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Fungal tolerance towards copper-based wood preservatives

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When wood preservatives against soft rot are used, metal tolerance ability becomes an important fungal peculiarity in the struggle for this ecological niche. Forty two fungal strains belonging to 27 different fungal species from 20 genera were isolated from decaying wood samples. It has been estimated that 81.0% of all strains studied show cellulase (endoglucanase activity) and 57.1% oxidase activity, which are important in assimilating an organic substratum such as wood. Organic acid production was characteristic of only 17.8% of the strains studied, and nearly all of them belonged to the genus *Penicillium*. Fungal tolerance to copper and resistance to copper-based wood preservatives was estimated. Only three of the strains studied (*Amorphotheca resinae* 0505, *Cladosporium sphaerospermum* 9–2, *Penicillium funiculosum* 0519) grew at 10 mM Cu^{2+}, but their growth was weak. The results showed that susceptibility of the test fungi to wood preservatives as well as their tolerance to copper ions were different and these properties didn't coincide. The response of strains estimated as the most copper-tolerant to various copper-based preservatives was different: the growth of *Amorphotheca resinae* 0505 was completely stopped by all the test preservatives, while *Cladosporium sphaeropermum* 9–2 was completely suppressed only by Impralit-CCO, as was also *Penicillium brevicompactum* H5–9.

Key words: soft rot fungi, copper tolerance, wood preservatives, cellulase, oxidase, medium acidification

INTRODUCTION

Microfungi can damage wood causing soft rot in natural environments or in wooden buildings both outdoors and indoors. The soft rot fungi *Ascomycetes* and *Deuteromyceetes* are not as active destructors of the lignin–cellulose complex as are *Basidiomycetes*. This kind of wood rot is distinguished by and is dangerous because it occurs not only in moist conditions, but also in comparatively dry sites. And the main thing is that it prevails both in wood with a high concentration of natural rot resistant compounds and in wood treated with various synthetic preservatives. Soft rot can happen in the sites that are too severe for white or brown rot fungi (too moist or too dry) and on substrata unfavorable for *Basidiomycetes* growth [1–6].

In wood preservation, attention is focused to white or brown rot fungi – macrofungi. The use of various chemical substances based on Ar, B, Cu, Cr, Hg or various phenol compounds successfully stops wood rot of this kind. Nevertheless, sometimes wood is damaged despite the application of chemical preservatives. The reason for this phenomenon appeared to be metal tolerance of the fungi. De Groot, Woodward [7] and Illman et al. [8] demonstrated that metal-tolerant macrofungi are capable of causing the rot of preservative-treated wood. There are data that microfungi could develop on treated wood, destroy toxic matters and neutralize their action [9]. Some species of microfungi were detected on wood treated with copper-based preservatives after its contact with soil [10]. Furthermore, facts are known when *Basidiomycetes* were more sensitive to preservatives than microfungi. Vasiliauskas et al. reported that chemical substances used for rot protection in natural environments stimulated stump colonization by *Ascomycetes* and *Deuteromycetes*, though decreased markedly the amount of *Zygomycetes* and almost completely eliminated *Basidiomycetes* [11].

Copper-based compounds have been among the main biocides of wood preservatives for nearly 200 years. In this period, wood decay fungi developed a mechanism that allowed them to survive in copper-treated wood. Fungi able to grow on the substrata with copper concentration higher than 1.6 mmo l^{-1} are considered to be copper-tolerant [12]. The phenomenon of copper tolerance has been known for more than 50 years [13], but the exact mechanism of copper tolerance and copper toxicity is not completely understood [9, 14–16].

Copper is a trace element necessary for fungal functioning, but it may be toxic at high concentrations [17]. Fungal tolerance towards copper and other heavy metals depends on many peculiarities of the organism defense mechanism, such as synthesis of metabolites able to bind metal ions, ability to absorb different amounts of metal ions into cell wall and to localize them in inner cellular structures, ion transport into cell, etc. [18–20].

Rabanus (1931) and Shimazono et Takubo (1952) suggested a hypothesis that copper tolerance of brown-rot fungi is linked to oxalic acid production, which presumably precipitates copper

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into a insoluble form of the oxalate, rendering the copper metabolite inert [21].

Metal tolerance of wood decay fungi rise new claims for synthetic preservative production and their efficiency evaluation, although this quality could be used for solving the ecological problem of the bioremediation of treated wood. In this field, some investigations have been carried out with *Basidiomycetes* [21–23].

