prasinellum* – PRM 587577, 587580, 587581, 587583). – Distr. Blansko, near Tišnov, on Květnice hill, on a boulder, c. 450 m, 6664; 12.3.1919, coll. J. Suza. (sub *T. prasinellum* – PRM 587600). – Distr. Blansko, Tišnov, in the village Borač on wood of an old roof, 400 m, 6664; 1931, coll. J. Suza (J. Suza: Lichenes Bohemoslovakiae, no.164, sub *T. prasinellum*; hb. Vězda, M 10608, PRC). – Distr. Blansko, Tišnov, on wood of an old fence in the village Střemchoví, 300 m, 6664; 8.1929, coll. J. Suza (PRM 587576). – Northern Moravia, Silesia Superior, Rychlebské hory Mts., near Mt. Falkenberg (Sokolí vrch), in pine forest, on basalt stones, 5768; 1884, coll. J. Plosel jr., (PRM 587599, sub *T. epilithellum*). – Silesia Superior, Rychlebské hory Mts., near Mt. Falkenberg (Sokolí vrch), in pine forest, on basalt and granite stones, 5768; 1886, coll. J. Plosel jr., (H. Lojka: Lichenotheca Universalis, no. 197 sub *T. epilithellum*, PRM 587592, 587594, 587596). – Jeseníky Mts., Rýmařov, between Skřítek and Klepáčov, in drain by roadside, on stones, about 800 m, 5968; 3.10.1974, coll. A. Vězda (hb. Vězda). – Jeseníky Mts., in the valley Velká kotlina, on a gneissaceous boulder, 1300 m, 5969; 23.9.1994, coll. J. H. (PRM 887022). – Southern Moravia, Brno, at margin of forest near Mokrá Hora, on wooden fence, 250 m, 6765; 4.1962, coll. A. Vězda (hb. Vězda). – Brno, near Jundrov, in garden, on wood, 270 m, 6765; 3.1991, coll. A. Vězda (hb. Vězda). – Brno, Botanic Garden of Masaryk University, on wooden fence in Kotlářská ul. street, about 280–300 m, 6765; 3.8.1932, coll. J. Suza (sub *T. prasinellum* – PRM 587579). – Eastern Moravia, Hostýnské vrchy Mts., near Košovy-Věletín, among tributary of the Juhyně brook near the cottage "Zálesák", by forest track, on roots of stump of cf. *Alnus* sp., 460 m, 6672; 11.5.1995, coll. J. H. (PRM 890825). – Bílé Karpaty Mts., "Hutě", near Žitková, on wall, on sandstone, 7073; 10.7.1996, coll. B. Gruna and M. Hájek (hb. Gruna). – Beskydy Mts., below Mt. Ropička, on a wooden fence, 800 m, 6377; 10.1956, coll. A. Vězda (hb. Vězda).

Slovak Republic: Northern Slovakia, High Tatra Mts., Tatranská Lomnica, on balcony on the third floor of a hotel, on wood, about 890 m, 22.9.1993, coll. A. Vězda (PRM 890522). – The Low Tatra Mts., Liptovský Mikuláš, near Lazisko, at the gamekeeper's lodge Pod Dobákom, on wooden fence, 750 m, 11.7.1990, coll. J. H. (PRM 887023).

Thelocarpon lichenicola (Fuckel) Poelt et Hafellner (Figs 3, 19–22, 26)

The species is known from many localities in the Czech Republic, where it has been found from the lowlands to the mountains at altitudes between 230–1300 m above sea level. It is recorded for the first time from the Slovak Republic.

Although it is considered a mountain species, it has been found in several localities at altitudes between 230 and 450 m above sea level. All these finds were made at sites of inverse climate conditions mostly in close and deep valleys in the Křivoklátsko area, Hostýnské vrchy Mts. and Český ráj. The inversion of the open area around Jestřebí (230 m), characterized by numerous peat bogs, is also

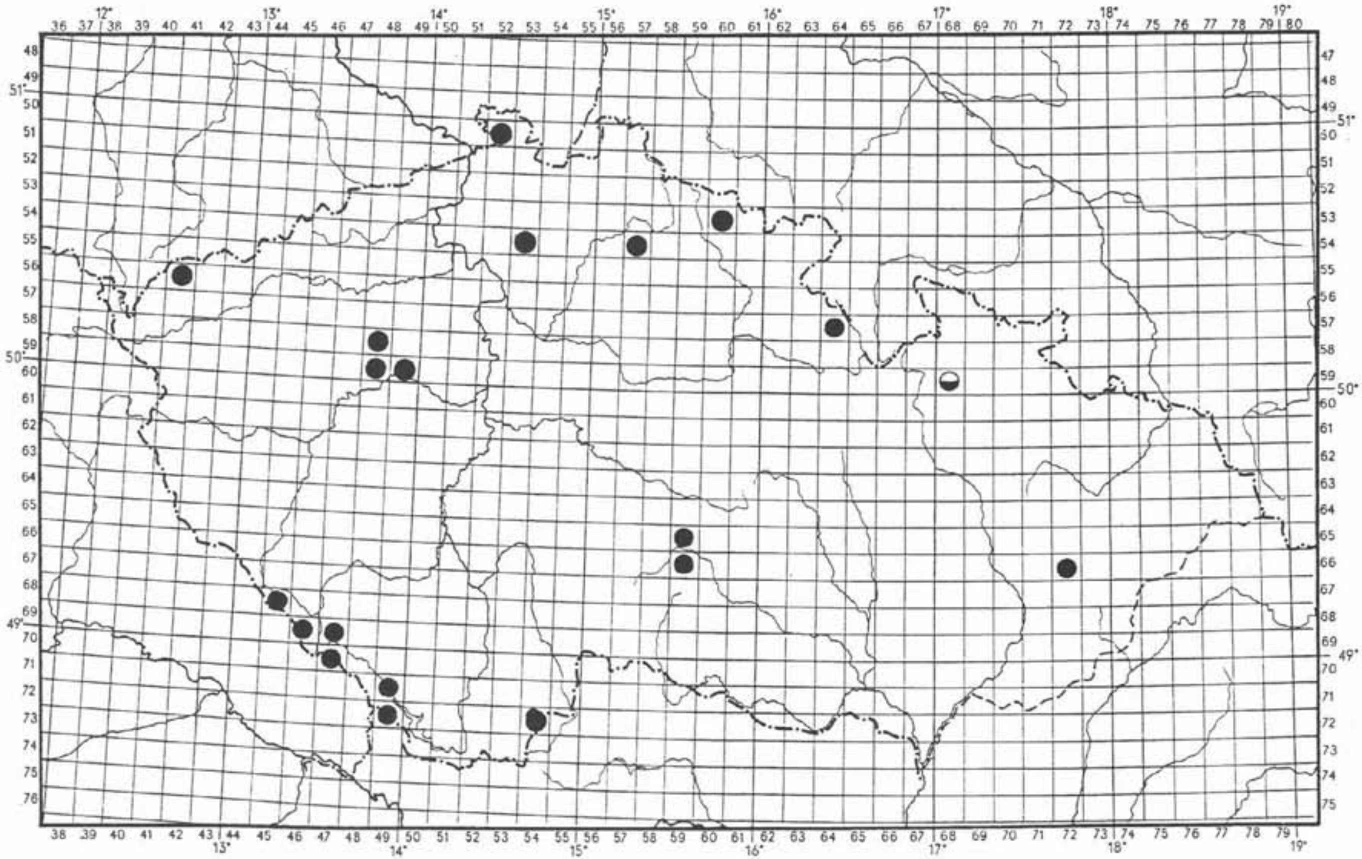


Fig. 26. Distribution of *Thelocarpon lichenicola* (Fuckel) Poelt et Hafellner in the Czech Republic.

Table 4.
Species of lichens and bryophytes accompanying *Thelocarpon lichenicola* according to substrata.

substrata	decaying wood	soil	saxicolous	Osmoporus odoratus	dead bryophytes	peat
lichens	*Absconditella lignicola* *Absconditella* sp. *Cladonia* sp. *Micarea prasina* *Placynthiella icmalea* *Steinia geophana* *Thelocarpon epibolum* *Vezdaea* sp.	*Baeomyces roseus* *Cladonia* sp. *Trapeliopsis granulosa*	cf. *Baeomyces rufus*	*Cladonia* sect. *cocciferae* *Micarea prasina*		*Placynthiella icmalea*
bryophytes	*Cephalozia bicuspidata* *Chiloscyphus profundus* *Hypnum cupressiforme* *Tetraphis pellucida*	*Atrichum undulatum* *Barbula unguiculata* *Ceratodon purpureus*	*Ceratodon purpureus* *Dicranella heteromalla*	*Chiloscyphus profundus* *Lophozia longiflora*	*Dicranum scoparium* *Sphagnum capillifolium*	*Cephalozia connivens* *Cephaloziella divaricata* *Chiloscyphus profundus* *Mylia anomala* *Sphagnum capillifolium* *Sphagnum flexuosum* *Sphagnum magellanicum*

confirmed by recent faunistic investigations and documented by many mountain species of spiders (Kůrka 1997) and insects (Honců and Vonička 1997).

Thelocarpon lichenicola occurs on decaying wood, rocks, stones or soils that are wet for a long time, on peat and dead bryophytes. It was also found on a dead basidiocarp of *Osmoporus odoratus*, on an old cup of acorn and lichenicolous on squamules of *Baeomyces rufus* in initial stage of ontogeny. As accompanied cryptogams the species listed in the Table 4 were found. Most often it was associated with *Absconditella lignicola, Placynthiella icmalea, Chiloscyphus profundus* (syn. *Lophocolea heterophylla*) and *Sphagnum capillifolium*.

