ISSN 1392–1320 MATERIALS SCIENCE (MEDŽIAGOTYRA). Vol. 9, No. 4. 2003

Evaluation of the Efficiency of Some on Offers Wood Preservatives

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Received 30 September 2003; accepted 18 October 2003

Recent investigations have shown microfungi to be prevalent in woods with high concentrations of naturally decay-resistant compounds and woods preserved with various chemicals. Microfungi may have metal leaching ability or be able of degrading a toxic compound into a less potent form, and in such a way neutralize wood preservatives. The efficiency of three wood preservatives (Asepas-1, Dispersan 1.1.1 and Arlits), containing copper salts, was tested in contact with agricultural and forest soil. Microbiological infection and weight loss of treated wood blocks were chosen as criterion for wood preservative efficiency evaluation. No representatives of *Basidiomycetes* that are responsible of wood major decay were isolated from treated samples even after 18 months. Nevertheless the infection of all treated wood blocks by microfungi was noticed and weight losses of wood samples were fixed: the efficiency of preservatives was depended on environment. The greatest weight loss among all tested preservatives in the agricultural soil was of Dispersan 1.1.1 treated wood (7.29 %) but of Arlits – in the forest soil (12.62 %). Species of the *Mucor* genus dominated on samples from forest soil and representatives of *Fusarium* genus were isolated from all treated wood blocks buried in agricultural soil after 18 months of burial.

Key words: wood, wood preservatives, preservative efficiency, soil fungi.

INTRODUCTION

An extremely wide variety of chemicals have been suggested as wood preservatives. Many of them were by-products of industrial processes. Coal-tar creosote has been used effectively for over 150 years. Early water-borne preservatives (metallic salts dissolved in water) were liable to be leached out if exposed to rain. The best of those developed recently, however, contain appreciable amount of alkaline chromate, which fixes other chemicals rendering them less liable to leaching. These copper-chrome-arsenic (CCA) mixtures have been shown highly effective against fungal decay in external structural timber. The salts most commonly used for treating green timber include borates, sodium fluoride, chrome and arsenic compounds. Copper sulphate is intended for use in the sap replacement. A variety of non-water-based solvents have been employed with quite a wide range of toxic compounds that provide protection usually against both fungi and insects [1].

Wood is light, strong, easy to work with and aesthetically pleasing, and as such it is still one of the most useful and most commonly used structural materials available. In spite of creations of more and more new wood preservatives, the desirable result not always could be achieved. Recent investigations have shown microfungi to be prevalent in woods with high concentrations of naturally decay-resistant compounds and woods preserved with various chemicals. Often microfungi that have wide adaptation ability could be the reason of wood damage and cause, so called, soft-rot. These microorganisms could be more tolerant to various chemical compounds than *Basidiomycetes* – main wood-destroyers. Besides they may have metal leaching ability or be able of degrading a toxic compound into a less potent form, and in such a way neutralize wood preservatives. Studies of fungal growth on media with metal ions showed that they could be accumulated in fungal hyphae or conidia. It was estimated that their adaptation associated with induction of new enzymatic systems [2, 3].

In recent years cases were stated when CCA have been used as preservatives but have been associated with soft-rot problems in hardwood in Australia and *Aureobasidium pullulans, Phialophora spp.* have been able to detoxify TnBTO (tri-n-butyl-tin oxide), *Penicillium roquefortii* reduced the efficiency of phenyl mercury acetate by absorbing biologically active mercury [1, 3]. Metal-tolerant or metal leaching fungi might have utility in bioremediation of treated wood after it is withdrawn from service. In this field laboratory studies were fulfilled with brown- and white-rot wood decay fungi and bacteria [4 – 6].

The fungi themselves damage the structure of wood slightly in comparison with *Basidiomycetes* causing brown or white rot. Fungi causing soft-rot grow exclusively in the cellulose part of wood cell wall known as S2 layer and utilize it. Furthermore some of them could decompose lignin and keratin [7].

Particularly great risk of wood damage is in contact with soil because of especially great fungal species variety here.