The aim of the present investigation was to estimate the copper tolerance degree and resistance to copper-based preservatives among soft-rot fungi isolated from processed wood and to find out whether copper tolerance of fungal strains corresponds to their resistance to copper-based preservatives. The function of fungi in an ecological niche is defined by their physiology. For this reason, a primary cellulase and oxidase activity was investigated and the ability to produce organic acids was checked.

MATERIALS AND METHODS

Fungi were isolated from several sites: waste wood (wooden boards) kept outdoors, outdoor wall of a wooden house under damaged pluvial run; wooden floor and untreated wooden wall indoors; wooden support poles, loft balks and joists; wooden painted windowsill outdoors; decorative board indoors. Samples were taken from visually noticeable decay signs (foxy, grayed, softened, slivered, cracked or crumbly). They were washed under tap water, then with sterile water and sterilized by carrying through flame. Small pieces of wood samples (about $2 \times 2 \times 10$ mm) were placed on Czapek agar or malt extract medium with antibiotic addition in Petri dishes or kept in a moist chamber (99% air humidity) until fungal growth was noticed. The cultures isolated were purified and identified according to their macro- and micro-morphological features [24–28].

For cellulase (endoglucanase, CM-cellulase) activity estimation, fungi were cultivated on modified Czapek medium: $NaNO_3$ – 2 g; KH_2PO_4 – 1 g; $MgSO_4 \cdot 7H_2O$ – 0.5 g; KCl – 0.5 g; $FeSO_4$ – 0.01 g, carboxymetylcellulose (CMC) – 10 g; agar –20 g; H_2O – 1000ml, at 24 ± 2 °C for 3–5 days (depending on fungal growth rate). Endoglucanase activity was detected by flooding of a 0.1% aqueous solution of Congo Red and by a pale orange zone around the colony. The activity was evaluated by the width of this zone [29].

Oxidase activity was estimated according to the Baverdamm method. Fungal colonies were grown on a solid Czapek agar medium with addition of 0.2% of gallic acid. Oxidase activity was evaluated by the width of the brown zone around the colony [30].

Fungal ability to produce organic acids (to acidify the medium) was tested on a solid Czapek agar medium with 5% of glucose. A yellow zone around the fungal colony showed the presence of organic acids in the medium, and the broader zone width corresponded to the greater amount of acids [31].

The fungal sensitivity to Cu^{2+} was assessed by evaluating the colony growth on a solid Czapek agar medium containing different amounts of $CuCl_2 \cdot 2H_2O$: 0.2; 0.5; 1; 2; 3; 5; 7 and 10 mM. As a control, fungal strains were grown on a Czapek medium without copper. The diameter of fungal colonies was measured 7 days after cultivation at 26 ± 2 °C. Copper inhibitory action was expressed as a relative colony diameter (%) and calculated as d_2 / d_1 100%, where d_1 is the fungal colony diameter (mm) on the control medium, and d_2 is the fungal colony diameter (mm) on the test medium. All tests were carried out in three replications.

Six copper-based wood preservatives with different additives were used for the evaluation of fungal resistance to chemicals: Anti-grybas (Unicell, Poland); Asepas-3 (Retrorega, Lithuania); Boramon (Altax, Poland); Wood Preserver Clear (WPC) (Cuprinol Limited, Great Britain), Impralit-CCO and Impralit-KDS (Rütgers, Germany). The assay was carried out by the agar-diffusion method in Petri dishes (90 mm in diameter). The zone width (mm) of suppressed fungal growth revealed the fungicidal action of the test preservatives and fungal resistance to the chemicals [32].

RESULTS

Forty-two fungal strains belonging to 27 different fungal species from 20 genera were isolated from decaying wood samples (Table). Most of them were anamorphs, and telemorphs were identified only for some of them (*Chaetomium fusiforme*, *C. globosum*, *Eurotium herbariorum*). There were more isolates that could have telemorphs (e. g., *Sporotrichum* sp. – from *Basidiomycetes*). The largest part of the isolates (23.8%) were representatives of the genus *Penicillium* (though not all of them were classed to certain species). Four isolates were from the genus *Alternaria*. The genera *Aspergillus*, *Chaetomium* and *Scytalidium* were represented by three species, and the genera *Cladosporium*, *Oidiodendron*, *Phialophora* and *Trichoderma* by two species each.