Exsiccata examined:

Czech Republic: Lichenes Selecti Exsiccati no. 1400 (sub *Ahlesia strasseri* PRM 801993, hb. Vězda).

Additional specimens examined:

Czech Republic: Western Bohemia, Krušné hory Mts., Distr. Sokolov, Přebuz, on path leading to "Rolavské" peat bog, on loose siliceous stone, 5641; 920 m, 20.10.1997, coll. Z. Palice and P. Uhlík (hb. Uhlík). – Šumava Mts., Distr. Klatovy, Železná Ruda, in glacier cirque of lake Černé jezero, below of the rock wall, on a loose slimy stone, 1200–1250 m, 6845; 12.10.1995, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Klatovy, Železná Ruda, glacier cirque of the lake Černé jezero, peaty overhangs on the wall, 1200 m, 6845; 24.5.1996, coll. Z. Palice (hb. Palice, with pycnidia!). – Šumava Mts., Distr. Klatovy, in the peat bog Hůrecká slať, on *Sphagnum* sp., 875 m, 6845; 21.7.1996, coll. J. Váňa, det. J. H. (PRM 890828, with pycnidia!). – Šumava Mts., Distr. Klatovy, Modrava, Mt. Smrkový vrch, on decaying wood, 1100 m, 6946; 21.6.1995, coll. Z. Palice (hb. Palice). – Northern Bohemia, Protected Landscape Area Labské pískovce, Distr. Děčín, Kyjov, on sandy bank by roadside, on pebbles, 360 m, 5052; 11.8.1997, coll. J. K. (PRM 891413, 891414). – Distr. Česká Lípa, near Jestřebí, in peat bog, on decaying wood, 230 m, 5453; 28.9.1995, coll. J. H. (PRM 890829). – Central Bohemia, Distr. Rakovník, Olešná, in peat bog near the village, on decaying wood of fallen trunk of *Pinus silvestris*, together with *Absconditella lignicola*, 340 m, 5848; 14.2.1998, coll. J. K. (PRM 891904, with pycnidia!). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, Skryje, near confluence of the brooks Ostrovecký potok and Houpačkový potok, on fallen decaying trunk, together with *T. epibolum*, 6048; 449 m, 21.6.1997, coll. J. K. (PRM 890830, with pycnidia!). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, 2 km SW of Račice, on slope by road, on an old cup of acorn, 420 m, 5949; 10.1.1998, coll. J. K. and P. K. (PRM 891897, with pycnidia!). – Distr. Rakovník, Protected Landscape Area Křivoklátsko, nature reserve Stříbrný luh, below western slope of the hill Hák, in valley of small brook, on decaying trunk of *Fagus*, together with *Vezdaea* sp., 350–370 m, 5949; 21.2.1997, coll. J. K. and P. K. (PRM, with pycnidia!). – Southern Bohemia, Šumava Mts., Distr. Prachatice, Kvilda, in peat bog near the nature reserve Jezerní slať and the stream Hamerský potok, on gneissaceous pebbles, on poorly developed squamules of *Baeomyces rufus*, 1070 m, 6947; 2.10.1990, coll. J. H. (PRM 887026). – Šumava Mts., Distr. Prachatice, Kvilda, 1 km SE of the village, on the right bank of the Vltava river, on mosses on rocks, 1000 m, 6947; 13.6.1997, coll. and det. R. Dětinský (hb. Dětinský). – Ibid.: on *Sphagnum magellanicum*, 6947; 13.6.1997, coll. Š. Bayerová (hb. Bayerová, together with *T. epibolum* on squamules of *Omphalina hudsoniana*). – Šumava Mts., Distr. Prachatice, 1 km N of the source of the Vltava river, in peat bog, on peat, about 1140 m, 7047; 5.11.1994, coll. Z. Palice and Š. Bayerová, det. J. H. (hb. Palice). – Šumava Mts., Distr. Prachatice, Černý Kříž, in pine forest near railway station, on burned rotting wood, together with *Absconditella lignicola*, 740 m, 7149; 20.4.1995, coll. Z. Palice, det. J. H. (hb. Palice). – Šumava Mts., Distr. Prachatice, Černý Kříž, in pine forest by Hučina stream near railway station, on rotting wood, 745 m, 7149; 30.4.1995, coll. Z. Palice, det. J. H. (hb. Palice). – Šumava Mts., Distr. Prachatice, in valley of the Vltava river, in nature reserve Houska, near the railway station Ovesná, on dying bryophytes, 735 m, 7149; 13.6.1997, coll. and det. R. Dětinský (hb. Dětinský). – Šumava Mts., Distr. Prachatice, on slope of Mt. Medvěd, by forest track, on humus, 1120 m, 7249; 21.6.1995, coll. J. H. (PRM 887033). – Šumava Mts., Distr. Prachatice, near Nová Pec, in glacier cirque of lake Plešné jezero, on plant debris, 1200 m, 7249; 1.6.1996, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, near Nová Pec, in glacier cirque of lake Plešné jezero, on avalanche track, on humus, about 1250 m, 7249; 19.5.1995, coll. Z. Palice (hb. Palice). – Šumava Mts., Distr. Prachatice, Nová Pec, in glacier cirque of the lake Plešné jezero, on a dead basidiocarp of *Osmoporus odoratus*, about 1300 m, 7249; 16.6.1996, coll. Z. Palice (hb. Palice). – Novohradské hory Mts., Distr. České Budějovice, in the valley Liščí důl, c. 3 km of the Černé údolí valley, on humus, 680 m, 7254; 2.9.1997, coll. and det. R. Dětinský (hb. Dětinský, with pycnidia!). – Eastern Bohemia, Distr. Semily, Protected Landscape Area Český ráj, Hrubá Skála, in Čertoryje valley, on rotten wood, about 330 m, 5457; 26.2.1995, coll. Z. Palice and P. Špryňar (hb. Palice). – Krkonoše Mts., Distr. Trutnov, Velká Úpa, in the valley Vavřincův důl, on old bridge of wooden logs over the brook Vavřincův potok, on decaying wood among mosses, 755 m, 5360; 4.5.1997, coll. JK. (PRM 890832, 891905, with pycnidia!). Ibid.: by forest track, on fallen decaying trunk, 810 m, 5360; 4.5.1997, coll. J. K. (PRM 890831). – Orlické hory Mts., Distr. Rychnov nad Kněžnou, between Kačerov and Uhřínov pod Deštnou, in long roadcurve, at roadside on the ground, 575 m, 5764; 18.4.1996, coll. Z. Palice, J. Horáková and Š. Bayerová (PRM 887771, hb. Palice). – Western Moravia, Distr. Jihlava, on slope of the hill Vysoký Kámen, on pebbles on ground among roots of a fallen tree, 640 m, 6559; 14.10.1996, coll. P. K., det. J. H. (PRM 890483). – Ibid.: on a rotten fallen trunk together with *Placynthiella icmalea*, 655 m, 6559; 14.10.1996, coll. J. H. (PRM 890827). – Distr. Jihlava, foot of Špičák hill, near the nature reserve Loučky, on boulder by a forest track, 620 m, 6659; 16.10.1996, coll. Š. Bayerová (hb. Bayerová). – Northern Moravia, Jeseníky Mts., Distr. Šumperk, along road between the peat bogs Skřítek and Klepáčov, on humid mossy ground, 800 m, 5968; 3.10.1974, coll. A. Vězda, Lich. Sel. Exs. no. 1400 sub *Ahlesia strasseri* (Zahlbr.) Keissl. ex H. Magn. (PRM 801993, hb. Vězda). – Eastern Moravia, Hostýnské vrchy Mts., Distr. Kroměříž, below the nature reserve Smrdutá, on stump near the brook Bystřička, 660 m, 6672; 13.5.1995, coll. J. H. (PRM 887027, with pycnidia!). – Hostýnské vrchy Mts., near

Košovy-Věletín, along tributary of the Juhyně brook near cottage "Zálesák", by a forest track, on soil, 460 m, 6672; 11.5.1995, coll. J. H. (PRM 890826).

SLOVAK REPUBLIC: Western Slovakia, West Tatra Mts., Oravice, in the valley Juráňova dolina, on rotten trunk lying in a tributary of the brook Juráňový potok, 960 m, 6784; 29.5.1990, coll. J. H. (PRM 887031). – Northern Slovakia, Low Tatra Mts., in the valley Krížská dolina, on rotten stump, 1160 m, 13.7.1990, coll. J. H. (PRM 887032, 887034). – Carpathians, Muráňská planina plateau, Hrdzavá valley, peat bog "V machoch", on top of rotting stumps, 760–800 m, 22.9.1995, coll. Z. Palice and Š. Bayerová, det. Z. Palice (hb. Palice).

***Thelocarpon olivaceum** B. de Lesdain (Fig. 27)

This sparsely in Europe distributed species was discovered for the first time by Z. Palice in the Czech Republic in the autumn of 1996 in a sedimentation basin of a power station on a siliceous stone. Now it is reported from two localities. According to Salisbury (1966) and Alstrup and Schting (1989) the species is so far known from Austria, France, Germany, Great Britain, Switzerland and Denmark. It is reported predominantly from stones, rocks and old leather; one collection was made on a brick. All the Czech collections were made on stones.

substrata	saxicolous
lichens	*Cladonia* sp.
	Porpidia crustulosa
	Verrucaria sp.
	Xanthoria sp.