The aim of the present work was to evaluate the efficiency of three on offers wood preservatives "Asepas-1", "Dispersan 1.1.1" and "Arlits" under natural conditions, when treated wood was in contact with soil, and determine the fungi participating in treated wood damage.

MATERIALS AND METHODS

Three water soluble wood preservatives of different destination and chemical composition on offers were chosen for the experiment: Asepas-1 used for wooden

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constructions protection against wood decay indoors and outdoors, Dispersan 1.1.1 – for wood, concrete, bricks and panels protection against moulds indoors and Arlits – for wood protection against decay outdoors. Copper salt was active matter of these preservatives.

Two sites of different ecological conditions for the experiment were chosen: agricultural soil and forest soil. Data of the environmental conditions were collected before experiment at each of the sites. Soil agrochemical characteristics were determined according to standard methods. Fungal infection was determined applying the dilution method by sowing soil suspension in 5 replications on the surface of standard media. The colony forming units (cfu) of fungi were calculated in a gram of dry soil. Fungal species were identified according to their cultural and morphological characteristics [8].

Microbiological infection and weight loss of treated wood blocks were chosen as criterion for wood preservative evaluation. Test was performed according to modified ENV 807:1993 method [9]. Blocks made of pine (*Pinus sivestris)* wood (10×20×20 mm) were treated with the preservatives according to the use instruction and buried at the depth of 20 cm and in a distance of 10 cm from one another in the agricultural and forest soils. Untreated wood blocks were used for the control. Periodically (after 6, 12, and 18 months) 3 replicate samples of each variant for microbiological studies (fungal infection tests) and 3 for weight loss were removed from the burial sites, brushed free of mycelium, washed first with tap water then with sterile distil water. The blocks washed were kept in damp chamber for 10 days in order to clarify the infection of samples by fungi. After visual examination, fungi were directly sowed from the surface onto the malt extract medium for microfungi and selective medium for *Basidiomycetes*: malt extract – 3 g, peptone – 0.5 g, 2.5 g o-phenylphenol – 0.006 g and distil water – 100 ml. Samples for weight loss test were brushed dried to constant weight and weight losses determined as percentage of initial weight.

RESULTS AND DISCUSSIONS

The examination of the environmental conditions of agricultural and forest sites before experiment showed different agricultural indicators (Table 1). The forest soil had slightly acid (pH_{KCl} – 5.04) environment that was favorable for fungal growth. The greater amount of humus, main nutrient elements such as phosphorus and potassium in the forest soil stimulate the development of microorganisms as well. Soil humidity is very important for fungal spores germination and mycelium growth. At the experiment initiation time it was greater in forest than in agricultural soil (29.4 and 13.6 better in the forest soil. The amount of fungal propagules % respectively). The amount of colony forming unites in 1 g of dry soil (cfu/g) confirmed that conditions for fungal development were was about 4 times higher here than in agricultural, though the number of different fungal species was nearly the same (15 different species – in agricultural soil and 16 – in forest soil). The analysis of species composition elucidated the differences of two sites in microbiological infection (Table 2).

Table 1. Environmental conditions of two burial sites

Variable	Sites	
	Agricultural	Forest
pH_{KCl}	6.75	5.04
Total N, %	0.133	0.425
Mobile P_2O_5, mg/kg	145.3	623.9
Mobile K_2O, mg/kg	173.5	165.0
Humus, %	2.38	7.74
Humidity, %	13.6	29.4
Amount of fungal propagules, cfu/g	$11.95 \cdot 10^4$	$47.21 \cdot 10^4$
Number of fungal species identified	15	16