Most of fungal species from the list presented in Table are mentioned in references as soft rot agents (*Alternaria alternata*, *Chaetomium globosum*, *Cladosporium sphaerospermum*, *Paecilomyces variotii*, *Phialophora malorum* and others) with cellulolytic or lignolytic properties. These fungi could be detected also in soil, on plant remnants and on decaying wood [27, 28, 33, 34]. Some fungal species isolated from wooden constructions were detected on untreated wood in natural conditions. Vasiliauskas et al. [6] from wood stumped in the forest isolated such fungal species as *Cladosporium herbarum*, *C. tenuissimum*, some representatives of the genera *Penicillium*, *Phoma* and *Phialophora*.

Soft rot is especially dangerous indoors because some of fungal species (e. g., *Cladosporium sphaerospermum*, *Ulocladium botrytis*, *Stachybotrys chartarum*, *Phoma*) could get into other substrata such as paints, wallpaper, gypsum boards and even plaster and cause much more damage [35].

The species composition of fungi isolated from wooden constructions is similar to that of wooden remnants in natural environments. However, some of them are considered not to be soft rot agents. For example, *Scopulariopsis candida*, *Torulomyces lagena* are usually isolated from soil and plant remnants. Nevertheless, *Torulomyces lagena* was detected on treated wood [28]. Besides, some more fungal species (*Alternaria alternata*, *Chaetomium globosum*, *Cladosporium sphaerospermum*, *Paecilomyces variotii*, *Phialophora malorum* and *Scytalidium lignicola*) isolated from samples in our study are mentioned among the species that could survive on treated wood [28]. *Cladosporium cladosporio-*

ides, *Paecilomyces variotii*, *Trichoderma harzianum* and *T. viride* were isolated during our previous investigations of treated wood in contact with soil [10].

These results force to turn more attention (during wood preservative efficiency evaluation) to *Alternaria alternata*, *Chaetomium globosum*, *Cladosporium cladosporioides*,

Table. **Fungal strains isolated from different sites and their oxidase activity, cellulase activity and organic acid production ability (– – negative reaction; + – coloured zone under the colony only; ++ – coloured zone up to 5 mm; +++ – coloured zone more than 5 mm); I – wooden floor of a first floor, Vilnius; II – waste wood (wooden boards) kept outdoors, Vilnius; III – wooden ceiling joist, Vilnius; IV – wooden decorative indoors boards, Vilnius; V – outdoor wall of a wooden house under damaged pluvial run, Vilnius; VI – wooden loft balk, Klaipėda; VII – wooden painted outdoor windowsill, Vilnius; VIII – wooden wall indoors, Vilnius; IX – wooden wall indoors, Trakai district; X – wooden support balk, Vilnius; XI – wooden decorative board in a cellar, Vilnius**

Fungus	Strain number	Isolation site	Oxidase activity	Cellulase activity	Organic acid production
Acremonium sp.	T–3	II	++	+	–
Alternaria alternata (Fr.) Keissl.	F 5–2	V	–	–	–
A. alternata (Fr.) Keissl.	H 4–6	IX	–	++	–
A. longipes (Ellis et Everch.) E. W. Mason	F6H–2	V	–	–	–
A. tenuissima (Kunze ex Pers.) Wiltshire	4 L	II	+	++	–
Amorphotheca resinae Parbery	0505	IX	+++	+++	–
Aspergillus candidus Link	3–2	X	++	+++	–
A. chevalieri (L. Mangin) Thom et Church	2–2	IX	–	++	–
Aspergillus sp.	3–4	X	++	+++	–
Chaetomium fusiforme Chivers	3–5	X	++	++	–
Ch. globosum Kunze	4–2	X	–	+	–
Ch. globosum Kunze	10–4	X	++	+	–
Cladosporium cladosporioides (Fresen.) G. A.	9–3	III	++	++	–
de Vries	9–2	III	++	+++	
C. sphaerospermum Penz.					
Eurotium herbariorum Link	1–4	X	++	+++	–
Humicola fuscoatra Traaen	F 9–3	VII	+++	++	–
Oidiodendron cerealis (Thüm.) G. L. Barron	7H	IV	+++	+++	–
Oidiodendron sp.	4–4	X	–	+++	–
Paecilomyces variotii Bainier	2–1	X	–	–	–
Penicillium brevicompactum Dierckx	H 5–9	IX	++	+++	++
P. funiculosum Thom	0519	VI	–	++	++
P. glabrum (Wehmer) Westling	T-2	I	–	–	+++
P. griseofulvum Dierckx	1–2	X	–	++	+
Penicillium sp.	T–1	I	+	–	+++
Penicillium sp.	2–1	IX	++	++	++
Penicillium sp.	H5–5	IX	+	++	++
Penicillium sp.	3–4	X	–	–	–
Penicillium sp.	1–1	X	–	++	–
Penicillium sp.	1–3	X	–	++	–
Phialophora malorum (Kidd et Beaumont)	F5H–1	V	++	++	–
McColloch					
Phialophora sp.	9	III	+	+	–
Phoma sp.	7–4	X	–	++	–
Scytalidium aurantiacum Klingström et L.	6–2	IV	+	+	–
Beyer					
S. lignicola Pesante	7–1	II	–	–	–
S. lignicola Pesante	F6–2	V	–	+	–
Scopulariopsis candida (Guég.) Vuill	6	X	++	++	–
Sporotrichum sp.	3–4	VI	+	+++	–
Stachybotrys chartarum (Ehrenb.ex Link) S.	F11–2	XI	++	–	–
Hughes					
Torulomyces lagena Delitsch	F2–1	VIII	++	++	–
Trichoderma harzianum Rifai	B5	IX	–	+	+
T. viride Pers.	4	IX	+	+	–
Ulocladium botrytis Preuss	3–3	VI	–	++	–