Table 5. Species of lichens accompanying *Thelocarpon olivaceum*, arranged according to substrata.

Exsiccata examined:

SWITZERLAND: H. Lojka: Lichenotheca Universalis no. 196. Mettmenstetten (Zürich). 1885, coll. Hegetschweiler (M 10571).

Zwackh: Lichenes exsiccati no. 869. Zwischen Riffersweil and Gossau (Zürich). 1873, coll. Hegetschweiler (M 10572).

GERMANY: F. Arnold: Lichenes exsiccati no. 1406. Bavaria, Pullach. 16.6.1888, coll. F. Arnold (M 10574).

Lichenes Monaccenses no. 261. Bavaria, Laufzorn near Munich. 6.12.1892, coll. F. Arnold (M 10575).

Additional specimens examined:

CZECH REPUBLIC: Central Bohemia, Brdy Mts., Distr. Příbram, on stony bank of the water basin Pilská, on stone, 660 m, 6349, 29.3.1997, coll. and det.

Š. Bayerová (hb. Bayerová). – Eastern Bohemia, valley of the river Labe, Chvaletice near Kolín, sedimentation basin near the power station, E of the village, on a loose siliceous stone, 220 m 5958; 18.10.1996, coll. Z. Palice (hb. Palice). – Ibid.: 14.2.1997, coll. Z. Palice and Š. Bayerová (hb. Palice).

***Thelocarpon pallidum** G. Salisb. (Fig. 27)

Until now the species had been considered endemic on British Isles. It is now also reported from the Czech Republic. No accompanying cryptogams were found in this specimen except of algal films. More detailed information about this collection will be published by the collector, Z. Palice (in prep.).

Specimen examined:

CZECH REPUBLIC: Northern Bohemia, Protected Landscape Area Kokořínsko, a former quarry "Újezd u Chcebuz", N of Újezd settlement, c. 1.5 km SE of Strachaly and 3 km NNE of Chcebuz, on loose stone on soil, 300 m, 5452; 27.11.1997, coll. Z. Palice (hb. Palice).

Additional specimen examined:

BRITISH ISLES: West Sussex, Coldwaltham, Waltham Brooks, 14.9.1978, coll. P. W. James (M).

Thelocarpon superellum Nyl. (Fig. 15–18, 27)

This species seems to be very rare because of the most conspicuous and the largest ascomata of all representatives of the genus. It had not so far been reported from the Czech Republic. Now it is known from three localities. Two specimens were collected on humid sandy soil in road verges, the third untypically on semi-immersed wood. In other countries the species is also known to occur on decaying algae on stones. The abundantly collected specimen in the locality in Northern Bohemia is prepared to be issued in Vězda's exsiccate collection Lichenes Rariores Exsiccati. It was collected there together with the above mentioned *T. lichenicola*, but this species was restricted only to siliceous pebbles. They were not found intermixed. Only accompanied bryophytes were found as listed.

substrata	rotten wood	soil
bryophytes	*Gymnocolea inflata*	*Dicranella rufescens*

Table 6. Species of bryophytes accompanying *Thelocarpon superellum*, according to substrata.

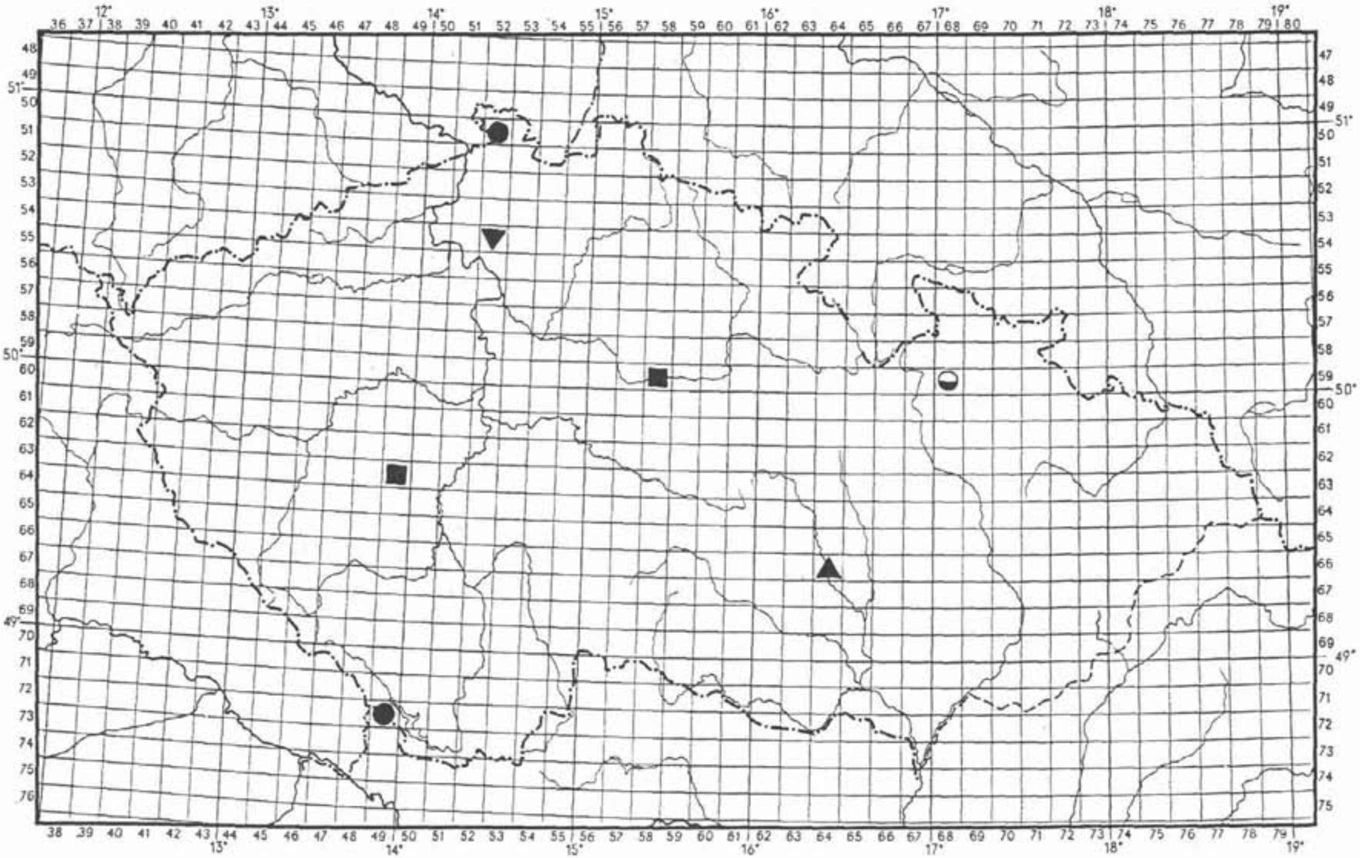


Fig. 27. Distribution of ▲ *Thelocarpon impressellum* Nyl., ■ *Thelocarpon olivaceum* B. de Lesd., ▼ *Thelocarpon pallidum* G. Salisbury and ● and ◒ *Thelocarpon superellum* Nyl. in the Czech Republic.

Specimens examined:

CZECH REPUBLIC: Northern Bohemia, Protected Landscape Area Labské pískovce, Kyjov, in denudated sandy roadverge, on humid soil, 360 m, 5052; 11.8.1997, coll. J. K. (PRM 891408, Vězda Lich. rar. exs. is prepared for distribution). – Southern Bohemia, Šumava Mts., Nová Pec, Plešné jezero lake, on bank beneath rock wall, on semi-immersed hard wood, 1090 m, 7249; 11.7.1997, coll. and det. Z. Palice (hb. Palice). – Northern Moravia, Jeseníky Mts., between the peat bogs Skřítek and Klepáčov, in roadverge, on humid soil, about 800 m, 5868; 3.10.1974, coll. A. Vězda (hb. Vězda).

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Screening for efficient organopollutant fungal degraders by decolorization

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Šašek V., Novotný Č. and Vampola P. (1998): Screening for efficient organopollutant fungal degraders by decolorization.– Czech Mycol. 50: 303–311

A set of cultures of wood-degrading Basidiomycetes was screened for the ability to decolorize model synthetic dyes with the aim of selecting strains with the highest activities of ligninolytic enzymes. Four decolorization patterns were observed; some species possessed no decolorizing ability, some decolorized on all the media, some decolorized only when fully grown, and only a part of them followed a typical behaviour described in *Phanerochaete chrysosporium* Burds., *i.e.* decolorized only on nutrient limited media. The strains with the highest decolorizing capabilities will be further studied with respect to biodegradation of aromatic organopollutants.

Key words: decolorization, synthetic dyes, ligninolytic enzymes, white rot fungi, biodegradation.

Šašek V., Novotný Č. a Vampola P. (1998): Použití dekolorizační metody pro testování kmenů hub aktivně degradujících organopolutanty. – Czech Mycol. 50: 303–311

Soubor kultur dřevokazných bazidiomycetů byl testován z hlediska jejich schopnosti odbarvovat modelová syntetická barviva s cílem vybrat kmeny s nejvyšší aktivitou ligninolytických enzymů. Byly pozorovány čtyři typy odbarvování; některé druhy neodbarvovaly vůbec, některé bez ohledu na použité medium, některé až po úplné kolonizaci agaru na misce, a pouze část druhů se projevovala způsobem, který je popsán u *Phanerochaete chrysosporium* Burds., tj. pouze na mediu s limitovaným obsahem živin. Kmeny s nejvyšší dekolorizační aktivitou budou dále využity při studiu biodegradace aromatických organopolutantů.