Table 2. Fungi isolated from different burial sites

Fungal species	Sites	
	Agricultural	Forest
Acremonium strictum W. Gams	+	+
Aspergillus penicilloides Speg.		+
Aspergillus spinulosus Warcup		+
Chrysosporium inops J.W. Carmich	+	+
Fusarium moniliforme J.Sheld	+	
Fusarium oxysporum Schltdl	+	
Gliocladium catenulatum J.C.Gilman et E.V. Abbott		+
Mortierella alpina Peyronel	+	
Mortierella humicola Oudem		+
Mortierella hyalina (harz) W. Gams	+	
Mortierella isabellina Oudem.		+
Mortierella lignicola Dixon-Stew.		+
Mucor circinelloides Tiegh.		+
Mucor hiemalis Wehmer		+
Oidiodendron echinulatum G.L. Barron	+	
Penicillium canescens Sopp	+	+
Penicillium decumbens Thom		+
Penicillium fellutanum Biourge	+	
Penicillium funiculosum Thom	+	
Penicillium godlewskii K.M. Zalessky	+	+
Penicillium lilacinum Thom	+	
Penicillium nalgiovense Laxa	+	
Sepedonium chrysosporium (Bull.) Link ex Fr.		+
Trichoderma harzianum Rifai	+	
Trichoderma koningii Oudem.		+
Verticillium album (Preuss) Pidopl.	+	+
Mycelia sterilia	+	+

Some species from *Acremonium, Mortierella, Penicillium, Trichoderma* and *Verticillium* genera were detected in both experimental sites. Fungi from *Chrysosporium, Fusarium* and *Oidiodendron* genera were characteristic only for the studied agricultural soil and representatives from *Aspergillus, Gliocladium, Mucor* and *Sepedonium* – only for the studied forest site. All of them

are known to be potential cellulose and lignin (in less degree) decomposers and in this sense they could be dangerous for wood blocks [10]. *Mycelia sterilia* was detected in both variants. Its systematic position wasn't defined because of unformed conidia.

The evaluation of two burial sites showed that wood blocks have been exposed at the different ecological conditions. It was likely that forest site could be defined as more aggressive for wood blocks because of environmental conditions more favorable for fungal development and greater amount of fungal propagules.

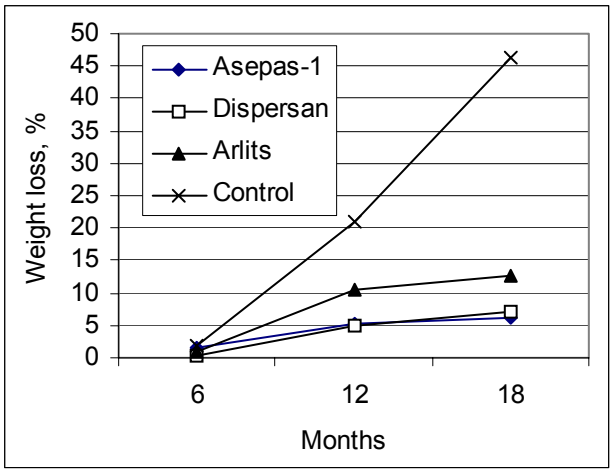

Fig. 1. Weight loss (%) of wood blocks treated with wood preservatives Asepas-1, Dispersan 1.1.1 and Arlits after 6, 12 and 18 months burial in forest soil

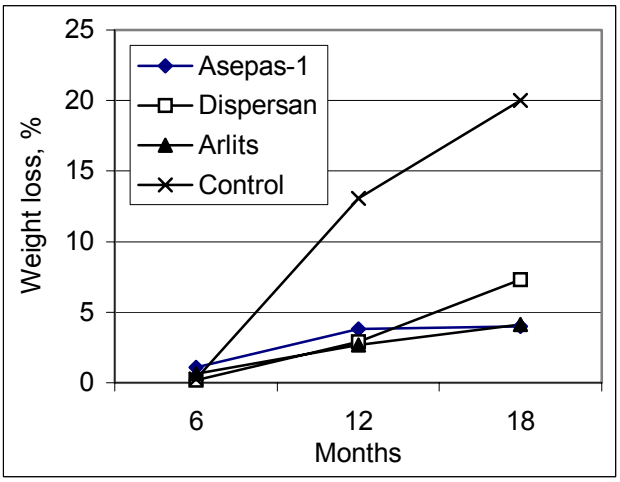

Fig. 2. Weight loss (%) of wood blocks treated with wood preservatives Asepas-1, Dispersan 1.1.1 and Arlits after 6, 12 and 18 months burial in agricultural soil