C. sphaerospermum, *Paecilomyces variotii*, *Phialophora malorum* and other fungi usual on wood in natural and man-made environments, especially on treated wood. In this sense, a great variety of fungal species being present on damaged wood, accumulation of ecological research data is very significant. It would allow generalizations and concentrate on certain fungal species during the search of efficient wood preservatives.

The physiological characteristics define fungal distribution on a substrate of one or another kind. Most of the fungal strains isolated from decaying wood showed cellulase (endoglucanase) (81.0% of all strains studied) and oxidase (57.1%) activity which is important in assimilating wood as an organic substratum (Table). The initial evaluation of cellulase activity showed that the *Amorphotheca resinae* 0505, *Aspergillus candidus* 3–2, *Aspergillus* sp. 3–4, *Cladosporium sphaerospermum* 9–2, *Eurotium herbariorum* 1–4, *Oidiodendron cerealis* 7H, *Oidiodendron* sp. 4–4, *Penicillium brevicompactum* H5–9 and *Sporotrichum* sp. 3–4 to be the most active cellulose destructors. Among them, only *Amorphotheca resinae* 0505 showed a high oxidase activity as compared with the other strains studied. Moreover, wide coloured zones indicating oxidase activity were formed by *Humicola fuscoatra* F9–3 and *Oidiodendron cerealis* 7H strains. Among strains of the same species, a different enzymatic activity was noted in all cases, although all of them were isolated from the same kind of substratum (wood). Cellulase activity was characteristic of *Alternaria alternata* H4–6 strain, while F5–2 strain of the same species didn't show this activity; cellulase and oxidase activity was characteristic of *Chaetomium globosum* 10–4 strain, while *C. globosum* 4–2 strain showed only cellulase activity.

Wildman [36] states that fungal populations occupying various ecological niches are more variable than those of a narrow occurrence. This conclusion was drawn from an investigation of secondary metabolite production by *Fusarium moniliforme* and *F. proliferatum strains*. So, in our case attention should be focused on fungal species isolated from decaying wood and often present on other substrata, such as paint, masonry, etc., i. e. to *Cladosporium sphaerospermum*, *Stachybotrys chartarum*, *Phoma* sp., *Ulocladium botrytis*. Therefore, studies of fungal physiological and ecological characteristics are necessary because their susceptibility to various antiseptics could be different, and its knowledge is important in selecting efficient wood preservatives.

When wood preservatives against soft rot are used, metal tolerance ability becomes an important fungal peculiarity in the struggle for this ecological niche. As mentioned above, this characteristic concerns fungal ability to produce organic acids. This ability was characteristic only of 17.8% of the strains studied, and nearly all of them belonged to the genus *Penicillium* (Table). *Penicillium glabrum* T–2 and *Penicillium* sp. T–1 acidified the medium most, but their enzymatic activity was hardly noticed (only a weak oxidase activity of *Penicillium* sp. T–1 was ascertained). These results indicate that the latter organic acid producers could be important in preservative suppression but not in the wood damage process.