Polymeric dyes like Poly R-478, Poly B-411 or Poly Y-606, which are not taken up by cells, can serve as suitable substrates for the detection of some enzymatic components of the fungal ligninolytic system. Degradation of those dyes correlates with the start of the lignin metabolism in white rot fungi and probably reflects a combined effect of peroxidases and H_2O_2-producing oxidases (Glenn and Gold 1983, 1985, Kuwahara et al. 1984; Paszczynski et al. 1991). Therefore, the dye-decolorizing test started to be used as a possible, easily usable and inexpensive alternative to radiolabelled lignin as a substrate in lignin biodegradation studies.

Recent investigations have shown that the enzymes of the ligninolytic complex are not only capable of efficeint degradation of lignin but also (probably) take part in the biodegradation of various recalcitrant xenobiotics, whose removal from contaminated soil and water is very difficult (Hammel 1989, Field et al. 1993).

Some studies demonstrated a good correlation between biodegradation of aromatic pollutants and decolorization of polymeric dyes (Field et al. 1992, 1993) and, therefore, this quick screening method can be applied for the search of prospective fungal degraders that can be used in soil remediation processes.

In this study, 39 new strains of wood-degrading fungi isolated from fruit bodies and decayed wood collected in forests in central Europe were tested on their efficiency to decolorize Poly R-478 and Remazol Brilliant Blue R dyes under various nutrient conditions in a solid agar medium.

MATERIAL AND METHODS

List of fungal strains tested

The cultures used in the study were isolated either from decayed wood or from the fruit bodies collected and identified by P. Vampola in forests in the Czech Republic in 1993.

Aurantioporus croceus (Pers.: Fr.) Murrill, strain 422/93, collected 7. IX. 1993 on a lying trunk of *Quercus robur* in Náměšť nad Oslavou, a nature reserve near Třebíč.

Bjerkandera adusta (Willd.: Fr.) P. Karst, strain 606/93, collected 21. X. 1993 on a lying trunk of *Populus tremula*, marches of Soběslav, Komárov, district Tábor.

Ceriporia metamorphosa (Fuckel) Ryv. et Gilbn., strain 193/93, collected 19. VI. 1993 on a lying trunk of *Quercus robur,* Ranšpurk nature reserve, Lanžhot, district Břeclav.

Daedaleopsis confragosa (Bolt.: Fr.) Schroet., strain 491/93, collected 24. IX. 1993 on a lying branch of *Fraxinus excelsior*, Polom nature reserve near Chotěboř, Horní Bradlo.

Ganoderma applanatum (Pers.) Pat., strain 164/93, collected 8. VI. 1993 on a lying trunk of *Cerasus avium*, valley of the Výrovka river, Radim, district Kolín.

Ganoderma lucidum (W. Curt.: Fr.) P. Karst., strain 530/93. collected 6. X. 1993 on a dead root of *Quercus robur*, Ranšpurk nature reserve, Lanžhot, district Břeclav.

Grifola frondosa (Dicks.: Fr.) S. F. Gray, strain 534/93, collected 6. X. 1993 on the base of a dead trunk of *Quercus robur,* Ranšpurk nature reserve, Lanžhot, district Břeclav.

Hapalopilus nidulans (Fr.) P. Karst., strain 166/93, collected 8. VI. 1993 on a lying trunk of *Prunus padus*, valley of the Výrovka river, Radim, district Kolín.

Hymenochaete tabacina (Sow.: Fr.) Lév., strain 227/93, collected 6. VII. 1993 on a dead branch of *Ribes nigrum*, garden near the village Vysoká near Jihlava.

Inonotus nidus-pici Pil., strain 189/93, collected 18. VI. 1993 in cavity of a living trunk of *Quercus cerris,* Randezvous nature reserve, Valtice near Břeclav.

Inonotus obliquus (Pers.: Fr.) Pil., strain 615/93, collected 21. X. 1993 on a living trunk of *Betula pendula*, marshes of Soběslav, Komárov, district Tábor.

Inonotus radiatus (Sow.: Fr.) P. Karst., strain 402/93, collected 7. IX. 1993 on a lying branch of *Carpinus betulus,* nature preserve near Třebíč, Náměšť nad Oslavou.

Irpex lacteus (Fr.: Fr.) Fr., strain 617/93, collected 21. 10. 1993 on a lying trunk of *Populus tremula,* Komárov, marches of Soběslav, Komárov, district Tábor.

Mycoacia sp., strain 446/93, collected on a lying trunk of *Abies alba*, Žákova hora nature reserve, Cikháj near Žďár nad Sázavou.

Pachykytospora tuberculosa (Fr.) Kotl. et Pouz., strain 505/93, collected 25. IX. 1993 on a dead branch of *Quercus* sp., nature reserve near the village Hrádek, district Hradec Králové.

Phaeolus schweinitzii (Fr.) Pat., strain 261/93, collected 17. VII. 1993 on a living trunk of *Cerasus avium,* Maršov, alley located 11 km north-west of Jihlava.

Phellinus alni (Bond.) Parm., strain 16/93, collected 21. I. 1993 on a living trunk of *Alnus incana,* valley of the Rozkošský brook, Havlíčkův Brod.

Phellinus alni (Bond.) Parm., strain 28/93, collected 21. I. 1993 on a living trunk of *Sorbus aucuparia*, field alley near the village Věž, Bezděkov, district Havlíčkův Brod.

Phellinus contiguus (Fr.) Pat., strain 141/93, collected 8. V. 1993 on a lying branch of *Sambucus racemosa,* Bradlo forest, Horní Kosov near Jihlava.

Phellinus contiguus (Fr.) Pat., strain 427/93, collected 8. IX. 1993 on a living trunk of *Fraxinus excelsior,* nature reserve Zásmuky near Kolín.

Phellinus hartigii (Allesch. et Schnabl) Bond., strain 609/93, collected 21. X. 1993 on a stump of *Picea abies,* marches of Soběslav, Komárov, district Tábor.

Phellinus hartigii (Allesch. et Schnabl) Bond., strain 249/93, collected 10. VII. 1993 on a lying trunk of *Abies alba,* Kloc nature reserve, Třešť.

Phellinus ex aff. *igniarius* (L.: Fr.) Quél., strain 619/93, collected 21. X. 1993 on a dead trunk of *Salix cinerea,* marches of Soběslav, Komárov, district Tábor.

Phellinus igniarius (L.: Fr.) Quél., strain 138a/93, collected 7. V. 1993 on a living trunk of *Salix fragilis,* valley of the Žabinec brook, Petrkov near Havlíčkův Brod.

Phellinus nigricans (Fr.) P. Karst. *sensu* Černý 1989, strain 248/93, collected 10. VII. 1993 on a lying trunk of *Fagus sylvatica,* Kloc nature reserve, Třešť.

Phellinus pilatii Černý, strain 196a/93, collected 19. VI. 1993 on a lying branch of *Populus canescens,* Ranšpurk nature reserve, Lanžhot, district Břeclav.

Phellinus pini (Thore: Fr.) A. Ames, strain 614/93, collected 21. X. 1993 on a living trunk of *Pinus uncinata,* marshes of Soběslav, Komárov, district Tábor.

Phellinus pomaceus (Pers.) Maire, strain 47/93, collected 20. VI. 1993 on a dead branch of *Prunus domestica,* Zásmuky near Kolín.

Phellinus pseudopunctatus A. David, Dequatre et Fiasson, strain 538/93, collected 6. X. 1993 on a living trunk of *Carpinus betulus,* Cahnov nature reserve, Lanžhot, district Břeclav.

Phellinus punctatus (Fr.) Pil., strain 421/93, collected 7. IX. 1993 on a lying trunk of *Carpinus betulus,* nature reserve near Třebíč, Náměšť nad Oslavou.

Phellinus robustus (P. Karst.) Bourd. et Galz., strain 204/93, collected 17. VI. 1993 by L. Hagara on a lying trunk of *Quercus* sp., forest near Adamov motel, Kbely, district Senica.

Phellinus tremulae (Bond.) Bond. et Borissov in Bond., strain 209b/93, collected 23. VI. 1993 on a lying trunk of *Populus tremula,* scrub on the Lužný pond, Vysoká near Jihlava.

Pilatoporus ibericus (Melo et Ryv.) Kotl. et Pouz., strain 190/93, collected 19. VI. 1993 on a lying trunk of *Carpinus betulus,* Ranšpurk nature reserve, Lanžhot district Břeclav.

Pleurotus ostreatus (Jacq.: Fr.) Kumm., strain 670/93, collected 7. XI. 1993 on the base of a dead trunk of *Acer pseudoplatanus*, the so-called old park on the right bank of the Jihlava river, Jihlava – Staré Hory.

Spongipellis spumeus (Sowerby: Fr.) Pat., strain 453/93, collected on a living trunk of *Acer campestre,* exhibition ground, Brno-Pisárky.

Stereum rugosum (Pers.: Fr.) Fr., strain 210/93, collected on a dead trunk of *Corylus avellana*, scrub on bank of the Lužný pond, Vysoká near Jihlava.

Trametes versicolor (L.: Fr.) Pil., strain 167/93, collected on a lying trunk of *Cerasus avium* by M. Procházková, southern slope of Vrchy hill near Pelhřimov, Vyskytná.

Tyromyces chioneus (Fr.: Fr.) P. Karst., strain 616/93 on a lying branch of *Betula pendula,* marshes of Soběslav, Komárov, district Tábor.