These conclusions were confirmed directly by the results of weight loss assessment of untreated wood blocks (control) (Fig. 1, 2). The weight losses of control blocks after 18 months of expose were more than twice greater in forest soil than in agricultural soil (46.21 and 20.00 % respectively). This tendency was noticed for treated blocks as well, thought their weight losses were of distinctly less degree. For example, weight loss of wood blocks treated with Asepas-1 was 4.00 % in the agricultural soil and 6.22 % in the forest soil after 18 month of burial. Besides, the greatest weight loss among all tested preservatives in the agricultural soil was of Dispersan 1.1.1 treated wood (7.29 %) but of Arlits – in the forest soil (12.62 %).

The results of microbiological studies of tested wood blocks helped to analyze deeper the reasons of different weight losses and degree of wood protection. All tested preservatives were effective enough to protect wood from major decay in the cause of the experiment. No representatives of *Basidiomycetes* that are responsible of wood major decay were isolated from treated samples even after 18 months (Table 3). This fungus wasn't detected on untreated blocks in agricultural soil either and we estimated lower decrease of block weight loss than of those buried in forest soil.

Nevertheless the infection of all treated and untreated wood blocks by microfungi was noticed. Fungal species belonging to 17 genera were isolated from all tested wood blocks on various stages of exposition. Some of genera were represented by several species, for example, 4 species belonging to *Fusarium* genus, 4 – to *Mortierella* and 7 – to *Penicillium*. Many of isolated species used to be found on wooden substrates and consider be wood damagers, such as *Acremonium strictum, Cladosporium herbarum, Mortierella isabellina, Mucor racemosus, Penicillium miczynskii* and *Trichoderma viride* [11, 12]. Other investigates have presented alike data [13, 14]. Such fungi as *Epicoccum purpurescens, Fusarium solani, Gliocladium viride,* and various species of the genera *Penicillium and Trichoderma* were isolated from buried untreated wood. Cellulase and peroxidases activity characteristic of all these species allows them to use wood as a substratum.

Furthermore the studies showed that the same species could develop on treated and untreated wood blocks. This elucidates that strains of certain species are at least tolerant to preservatives used (copper compounds). *Cladosporium herbarum* and some species from *Mucor* and *Fusarium* genus are known to be very resistant to chemical agents [3, 11]. Fungal strains isolated from treated wood could be capable of metabolizing toxic chemicals into less potent derivatives and detoxify the preservatives thereby facilitating the entry and spread of others capable to develop on wooden substrate. Duncan and Deverall proved by chemical analysis the degradation of arsenic and pentachlor-o-phenol by *Phoma* and *Grafium* species [3]. Consequently the fungal strains developing on treated wood are responsible for preservative degradation and are the reason of preservative inefficiency.

Every new-created wood preservative can be treated as new substrate for microorganisms. Because of their variability fungi gradually adapt to new substrate. Deep inner functional changes take place in fungal cells, some enzymatic systems are suppressed, and others are induced and became more powerful [10].

The different occurrence moment of certain species shows the formation of fungal association. In early stages of block exposition (after 6 and 12 months) the greater variety of fungal species was noticed. For example, *Acremonium strictum* was noticed only on wood blocks treated with Asepas-1, *Penicillium canescens* – only on Dispersan 1.1.1 in forest soil and *Fusarium oxysporum* – only on Arlits in agricultural soil.

Table 3. Fungi isolated from wood blocks that have been treated with preservatives Asepas-1 (As.), Dispersan 1.1.1 (Dis.) and Arlits (Ar.) after 6 (I), 12(II) and 18 (III) months burial in forest and agricultural soil