For fungal copper tolerance studies, strains of different physiological features and from different taxonomic groups were chosen among them strains producing organic acids and showing or not cellulase and oxidase activity (*Penicillium brevicompactum* H5–9, *P. funiculosum* 0519, *P. griseofulvum* 1–2), showing cellulase and oxidase activity and belonging to *Dematiaceous* (*Amorphotheca resinae* 0505, *Cladosporium sphaerospermum* 9–2, *Humicola fuscoatra* F9–3, *Oidiodendron* sp. 7H, *Phialophora* sp. F5H–1) or *Ascomycetes* (*Chaetomium fusiforme* 3–5, *Eurotium herbariorum* 1–4) and *Sporotrichum* sp. 3–4 – anamorph of *Basidiomycetes*.

The fungal strains studied showed a different susceptibility to copper (Fig. 1). Low concentrations (0.2 or 0.5 mM Cu^{2+}) slightly suppressed the growth of most fungi and even remotely stimulated some of them (*Cladosporium sphaerospermum* 9–2, *Penicillium funiculosum* 0519). The copper concentration increasing in the medium, the fungal metal tolerance decreased gradually and at a concentration of 7–10 mM Cu^{2+} the greater part of the strains studied stopped growing at all. Nevertheless, some strains (*Penicillium funiculosum* 0519, *Amorphotheca resinae* 0505, *Cladosporium sphaerospermum* 9–2) developed in the medium even at a concentration of 10 mM Cu^{2+}. It should be noted that the growth of *Penicillium funiculosum* 0519 strain up to the 5 mM Cu^{2+} concentration was suppressed least. The high adaptive possibilities of this fungus to heavy metals present in their environment have been pointed out by other investigators as well [37].

A different sensibility of different strains of the same genus was noted. Three strains of the genus *Penicillium* responded differently to copper addition: *Penicillium funiculosum* 0519 was the most tolerant, *P. brevicompactum* H5–9 – very sensitive as its growth at 2 mM Cu^{2+} was only 11.8%, and addition of 3 mM Cu^{2+} inhibited completely its development, whereas *P. griseofulvum* 1–2 took an intermediate position – the concentration of 7 mM Cu^{2+} stopped completely its growth.

With increasing the concentration of copper ions, the tolerance of most fungi decreased gradually. Only the tolerance of *Chaetomium fusiforme* 3–5 strain dropped very sharply when the copper concentration reached 3 mM.

In regard to all fungi studied, copper slowed down the colony growth rate very distinctly. Sometimes another way of inhibition was noted as well. For example, when copper ion concentration reached 2 mM, the colony diameter of *Phialophora malorum* F5H–1 strain even increased a little, but the colony mycelium was very thin, the colony density was sparse and pigmentation changed unusually. A similar reaction was shown by *Sporotrichum* sp. 3–4 at 1–2 mM copper concentrations when fungal colonies were larger but their density was evidently sparser.

Up to 1 mM Cu^{2+} in the medium, the growth of most fungi was slightly suppressed. With the concentration increased to 2 mM, the growth of two fungal strains, *Penicillium brevicompactum* H5–9 and *Eurotium herbariorum* 1–4, was inhibited most markedly (11.8% and 10.3%, respectively). When the metal concentration was increased to 7 mM, most fungi didn't develop at all. Only three of the strains studied grew at 10 mM Cu^{2+}, but their growth was weak. Two of them belonged to Dematiaceous fungi (*Amorphotheca resinae* 0505, *Cladosporium sphaerospermum* 9–2), and one of them (*Penicillium funiculosum* 0519) was able to acidify the medium.