Screening Method

Decolorization of Poly R-478 and Remazol brilliant blue R (RBBR) was estimated on agar plates where a plug cut out of a malt extract agar culture (5 g/l malt extract Oxoid, U. K.,10 g/l glucose, 20 g/l agar, pH 4.5) of the fungus tested was inserted in a well (1 cm diameter) cut in the agar medium containing 20 g/l agar and 200 mg/l poly R-478 dye or RBBR (both Sigma, USA) and incubated at 28 °C. The growth media employed are listed below. Formation and development of a decolorized zone were observed at regular time intervals to determine the period (in days) from the inoculation to the appearance of a decolorized zone and/or a complete decolorization of the agar medium.

In addition to the decolorization capacity, also the growth of strains on the agar media was also estimated by measuring the colony diameter on an agar plate.

The following growth media were used: a mineral, low nitrogen medium (NMM) according to Tien Kirk (1988), a malt extract/glucose medium (MEG) containing per litre 5 g malt extract (Oxoid, U. K.) and 10 g glucose, pH 4.5, and a complex, high nitrogen medium (YEPG) containing 10 g glucose, 5 g peptone, 2 g yeast extract (Difco, USA), 1 g KH_2PO_4 and 0.5 g $MgSO_4 . 7H_2O$ per litre, pH 4.5.

Results and Discussion

A set of 39 strains of wood destroying Basidiomycetes studied. With the exception of two species (*Phaeolus schweinitzii* and *Pilatoporus ibericus*) causing brown rot of wood, all other strains represented white rot fungi possessing the ability to degrade lignin efficiently. In all 39 strains the ability to decolorize Poly R-478 was screened and in 33 strains of them decolorization of RBBR as well.

The two dyes tested showed a similar decolorization pattern (*cf.* Tab. 1 and 2), even though they represent chemically different types (Poly R-478 is a polymeric anthrapyridone dye with a poly(vinilamine) sulfonate backbone, RBBR is a non-polymeric vinylsulfonyl anthraquinone dye). Brown rot species did not show any decolorizing ability nor did some white rot species (*Inonotus nidus-pici, I. radiatus, Phellinus tremulae*). With other white rot species the pronounced differences were observed. Several species (*Grifola frondosa, Phellinus hartigii, P. alni, P. nigricans,*) showed only a modest decolorizing ability. Using Poly R-478 the highest number of positive decolorizing strains was detected in the MEG medium, whereas using RBBR the highest decolorization scores were observed on NMM. With the YEPG medium, using peptone as the nitrogen source which is rich in nutrients, only several strains (*Bjerkandera adusta, Inonotus obliquus, Irpex lacteus, Mycoacia* sp., *Phellinus pseudopunctatus, Pleurotus ostreatus, Trametes versicolor*) gave positive results. These strains also effectively decolorized the dyes in the other two media. This observation suggested that those strains, contrary to *Phanerochaete chrysosporium*, did not need a nutrient limit to manifest their ligninolytic activities. The decolorization of RBBR on the YEPG medium caused by *Inonotus obliquus, Phellinus pseudopunctatus* and *Trametes versicolor* occurred separated from the development of the mycelium, i.e. these fungi first produced a dense and abundant mycelium that covered the agar surface, and only then a sudden decolorization of almost the entire agar layer took place. However, most strains (typically *Aurantioporus croceus, Ceriporia metamorphosa, Hapalopilus nidulans, Phellinus* ex aff. *igniarius, Stereum rugosum, Tyromyces chioneus*) followed the *Phanerochaete chrysosporium* pattern and decolorized only on low nutrient media. This observation indicates that the general condition causing the start of ligninolitic enzyme production triggered by nutrient limitation is widely spread but cannot be applied to all white rot fungal species.

Table 1
Decolorization of agar media containing Poly R-478 dye during growth of selected wood-rot fungal strains at 28 °C on three different media

Fungal strain	Medium					
	YEPG		MEG		NMM	
	A	B	A	B	A	B
Aurantioporus croceus 422/93		−(32)	+(10)	D(15)		−(22)
Bjerkandera adusta 606/93	+(4)	D(7)	+(4)	D(6)	+(3)	D(11)
Ceriporia metamorphosa 193/93		−(32)	+(4)	D(8)	+(7)	D(13)
Daedaleopsis confragosa 491/93	++(5)	D(7)	++(5)	D(7)		−(22)
Ganoderma applanatum 164/93		−(22)		−(22)		−(22)
Ganoderma lucidum 530/93		−(32)	+(6)	D(10)	+(8)	D(14)
Grifola frondosa 534/93		−(32)	++(11)	D(24)		−(22)
Hapalopilus nidulans 166/93		−(28)	+(11)	D(28)	+(7)	D(22)
Hymenochaete tabacina 227/93		−(32)		−(32)		−(22)
Inonotus nidus-pici 189/93		−(32)		−(28)		−(22)
Inonotus obliquus 615/93		−(32)	+(17)	++(19–32)		−(22)
Inonotus radiatus 402/93		−(32)		−(32)		−(22)
Irpex lacteus 617/93	+(4)	++(5–32)	+(5)	D(9)	+(2)	D(11)
Mycoacia sp. 446/93	+(9)	+(9–32)	+(8)	+++(21–32)	+(16)	+(19–22)
Pachykytospora tuberculosa 505/93	++(13)	++(13–32)	+(5)	D(13)		−(22)
Phaeolus schweinitzii 261/93		−(32)		−(32)		−(22)
Phellinus alni 16/93		−(32)	+(15)	++(17–32)		−(22)
Phellinus alni 28/93		−(22)	+(14)	+(14–22)		−(22)
Phellinus alni 97/93		−(22)		−(22)		−(22)
Phellinus contiguus 141/93		−(22)		−(22)		−(22)
Phellinus contiguus 427/93	+(7)	+(7–22)		−(22)		−(22)
Phellinus hartigii 609/93		−(22)		−(22)		−(22)
Phellinus hartigii 249/93		−(22)		−(22)		−(22)
Phellinus ex aff. igniarius 619/93		−(22)	+(9)	D(16)	±(9)	±(9–22)
Phellinus igniarius 138A/93		−(22)	+(5)	D(14)		−(22)
Phellinus nigricans 248/93		−(22)		−(22)	++(22)	++(22)
Phellinus pilatii 196A/93		−(22)	+(17)	+++(22)	+(8)	(19–22)
Phellinus pini 614/93		−(22)		−(22)	+(8)	D(19)
Phellinus pomaceus 47/93		−(22)	+(16)	+++(22)	+(6)	+(6–22)
Phellinus pseudopunctatus 538/93	+(7)	+++(11–22)	+(7)	D(11)	+(8)	(14)
Phellinus punctatus 421/93	+(9)	+++(22)	+(7)	D(16)	+(8)	D(14)

Fungal strain	Medium					
	YEPG		MEG		NMM	
	A	B	A	B	A	B
Phellinus robustus 204/93		–(22)		–(22)		–(22)
Phellinus tremulae 209b/93		–(22)		–(22)		–(22)
Pilatoporus ibericus 190/93		–(22)		–(22)		–(22)
Pleurotus ostreatus 670/93	+(8)	+++(21–32)	+(5)	D(19)	+(5)	D(9)
Spongipellis spumeus 453/93		–(32)		–(28)		–(22)
Stereum rugosum 210/93		–(32)	+(10)	D(13)	+(7)	+++(16–22)
Trametes versicolor 167/93	+(3)	D(6)	+(2)	D(6)	+(4)	++(7–22)
Tyromyces chloneus 616/93		–(22)	+(7)	D(16)	+(6)	D(14)

Comments to Table 1
Estimation of decolorizing activity: decolorization of 1/3 of the agar plate (+), of 2/3 (++), almost total decolorization (+++), complete decolorization without any shade of red or pink (D), no decolorization (–), only slight indication of decolorization (±).
Decolorization started (A) – the corresponding growth time (days) given in parentheses; maximal degree of decolorization observed (B) – the corresponding growth time (days) needed to reach the maximal degree of decolorization or the period from maximal decolorization until the end of the experiment without any further decolorization are given in parentheses.
YEPG – yeast extract/peptone/glucose medium;
MEG – malt extract/glucose medium;
NMM – nitrogen limited medium.
Brown rot species.

Based on this screening, the strains with the most powerful decolorizing capacities were selected and they will further be used in tests of degradation of polycyclic aromatic hydrocarbons. Synthetic dyes themselves, however, represent serious environmental pollution because of their xenobiotic character, toxicity, and the fact that they have been produced and used in great amounts, mainly in the textile industry. Therefore, there is great interest in decreasing the negative impact of synthetic dyes on the environment (see Banat et al. 1996 and references therein). In this respect, the selected species with highest decolorizing capabilities also represent prospective microorganisms to be applied for the remediation of effluents and soils polluted with synthetic dyes.

