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The susceptibility of some acetylated hardwood species to mould fungi attack – An attempt to objectify the assessment

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ABSTRACT

The resistance of natural and treated with preservative wood to filamentous (mould) fungi (*Ascomycota*, *Fungi Imperfecti*) and the efficacy of fungicides in terms of control of discolourations and disfigurements caused by the fungi growing on the wood surface over a very short time is determined by several, mainly descriptive, laboratory methods, very often using a subjective visual grading base. The aim of the research was to evaluate the growth of filamentous fungi on wood samples by comparing a descriptive method and instrumental methods, to attempt to quantify and objectify the assessment of wood susceptibility to mould fungi using as both an example and a model wood acetylated with acetic anhydride and control wood samples of the same species. For 4 weeks samples of beech (*Fagus sylvatica*), birch (*Betula pendula*) and poplar (*Populus nigra*) wood were exposed to a mixture of pure cultures of: *Aspergillus niger*, *Penicillium funiculosum*, *Paecilomyces variotii*, *Trichoderma viride* and *Alternaria alternata* fungi or only to *Chaetomium globosum* fungus. The growth of fungi on the surface of the test samples was evaluated using descriptive (4-grade accepted scale) and instrumental (total colour changes and ergosterol content measurements) methods. The mean descriptive grade of fungal growth was from 2.3 to 3.0 and the instrumental evaluation of the growth was between 8.1 and 31.0 in terms of total colour change and from 152.3 to 437.5 μg 10 cm^{-2} of ergosterol content. Measurements of the colour changes of the wood surface and ergosterol content show a greater differentiation of wood infestation grades than the evaluation based on descriptive methods.

1. Introduction

Throughout the world filamentous (mould) fungi belonging to the *Ascomycota* and *Fungi imperfecti* group are known agents causing a decrease in the trade value of building wood elements. The nutritional requirements of the fungi are very limited. The moulds very often occur on the surfaces of wooden elements, especially on wood species of class 1 – non- resistant to fungi (Ważny, 1994; Clausen and Yang, 2003; Fojutowski et al., 2007, 2009, 2010, 2011). In favourable conditions of high air humidity and moisture content the fungi may cause the disfigurement of wood in a very short time, and with the strong prolongation of their growth, some of them may even cause the soft rot of lignocelluloses materials. This is due to the fact that some of them (e.g. *Trichoderma* spp., *Paecilomyces* sp., *Papulospora* sp., *Chaetomium globosum*, *Humicola grisea*, *Trichurus spiralis*) (Zabel and Morrell, 1992; Hart et al., 2003; ENV 807) produce large quantities of cellulolytic enzymes (cellulases and hemicellulases), which can cause the cellulose and other polysaccharides contained in wood to decay. Protection of wood with fungicides is one of the easiest ways to increase wood resistance to fungi attack. The use of fungicides may however be connected with damage to health and the environment. The acetylation of wood with the anhydride of some organic acids is considered in terms of ecology a more friendly method of enhancing wood resistance to fungi. Expertise in the susceptibility of wood to mould fungi attack is important not only from the point of view of wood quality, but also on account of environmental and health hazards to humans. These hazards are due to spores, conidia and mycotoxins emitted by fungi, which may be the cause of various allergies and infections (Wiszniewska et al., 2004). The resistance of wood to filamentous fungi and the efficacy of fungicides in terms of control of the rot and destruction of wood caused by the fungi over a long period of time is determined through measurement of mass loss of wood in laboratory condition as a

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basic objective method (Zabel and Morrell, 1992; ENV 807) similar to the method used in tests with basidiomycetes fungi (EN 113, CEN/TS 839). The resistance of natural wood and the efficacy of fungicides to filamentous (mould) fungi (*Ascomycota*, *Fungi Imperfecti*) in terms of control of discolourations and disfigurements caused by the fungi growing over a very short time on the wood surface is however determined by several mainly descriptive laboratory methods, sometimes on a subjective visual grading base. A descriptive rating scale proposed in the methods of assessment of building materials' resistance to mould fungi may be considered subjective to some extent (Instrukcja 355/98, 1998). There were some attempts to use instrumental methods such as a leucometer, a photometer, or a spectrophotometer (Grant, 1972; Ehlert and Pantke, 1975; Ważny et al., 1989, 1991) to facilitate the resistance evaluation and make it more objective. The problem of the quantification and objectivity of the methods used to assess the resistance to mould fungi of wood and wood-based materials and the efficacy of their anti-fungal protection may be of great importance. The determination of ergosterol content in wood infected with mould fungi seems to be a promising method as well. Ergosterol is a component of the cellular membrane of fungi present in all phases of fungi development including conidia and spores (Seitz et al., 1977; Gutarowska, 1999; Żakowska et al., 1999). Earlier tests produced some positive results in this matter in the case of natural Scots pine wood (Fojutowski et al., 2012). The aim of the research was to evaluate the growth of filamentous fungi on wood samples by comparing a descriptive method and instrumental methods, to attempt to quantify and objectify the assessment of wood susceptibility to mould fungi using as both an example and a model wood acetylated with acetic anhydride and control wood samples of the same species. Besides the standard subjective visual grading of mould fungi growth on the wood surface, instrumental measurement of total colour change of the wood and a determination of the ergosterol content in the wood were carried out in order to assess the wood's resistance to filamentous fungi.