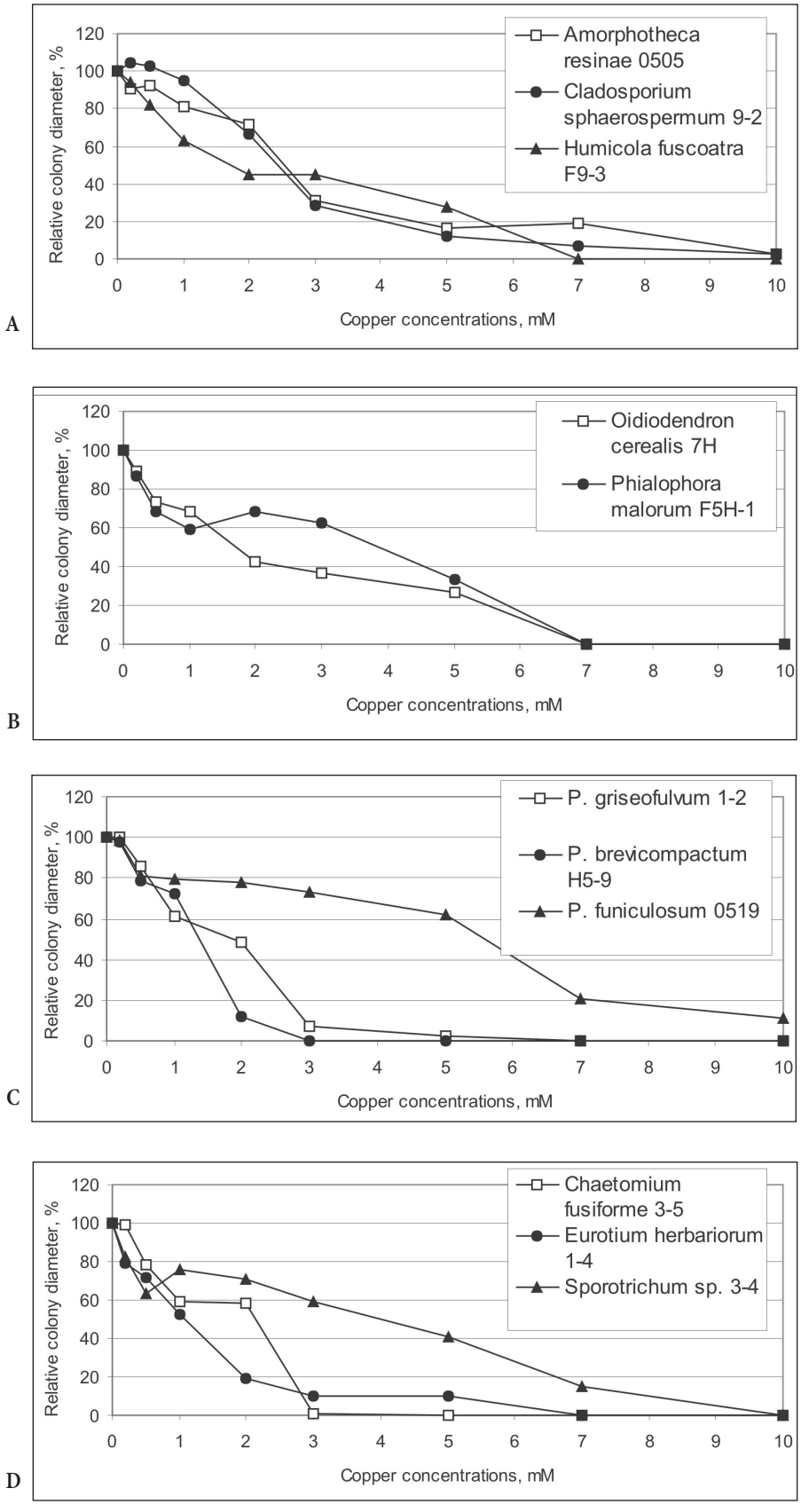

Fig. 1. Relative fungal colony diameter (%) on Czapek agar supplemented with Cu^{2+} at different concentrations: A – *Amorphotheca resinae* 0505, *Cladosporium sphaerospermum* 9–2 and *Humicola fuscoatra* F9–3; B – *Oidiodendron cerealis* 7H and *Phialophora malorum* F5H–1; C – *Penicillium griseofulvum* 1–2, *P. brevicompactum* H5–9 and *P. funiculosm* 0519; D – *Chaetomium fusiforme* 3–5, *Eurotium herbariorum* 1–4 and *Sporotrichum* sp. 3–4