Acknowledgements

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Table 2
Decolorization of agar media containing RBBR dye during growth of selected wood-rot fungal strains at 28 °C on three different media

Fungal strain	Medium					
	YEPG		MEG		NMM	
	A	B	A	B	A	B
Aurantioporus croceus 422/93		–(20)		D(20)	+(3)	D(24)
Bjerkandera adusta 606/93	+(6)	D(9)	+(3)	D(9)	+(3)	D(17)
Cerioporia metamorphosa 193/93		–(20)	++(5)	D(7)	++(5)	D(7)
Daedaleopsis confragosa 491/93	±(4)	D(21)		–(27)		–(27)
Ganoderma lucidum 530/93	+(13)	D(21)	+(3)	D(14)	+(4)	D(17)
Grifola frondosa 534/93		–(20)		–(20)		–(27)
Hapalopilus nidulans 166/93		–(20)	+(5)	D(20)	+(3)	D(17)
Hymenochaete tabacina 227/93		–(20)	+(3)	+++(20)	+(3)	+++(20)
Inonotus nidus-pici 189/93	+++(19)	D(21)	+(3)	D(24)	+(3)	D(19)
Inonotus obliquus 615/93	D(14)	D(14)	+(3)	+++(20)	+(3)	D(20)
Inonotus radiatus 402/93		–(20)		–(20)		–(20)
Irpex lacteus 617/93	+(6)	D(9)	+(3)	D(7)	++(3)	D(7)
Mycoacia sp. 446/93	+(9)	D(14)	+(4)	D(14)	+(3)	D(14)
Pachykytospora tuberculosa 505/93		–(20)		–(20)	+(3)	+++(20)
Phaeolus schweinitzii 261/93		–(20)		–(20)		–(20)
Phellinus alni 16/93		–(20)		–(20)		–(20)
Phellinus alni 28/93		–(20)	+(14)	–(20)		–(20)
Phellinus alni 97/93		–(20)		–(20)		–(20)
Phellinus hartigii 609/93		–(20)		–(20)	+(3)	D(20)
Phellinus ex aff. igniarius 138A/93		–(27)		–(20)		–(27)
Phellinus igniarius 619/93		–(20)		–(20)	+(3)	D(24)
Phellinus nigricans 248/93		–(20)		–(20)		–(20)
Phellinus pilatii 196A/93		–(20)	+(3)	D(14)	+(3)	D(14)
Phellinus pini 614/93	±(17)	+++(20)	+(11)	+++(20)	+(3)	D(20)
Phellinus pomaceus 47/93		–(19)	+(4)	D(19)	++(6)	D(19)
Phellinus pseudopunctatus 538/93	+++(17)	D(19)	+(3)	D(14)	+(3)	D(14)
Phellinus punctatus 421/93	+++(14)	D(17)	+(6)	D(16)	+(3)	D(17)
Phellinus tremulae 209b/93		–(20)		–(20)		–(20)
Pilatoporus ibericus 190/93		–(20)		–(20)		–(20)
Pleurotus ostreatus 670/93	+(8)	D(10)	+(3)	D(9)	+(3)	D(20)
Stereum rugosum 210/93		–(20)	+(10)	D(16)	+(7)	D(20)
Trametes versicolor 167/93	+++(7)	D(10)	++(3)	D(7)	++(3)	D(10)
Tyromyces chioneus 616/93	+(13)	D(27)	+(3)	D(10)	+(3)	D(14)

Comments see Table 1

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Determination of toxigenic Fusarium spp. in the domestic wheat – using the ICFM methodological recommendation

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Ostrý V., Ruprich J. and Kožíšek J. (1998): Determination of toxigenic Fusarium spp. in the domestic wheat – using the ICFM methodological recommendation. – Czech Mycol. 50: 313–321

Fifty one food wheat samples from three production regions in the Czech Republic have been mycologically examined in this study. *Fusarium* species were isolated by the method of grain rinse with sterile 0.1 % pepton in water and by the method of direct plating of grains after their surface sterilization. Czapek Dox Iprodione Dichlorane Agar (CZID) was used for cultivation. The methodological procedures used issued from the recommendation of the International Commission of Food Mycology (ICFM). The identification of the isolated strains has been done according to Nelson et al. (1983) and by comparing with collection strains of the genus *Fusarium* (Czech Collection of Microorganisms in Brno – CCM). Standardization of the above-mentioned mycological methods in food mycology is necessary for making collaborative studies and also for possibilities of comparison of results obtained in different time. *Fusarium* spp. isolated from food wheat samples of showed the greatest frequency in the following order: *Fusarium graminearum, F. avenaceum, F. sporotrichioides, F. reticulatum and F. solani*. The methodological procedure is recommended for determination of toxigenic *Fusarium* spp. in wheat and other cereals after a harvest and for comparison of results obtained both between individual production regions and in individual years.

Key words: *Fusarium* spp., wheat, isolation, identification, mycotoxins, food mycology

Ostrý V., Ruprich J. a Kožíšek J. (1998): Stanovení toxinogenních mikroskopických hub rodu Fusarium v tuzemské pšenici – použití metodického doporučení ICFM. – Czech Mycol. 50: 313–321

V studii bylo mykologicky vyšetřeno 51 vzorků potravinářské pšenice ze tří produkčních oblastí v České republice na přítomnost toxinogenních fusárií. Druhy rodu *Fusarium* byly izolovány metodou oplachu zrn sterilní peptonovou vodou a metodou přímé kultivace zrn po jejich povrchové sterilizaci. Byla použita kultivační živná půda – Czapek Dox Iprodione Dichloran Agar (CZID). Použité metodické postupy vycházely z doporučení International Commision of Food Mycology (ICFM). Identifikace izolovaných kmenů byla provedena dle Nelsona et al. (1983) a porovnáním se sbírkovými kmeny rodu *Fusarium* z České sbírky mikroorganismů v Brně. Standardizace uvedených kultivačních metod v oblasti mykologie potravin je v současné době nezbytná pro provádění kolaborativních studií i možnosti srovnání získaných výsledků v různém čase. S největší frekvencí byly izolovány ve vzorcích potravinářské pšenice druhy rodu *Fusarium* v následujícím pořadí: *Fusarium graminearum, F. avenaceum, F. poae, F. sporotrichioides, F. reticulatum a F. solani*. Použitý metodický postup můžeme doporučit pro stanovení toxinogenních fusárií v pšenici a ostatních obilovinách po sklizni a pro srovnání získaných výsledků mezi jednotlivými produkčními oblastmi i v jednotlivých letech.

Introduction

Fusarium species are significant phytopathogens of cereals and other cultivated plants in our country (Šíp and Stuchlíková 1997), in the neighbouring countries (Mesterházy 1984, Chelkowski et al. 1984, Chelkowski et al. 1989, Rintelen 1985, Jimenez et al. 1993), and also in the world (Wilcoxson et al. 1988, Sayer and Lauren 1991). Many of them rank to the significant producers of mycotoxins (trichothecenes, zearalenone, moniliformin, fusarin C, fumonisins and others) (Ueno 1983, Chelkowski 1989, Miller et al.1991) which are hazardous for health of man, farm and wild animals.

From the historical point of view, there are many approaches of dividing the genus Fusarium into sections (groups), species and varieties. There is the conception of Wolenweber and Reinking (1935) with 26 species, through the approach of Snyder and Hansen (1945) with 9 species, Bilai (1955) with 26 species, Booth (1971a) with 44 species, Joffe (1974) with 33 species, Nelson et al. (1983) with 30 species, to Gerlach and Nirenberg (1982) with 78 species. The chemotaxonomic methods, which use dividing on the basis of detection and determination of secondary metabolites, have been developed and applied recently (Thrane 1990, Miller et al. 1991).

A qualitative and quantitative determination of the individual *Fusarium* spp. in cereals is made by means of procedures which are based on different methodics of mycological treatment of samples and on a type of culture medium used Wolenweber and Reinking (1935), Snyder and Hansen (1945), Bilai (1955), Booth (1971b), Joffe (1974), Nirenberg (1976), Gerlach and Nirenberg (1982), Nelson et al. (1983), Andrews and Pitt (1986) and Abildgren et al. (1987). Pitt et al. (1992) recommended methods for mycological examination of foodstuffs in food mycology.

The goal of this study was in particular testing of methodological approaches for isolation and determination toxigenic *Fusarium* spp. in food wheats according to the recommendation of the 1st – 3th International Workshops on Standardization of Methods for the Mycological Examination of Foods and the International Commission of Food Mycology (ICFM). These approaches can be used in practical activities of the National Reference Centre in evaluation of food safety of vegetable origin.

Material and Methods

A) Source of samples

In the course of August 1993 food wheat samples (*Triticum aestivum* L.) were taken in collaboration with the workers of purchasing and supplying agricultural

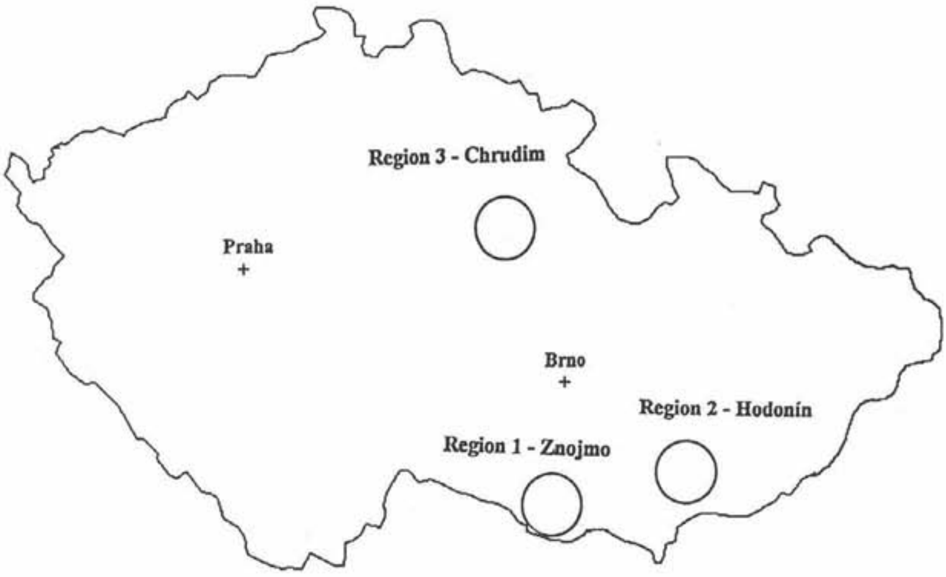

Fig. 1. Sampling regions

firms of shareholding companies a.s. Znojmo (18 samples), Hodonín (13 samples), and Chrudim (20 samples) (for localization see Fig. 1).