2. Experiments and methods

2.1. Materials and methods

The main materials used in the tests consisted of beech (*Fagus sylvatica* L.), birch (*Betula pendula* Ehrh.) and poplar (*Populus nigra* L.) control wood sample strips of dimensions 25 Radial(R)(thickness) × 100 Tangential(T) × 450 L(Length)mm and wood strips of the same dimensions acetylated with acetic anhydride. The strips were planed on four surfaces. The acetylation process was carried out using acetic anhydride by the vacuum-pressure method followed by final drying at 140 °C and characterized by anhydride net absorption of 476 kg m^{-3} for the beech wood, 416 kg m^{-3} for the birch wood and 330 kg m^{-3} for the poplar wood. The density of the control wood samples and the acetylated wood was similar for the beech wood – about 710 kg m^{-3} and for the birch wood – 650 kg m^{-3}. The density of the acetylated poplar wood was approximately 8% higher than the control wood samples, which amounted to 480 kg m^{-3}. Wood samples of the dimensions of 4 × 20 × 50 mm($R \times T \times L$) were cut out of the strips as a material used directly for mycological and all subsequent tests. The samples were conditioned for testing in normal conditions (20 °C/65%RH) until the equilibrium moisture content was reached and then sterilized with steam before the mycological test. A set of 6 samples was used for each variant of tested fungi and wood sample treatment.

A method adapted from building procedures (Instrukcja 355/98, 1998) was used for the mycological testing. The acetylated and control wood samples were exposed to a mixture of pure cultures of: *Aspergillus niger* van Tieghem (*An*) (DSM 12634), *Penicillium funiculosum* Thom (*Pf*) (DSM 2213), *Paecilomyces variotii* Bainier (*Pv*) (DSM 1961), *Trichoderma viride* Persoon:Fries (*Tv*) (DSM 63065) and *Alternaria alternata* (Fries:Fries) von Keissler (*At*) (DSM 6210) fungi or only to *C. globosum* Kunze:Fries (*Cg*) (DSM, 1962) fungus and incubated at a temperature of 27 ± 1 °C and relative humidity of 90%. The fungi were from DSMZ collection. A suspension of fungi with a density of 1×10^6 conidia cm^{-3} was sprayed on the surface of the tested wood samples which were placed individually on the surface of salt-agar medium in Petri dishes of 90 mm diameter and an outside height of 15 mm. After 4 weeks of incubation the growth of mycelium on the surface of the test samples was evaluated using as the main method for fungal growth assessment the following scale:

0 – no growth of fungi on the sample, visible under a microscope,

1 – trace growth of fungi on the sample, hardly visible to the naked eye but clearly visible under a microscope, or growth limited to the edges of the sample, visible to the naked eye,

2 – growth of fungi on the sample, visible to the naked eye, but less than 15% of the surface is covered with fungus or fungi,

3 – over 15% of the surface is covered with fungus or fungi visible to the naked eye.

The above standard evaluation was supplemented with an estimation of the percentage of the sample surface overgrown with mycelium and the intensity of fungi growth: 3 – strong, very thick mycelium; 2 – medium thick mycelium; 1 – weak, thin mycelium; 0 – lack of growth visible to the naked eye. This assessment method was added for better evaluation and differentiation of the state of fungi growth on the test samples and their resistance to filamentous fungi attack. An example view of the tested wood samples at the end of the test is shown in Fig. 1.

The final state of the samples' infestation with fungi was also assessed instrumentally by measuring the colour of the surface of the wood samples and content of ergosterol in the wood. We chose the methods taking into account earlier studies by others and our own (Grant, 1972; Ehlert and Pantke, 1975; Ważny et al., 1989, 1991; Fojutowski et al., 2012), commonly known colour changes on the surface of materials on which filamentous fungi grow, and the relation between the ergosterol content and fungal mass. The ergosterol content has been widely used as an estimate of fungal biomass in various environments, because a strong correlation was found between the ergosterol content and fungal dry mass (Schnürer, 1993; Suberkropp et al., 1993).

The colour measurements were conducted with the use of the Elrepho 2000 Data Color apparatus in CIE Lab system (Bekhta and Niemz, 2003) where the coordinates of colour L, a, b and the total colour change ΔE were determined according to the formula (Eq. (1)):

$$\Delta E = \left[(L_1 - L_o)^2 + (a_1 - a_o)^2 + (b_1 - b_o)^2\right]^{1/2} \quad (1)$$

L_1, a_1, b_1 – coordinates of the colour of the wood before the mycological test,

L_o, a_o, b_o – coordinates of the colour at the end of the mycological test.

The colour measurements were made on the same area of the samples before and after the fungal attack. The surface of wood samples after fungal attack was not cleaned off fungi to measure the results of fungus or fungi growth.

The ergosterol content was determined by the spectrophotometric method (Seitz et al., 1977; Gutarowska and Żakowska, 2002) after extraction from the mycelium. The ergosterol was determined in the whole timber sample, because part of fungi