Fungal taxon	Control	Preservatives			Control	Preservatives		
		As.	Dis.	Ar.		As.	Dis.	Ar.
	Forest soil				Agricultural soil			
Acremonium strictum W. Gams		I					III	
Aspergillus niger Tiegh.	I,II,				I			
Aspergillus versicolor (Vuill.) Tirab.				II			III	
Basidiomycetes	II,III							
Cladosporium cladosporioides (Fresen.) G.A. de Vries				III	III			
Cladosporium herbarum (Pers.) Link ex Gray							III	III
Epicoccum purpurecens Ehrenb. ex Schlecht.	I	I						
Fusarium equiseti (Corda) Sacc.							III	
Fusarium moniliforma J. Sheld					III	III	III	III
Fusarium oxysporum Schltdl.					III		III	II,III
Fusarium solani (Mart.) Appel et Wollenw.					II,III	I.II		I,II
Monodyctis levis (Wiltsh.) Hughes			I	I				
Mortierella hyalina (Harz) W. Gams	I		I	I				
Mortierella isabellina Oudem.		I		II				
Mortierella stylospora Dixon-Stew.				III				
Mortierella vinacea Dixon-Stew.			II	II				
Mucor albo-ater Naumov.		III						
Mucor hiemalis Wehmer	II,III	II,III	III		II,III			II
Mucor racemosus Fresen.	I,III	II	I,III	III	I		I	I
Mucor sp.								II
Paecilomyces variotii Bainier	II							
Penicillium canescens Sopp	II		I					
Penicillium decumbens Thom				II				
Penicillium frequentans Westling		III	III					
Penicillium miczynskii K.M. Zalessky							I	
Penicillium purpurogenum Stoll						III		
Penicillium spinulosum Thom							II	
Penicillium sp.	II					III	III	
Phoma lingam (Tode ex Fr.) Desm.	I		I					
Pleurothecium recurvatum (Morgan) Hohnel		I						
Scytalidium lignicola Pesante	I							
Trichocladium asperum Harz			I					
Trichoderma harzianum Rifai	II	II,III	III	I,II,III	II,III	I,III	III	,III
Trichoderma viride Pers.	I,III	I,II	II		I	I	I	I
Verticillium alboatrum Reinke et Berthold			I	I				
Wardomyces anomala Brooks et Hansford			I					

After 18 months dominating species clarified on all copper containing preservatives. *Trichoderma harzianum* was isolated from wood samples treated with every studied preservative in both sites. Various species of the *Trichoderma* genus produce volatile matters with antibiotic quality. Accordingly they could easily compete for nutrient source and space with other microorganisms. This antagonistic feature of the species is one more reason determining the predominance on the substrate.

The species variety correlation between natural micro flora of burial site and those developing on buried treated wood.

Representatives of *Fusarium* genus were isolated from all treated wood blocks buried in agricultural soil. They were found before the experiment in this site as well. Species of the *Mucor* genus dominated on samples from forest soil. Microbial associations, being numerous and varied are very complex and sensitive to slight changes in environment.

Meteorological conditions off course influence the formation of association on unnatural substrate as different fungal species have different require of moisture and temperature. *Aspergillus niger* could bear great water deficiency and temperature fluctuation [10]. In present experiment this fungal species was isolated only from untreated wood blocks. Apparently it's not resistant to all tested preservatives.

The laboratory efficiency tests of Asepas-1, Dispersan 1.1.1 and Arlits carried earlier were fulfilled under constant and favorable for fungi development conditions [15]. Wood preservatives were infected and damaged (the wood block weight losses were fixed) by a complex of fungi (*Alternaria tenuissima, Fusarium sp.,Sporotrichum olivaceum* and *Trihoderma harzianum*). After 3 months wood blocks treated with Asepas-1 sustain the heaviest losses compared with other tested preservatives.

The carried experiments shows not only that wood preservative efficiency depended greatly on environmental conditions it exposed but leads to search of active and resistant fungal strains for preservative bioremediation, as well.

CONCLUSIONS

Preservatives Asepas-1, Dispersan 1.1.1 and Arlits are effective enough to protect wood from major decay in the course of the experiment. No representatives of *Basidiomycetes* were detected on buried wood block treated with these preservatives.

Nevertheless the tested preservatives were inefficient to protect wood blocks from microfungi. Weight losses of all treated wood blocks were estimated and fungal development was noticed on them.

The carried experiments showed that wood preservative efficiency is dependent greatly on environmental conditions and microflora.

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