B) Mycological study

1. Determination of qualitative representation of *Fusarium* spp. in a rinse

Working procedure:

A rinse from the 30 g sample is performed with 270 ml sterile 0.1 % pepton in water in the 500 ml Erlenmeyer flask on a laboratory shaker for 5 minutes, and then the sample is let to stand for about 1 minute, and it will be used for other dilution. Samples were then serially diluted 1:10 (1+9). Spread plates are recommended over pour plates. Serially diluted samples (0.1 ml) were spread on CZID plates.

Culture medium used:

Czapek Dox Iprodione Dichlorane Agar (CZID) (Oxoid components), 20 ml per plate (Abildgren et al., 1987)

Incubation: 5 days at 25 °C in dark.

2. Determination of internal contamination of wheat grains with the *Fusarium* spp.

Working procedure:

100 wheat grains of each sample are surface-sterilized by immersion into 5 % NaClO (0.6 % chlorine) for the time of 2 minutes, and then it is rinsed three times with sterile distilled water. 100 surface – sterilized grains are placed on the Petri dishes (10 cm) with Czapek Dox Iprodione Dichlorane Agar per 10 grains on each Petri dish.

Incubation: 5 days at 25 °C in dark.

3. Identification of the isolated *Fusarium* spp.

Culture medium used:

(PDA) – Potato Dextrose agar with chloramfenicol selective supplement (Oxoid) (Booth 1971b)

(CLA) – Carnation-leaf agar (Fischer et al. 1982).

Young carnation (*Dianthus caryophyllus* L.) leaves are cultivated in laboratory conditions and are harvested from plants free from pesticide residues.

Identification of the isolates has been made according to Nelson et al. (1983) and by comparison with the collection strains *Fusarium* spp. of Czech Collection of Microorganisms in Brno (only supplemental method).

4. Statistical evaluation

A single-factor analysis of dispersion – ANOVA was applied with the use of the Borland Company software Quattro – Pro for Windows of the version 5.

Results and Discussion

Fig. 1 represents a percentual internal contamination by the representatives of *Fusarium* spp. in food wheat samples from three production regions. This indicator characterizes an extent of grain internal contamination. It is assumed that the samples of grain with a higher internal contamination by the representatives *Fusarium* spp. may contain also a higher contamination of wheat samples by *Fusarium* mycotoxins (Chelkowski 1989). In the testing food wheat samples from the 1987 harvest in the FRG it was determined internal contamination of grain in the range of 60–100 % in more than 80 % of the tested samples (Muller and Schwadorf 1993), and in testing of wheat samples from the 1987–1989 harvests in New Zealand it was determined internal contamination of grain in the range of 20–100 % in about 7 % of the tested samples (Sayer and Lauren 1991). The above-mentioned examples illustrate a considerable difference in the results achieved, which are dependent on the whole line of factors – climatic conditions, measures of farming technology, variety, region, etc. (Chelkowski 1989), but also

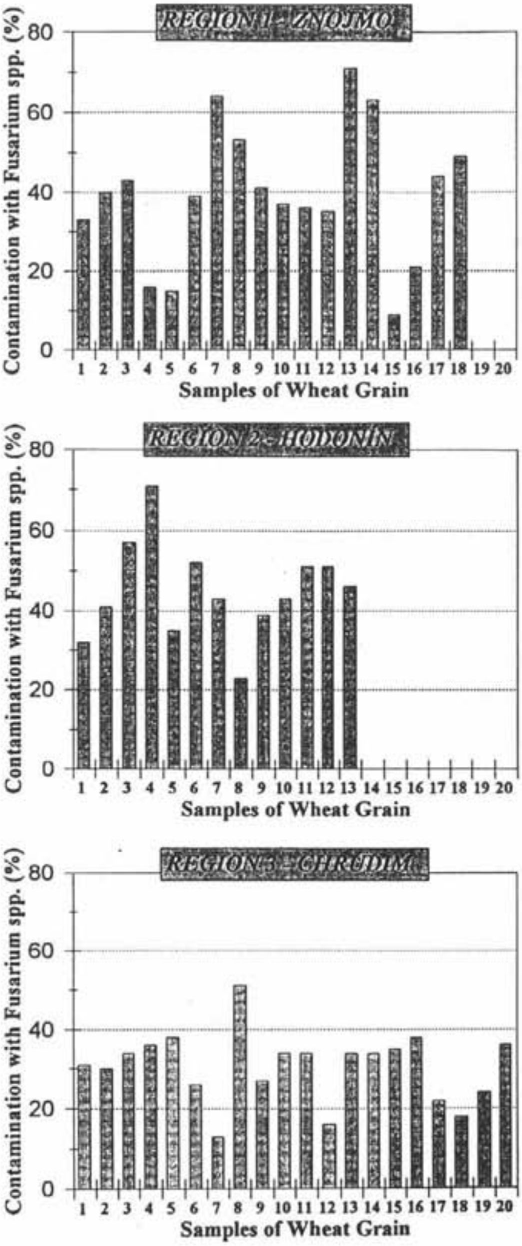

Fig. 1. Internal contamination of wheat grain with *Fusarium* spp.

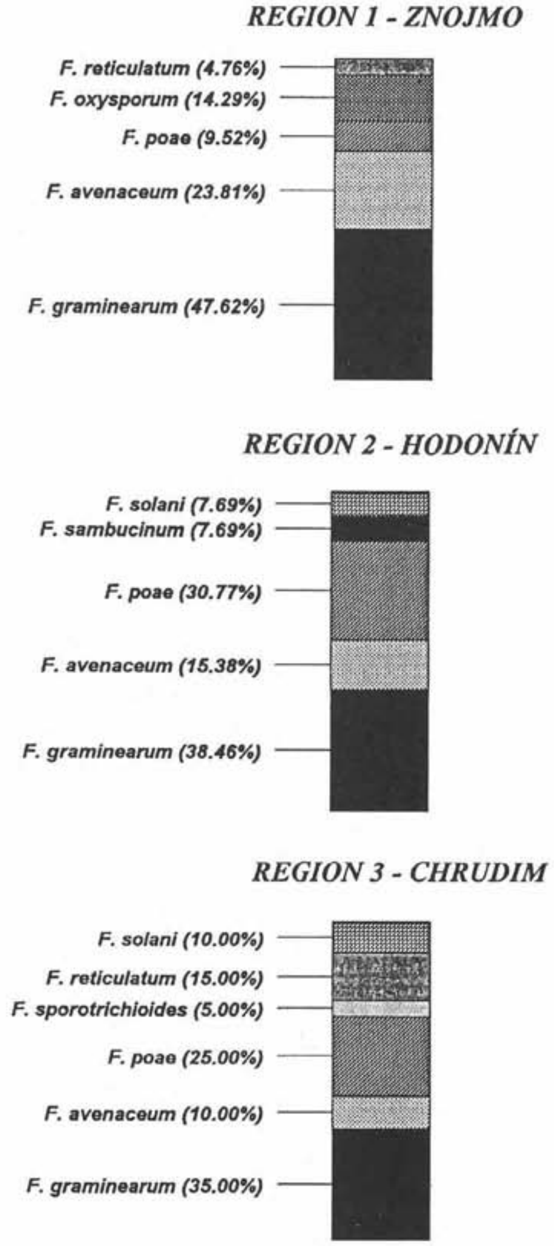

Fig. 2. Qualitative determination of *Fusarium* spp. from the surface of wheat grain from 3 regions

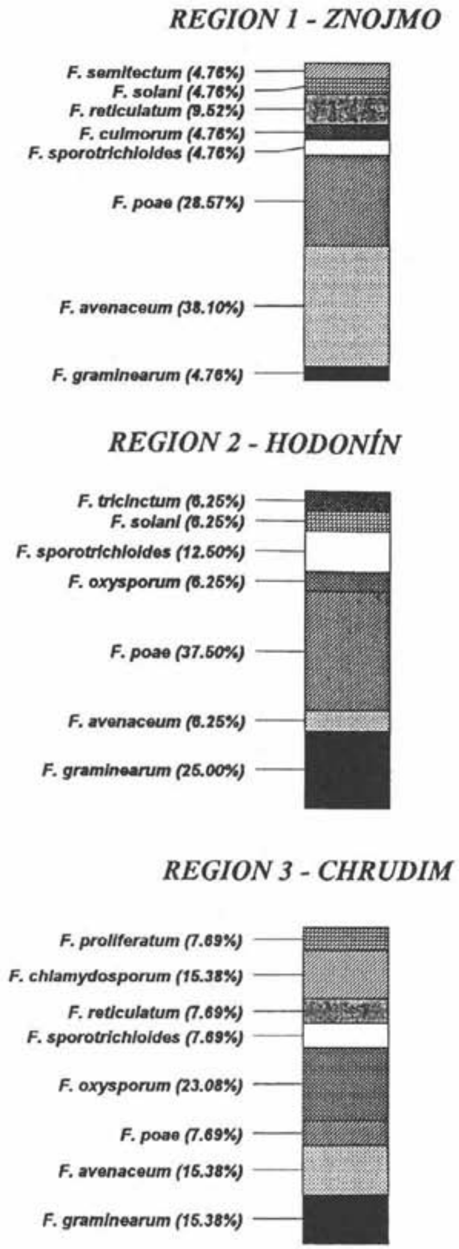

Fig. 3. Qualitative determination of *Fusarium* spp. after surface sterilization of wheat grain from 3 regions

on sampling and methodological approaches to determination of grain internal contamination, which may significantly affect a result of laboratory examination. Only for illustration it is stated that in the German treatise a 5 % solution of NaClO was used for surface sterilization for the time of 2 minutes, in the paper from New Zealand a 10 % solution of the preparation Janola (31.5 g NaClO for 1 l) was used for the time of 3 minutes; similarly, culture media were different as well. In our case, we have detected average % of grain internal contamination in food wheat in the region of Znojmo, which is 39.4 %, in the region of Hodonín 45 %, and in the region of Chrudim 30.6 %. With regard to a great dispersion of the results in individual sets, statistically significant differences between the regions were not, however, detected.