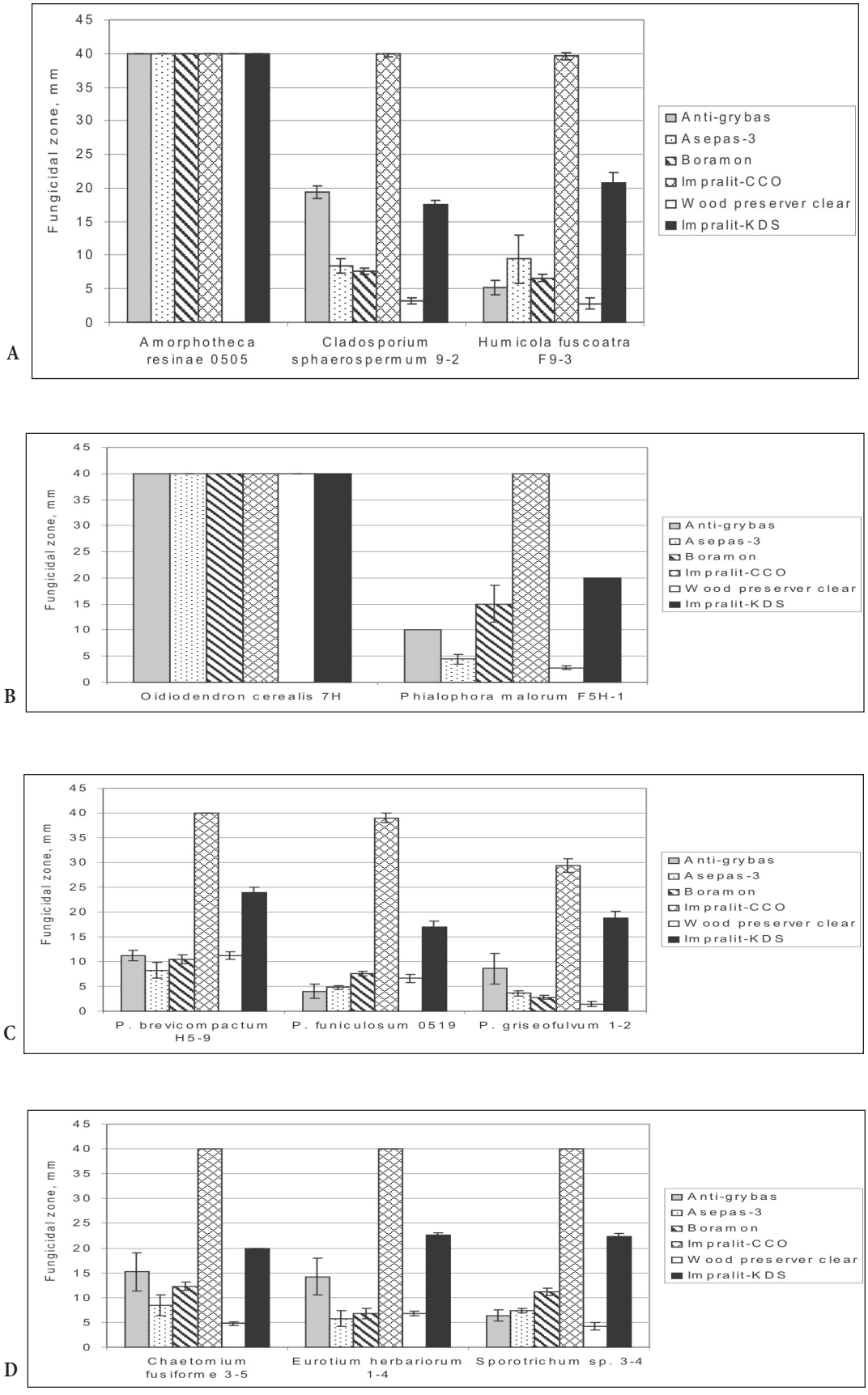

Fig. 2. Susceptibility of fungal strains to different wood preservatives: A – *Amrphotheca resinae* 0505, *Cladosporium sphaerospermum* 9–2 and *Humicola fuscoatra* F9–3; B – *Oidiodendron cerealis* 7H and *Phialophora malorum* F5H–1; C – *Penicillium brevicompactum* H5–9, *P. funiculosum* 0519 and *P. griseofulvum* 1–2; D – *Chaetomium fusiforme* 3–5, *Eurotium herbariorum* 1–4 and *Sporotrichum* sp. 3–4