Fig. 2 shows a percentual representation of the *Fusarium* spp. from a rinse of food wheat samples from three production regions. These results are important mainly from the view of comparison of qualitative representation of the genus *Fusarium* in a rinse, and in grains after surface sterilization.

Fusarium spp. in internal contaminated food wheat grains from three production regions is presented in Fig. 3. A qualitative representation of the isolated *Fusarium* spp. is important especially from toxicological view (possible production of *Fusarium* mycotoxins) and the phytopathological view (pathogenicity and invasivity).

Fusarium spp. isolated from food wheat samples showed the greatest frequency in the following order: *Fusarium graminearum, F. avenaceum, F. poae*. The potential toxigenic *Fusarium* spp. can produce the next mycotoxins (Table 1).

In the qualitative mycological examination of the internal contamination of wheat grain from the 1990–91 harvests in Hungary the following *Fusarium* spp. were determined, including their percentage representation (the names are presented as they appear in the original publication): *Fusarium equiseti* (Corda) Sacc. (35 %) and *Fusarium poae* (Peck) Wollenw. (35 %), *Fusarium graminearum* Schwabe (11.7 %), *Fusarium sporotrichioides* Sherb. (11.7 %), *Fusarium semitectum* Berk. et Rav. (5.85 %), *Fusarium culmorum* Sacc. (5.36 %), the other isolated *Fusarium* spp. (less than 5 %) (Toth et al. 1993). In the previous study (Mesterházy 1984) the prevalent strains of *Fusarium graminearum* and *Fusarium culmorum* formed 85 % of all isolated strains of the genus *Fusarium*, while *Fusarium equiseti* and *Fusarium poae* occurred only sporadically. In the qualitative mycological examination of internal contamination of wheat grain in Austria (Adler et al. 1990) nine *Fusarium* species were isolated: *Fusarium culmorum, Fusarium avenaceum, Fusarium graminearum, Fusarium poae, Fusarium equiseti, Fusarium sambucinum, Fusarium nivale, Fusarium oxysporum* and *Fusarium solani*. In the qualitative mycological examination of internal contamination of wheat grain from the 1987 – 1989 harvests in New Zealand four species with the greatest frequency of occurrence were isolated: *Fusarium graminearum*

Table 1 The isolated *Fusarium* spp. and their potential mycotoxins.

Producers	Mycotoxins*
Fusarium graminearum	DON, 3-acetyl DON, NIV, DAS, ZEA, FUS C, FUS X, BUT
Fusarium avenaceum	MON, FUS C
Fusarium poae	T-2 toxin, FUS C, DAS, NIV
Fusarium sporotrichioides	T-2 toxin, DAS, MON, FUS C, ZEA
Fusarium culmorum	DON, NIV, ZEA, MON, FUS C, FUS X, 3-acetyl DON, BUT
Fusarium oxysporum	MON, FUS C, WORT, ISOVER, ZEA
Fusarium reticulatum	?
Fusarium solani	NTCH, FUS A, TRICH
Fusarium proliferatum	MON, FUM
Fusarium tricinctum	FUS C, BUT
Fusarium semitectum	FUSP, DEOFUSP
Fusarium sambucinum	FUS C, ZEA, TRICH, BUT
Fusarium chlamydosporum	?

(*El-Banna et al. 1984, Frisvad 1988, Frisvad and Thrane 1993, Marasas 1994)

3-acetyl DON	3 – acetyl deoxynivalenol	NIV	nivalenol
DON	deoxynivalenol	MON	moniliformin
DAS	diacetoxyscirpenol	FUM	fumonisins
FUS C	fusarin C	ZEA	zearalenon
FUS X	fusarenon X	BUT	butenolide
FUSP	fusapyrone	DEOFUSP	deoxyfusapyrone
FUS A	fusaric acid	TRICH	trichothecenes
WORT	wortmannin	ISOVER	isoverrucarol
NTCH	naphtochinones		

Fusarium avenaceum, Fusarium culmorum, and *Fusarium poae* (Sayer and Lauren, 1991).

A fundamental problem inherent in *Fusarium* identification is that members of the genus vary widely in morphological and nonmorphological characteristics, including virulence and toxigenity and these criteria are used in taxonomic systems. Nomenclatural problems dealing with synanamorphs and generic typification and neotypification of species are very often discussed, so identification of the isolates has been made according to Nelson et al. (1983) in this study (Windels 1991).

Quantitative and qualitative finds of *Fusarium* spp. in cereals are dependent on climatic conditions, measures of farming technology, variety, sampling, methodics of mycological treatment of samples, and type of culture medium used. A standardization of the aforesaid mycological methods and introducing the principles of the Quality Assessment / Quality Control (QA/QC) into a laboratory work also in the field of food mycology is necessary for making collaborative studies and for possibilities of testing capability of laboratories for food mycology.

The selective substrate for *Fusarium* spp. Czapek Dox Iprodione Dichlorane Agar (CZID) was developed for use in cereal industry. This tested culture medium and selected methodological procedure of surface sterilization of grains are according to our practical experience applicable for quantitative determination of the "field" microscopic fungi *Fusarium* spp. in wheat and other cereals after a harvest with a following cultivation of isolated strains on media – Potato Dextrose agar (PDA) and Carnation-leaf agar (CLA).

Interpretation of the results obtained in collaborative studies, and possibility of comparison of the results with other studies (e.g. with phytopathological ones) are bound to similar methodological approaches and use of the same culture media for cultivation.

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Book review

H. Hartleb, R. Heitefuss, H. H. Hoppe

Resistance of Crop Plants against Fungi

Gustav Fischer Verlag, Jena, 1997. 544 pages. ISBN 3–437–35338–1. Price 189,– DM.

Interactions between plants and fungi are a very complicated phenomenon which can be considered at different levels of biological organisation (molecular, physiological, cytological, anatomical, individual, population, communities). During the last two decades there is a considerably increasing interest in research in this area because of its theoretical and practical importance. Only strongly interdisciplinary research may lead to new substantial knowledge. This approach is broadly and exactly analysed in the reviewed book.

Authors of international reputation have participated in the preparation of various chapters of this book under the guidance of three editors, well-known German plant pathologists. The book contents are trying to follow a general scheme, from basic and theoretical to practical aspects of the resistance of plants against fungi. This viewpoint is used for the division of book into four main parts.

The book starts with an explanation of general principles of host-parasite interactions. In the first part, a basic explanation of fungal mechanisms of recognition and attack of plants is given. Enzyme secretion and the role of toxins in pathogenesis are broadly discussed.

In the second part, information on mechanisms of resistance is summarised. This very comprehensive part is divided into six chapters according to different resistance mechanisms (e.g. preformed structural and chemical barriers, cell wall modifications, hypersensitive reaction, elicitors and suppressors, phytoalexins, molecular and biochemical aspects of host-pathogen interactions).

The third part deals with special types of resistance and their modification. Durable resistance, adult plants resistance, induced resistance, abiotic and biotic impacts on resistance are discussed.

The final and the most extensive part deals with epidemiological aspects and strategies of utilizing resistant cultivars. This part is divided into seven chapters dealing with the influence of resistance on disease epidemics, gene management, host resistance diversity, virulence analysis of host pathogen populations, resistance assessment, utilisation of resistant cultivars. In the last and very valuable chapter recent relevant literature on sources of resistance in most important crop plants is listed. At the end of the book, is a very well elaborated index of organisms and subjects.

The topic, concept and contents of the book can be considered as an extraordinary interesting, comprehensive and up-to-date review of recent progress in the area of crop plant resistance to pathogenic fungi. Each chapter is followed by the most recent references. The text is enriched with a lot of very clear tables, figures and graphs. The reviewed book could be an important source of information for plant pathologists, mycologists, geneticists, plant breeders and all those interested in the current progress in host-parasite interactions research.

Aleš Lebeda

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Fig. 3. *Pholiota aurivella* (Batsch: Fr.) P. Kumm. Fruitbody with prominent dark scales.
– Czech Republic, Praha, Máslovická rokle valley, *Salix fragilis*, 12 Oct.1994, JH 340/94 (PRM). Microcharacters of this fruitbody are depicted in Fig. 1. Photo J. Holec.

Fig. 4. *Pholiota aurivella* (Batsch: Fr.) P. Kumm. Typical robust fruitbody with thick dark scales and dry mat pileus surface.
– Czech Republic, Praha-Suchdol, Tiché údolí, *Salix fragilis*, 23 Oct.1997, JH 888/97 (PRM). Photo J. Holec.

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Accredited with the International Association for Plant Taxonomy for the purpose of registration of new names of fungi.

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