The susceptibility of the fungi mentioned above to wood preservatives was different as was their tolerance to copper ions, but these properties didn't coincide. The most susceptible strains were the slow-growing (respectively 2–3 and 1–1.5 cm per 10 days) Dematiaceous fungi *Amorphotheca resinae* 0505 and *Oidiodendron* sp. 7H (Domsch et al., 1980), and neither of them produced organic acids in our study. Their growth in Petri dishes was completely stopped by all preservatives studied (Fig. 2) Nevertheless, *Amorphotheca resinae* 0505 developed on a solid medium even at 10 mM of Cu^{2+} concentration (Fig. 1). The other Dematiaceous fungi studied were most susceptible to Impralit-CCO and least to Wood Preserver Clear (Fig. 2). The three *Penicillium* strains studied did not only show cellulolase activity, but also produced organic acids, although their reaction to different preservatives differed. *Penicillium brevicompactum* H5–9 was most susceptible to all preservatives studied. *Penicillium funiculosum* 0519 was most resistant to Anti-grybas and Impralit-KDS (fungicidal zone respectively 4 ± 1.4 and 17 ± 1.2 mm), and *Penicillium griseofulvum* 1–2 strain was most resistant to Asepas-3, Boramon, Impralit-CCO and Wood Preserver Clear among all the *Penicillium* strains studied (fungicidal zone made up respectively 3.6 ± 0.5, 2.8 ± 0.4, 29.4 ± 1.3 and 1.4 ± 0.5 mm), although, according to our data, the latter fungus acidified the medium less than did *P. funiculosum* 0519 or *P. brevicompactum* H5–9.

The fungal strains from *Ascomycetes* and one strain (anamorph) of *Basidiomycetes* were most susceptible to Impralit-CCO and Impralit-KDS, as were also the other fungal strains studied, except *Cladosporium sphaerospermum* 9–2 which was most susceptible to Impralit-CCO and Anti-grybas (Fig. 2).

Fungal growth suppression (fungicidal effect) wasn't the only way of preservative action. Growth depression demonstrated by a weaker sporulation of *P. funiculosum* 0519 or *P. brevicompactum* H5–9 was noted under the effect of WPC and abundant cleistothecium formation by *Eurotium herbariorum* under the effect of Asepas-3 and Boramon.

The strains estimated as most copper-tolerant responded to various copper-based preservatives differently: the growth of *Amorphotheca resinae* 0505 was completely stopped by all the test preservatives, while *Cladosporium sphaeropermum* 9–2 was completely suppressed only by Impralit-CCO, as was also *Penicillium brevicompactum* H5–9.

In one case (e. g., *Penicillium funiculosum* 0519 produced organic acids, was resistant to copper ions and copper-based preservatives) our results supported the statement of Green III and Clausen [22] that fungal copper tolerance depends on acid production ability, although other results (*Cladosporium sphaerospermum* 9–2 didn't acidify the medium but was quite tolerant to copper and copper-based preservatives) insist on the idea that the ability to survive in a heavy metal environment depends on the other biological peculiarities of the organism and that there are different means and ways of fungal defense against heavy metals and other unfavourable extreme conditions.

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GRYBŲ ATSPARUMAS VARIO TURINTIEMS ANTISEPTIKAMS

S a n t r a u k a

Naudojant medienos antiseptikus prieš minkštąjį puvinį, grybų atsparumas metalams tampa svarbia savybe. Keturiasdešimt dvi grybų padermės, priklausančios 27 skirtingoms grybų rūšims iš 20 genčių, buvo išskirtos iš pūvančios medienos ėminių. Nustatyta, kad 81,0% visų tirtų padermių buvo būdingas celiuliazinis (endogliukonazinis) aktyvumas, o 57,1% tirtų padermių – oksidazinis aktyvumas; abu yra svarbūs organinio substrato – medienos – asimiliacijai. Organines rūgštis sintetino tik 17,8% tirtų padermių ir beveik visos jos priklausė *Penicillium* genčiai. Ištirtas grybų atsparumas variui ir vario turintiems antiseptikams. Tik trys tirtos padermės (*Amorphotheca resinae* 0505, *Cladosporium sphaerospermum* 9–2, *Penicillium funiculosum* 0519) augo esant 10 mM Cu^{2+} koncentracijai, tačiau jų vystymasis buvo silpnas. Rezultatai rodo, kad tirtų grybų jautrumas medžio antiseptikams buvo skirtingas, kaip ir jų atsparumas vario jonams, tačiau abi šios savybės nesutapo. Labiausiai vario jonams atsparios padermės skirtingai reagavo į medienos antiseptikus: visi tirti antiseptikai visiškai sustabdė *Amorphotheca resinae* 0505 augimą, tuo tarpu *Cladosporium sphaeropermum* 9–2 ir *Penicillium brevicompactum* H5–9 padermių augimą visiškai užslopino tik Impralit-CCO.