

# FUNGAL STAINING OF PONDEROSA PINE SAPWOOD: EFFECTS OF WOOD PRECONDITIONING AND BIOPROTECTANTS<sup>1</sup>

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## ABSTRACT

Differences in stain susceptibility were evaluated on sterile and unsterile samples of freshly sawn, frozen and thawed, or oven-dried and rewetted ponderosa pine sapwood. Samples treated with sterile medium or with medium inoculated with either *Pseudomonas putida* or *Bacillus subtilis* were inoculated with selected wood-staining fungi. In general, fresh unsterile samples were less stained than either frozen or oven-dried wood. Sterilization by either steaming or gamma irradiation generally eliminated the differences in degree of stain noted in unsterilized specimens exposed to the various initial wood conditions. Sterilization alters either the microflora or the nutritional quality of the wood, enhancing fungal wood-staining. Therefore, unsterile, freshly sawn wood should be used in assessing the efficacy of bioprotectants.

*Keywords:* Staining, bacteria, fungi, bioprotection.

## INTRODUCTION

Evaluation of potential biocides or biological protection agents for fungal stain control frequently begins with small-scale laboratory tests (Chapman and Scheffer 1940; Verrall 1949; Hatfield et al. 1950; Kenega and Cowling 1959; Hulme and Thomas 1979; Unligil 1979; Cserjesi and Johnson 1982; Drysdale and Preston 1982; Eslyn and Cassens 1983; American Society for Testing and Materials 1984; Lewis et al. 1985; Tsunoda and Nishimoto 1985; Hong 1989; Micales et al. 1989; Tsunoda 1989; Presnell and Nicholas 1990; Laks et al. 1991). In such trials, the test substrate is often freshly sawn or frozen wood ex-

posed on glass rods in petri dishes or in plastic containers. However, studies in the literature are fairly divided with regard to the use of fresh versus frozen and sterilized versus unsterilized samples. Some methods even employ dried, rewetted specimens (Tsunoda and Nishimoto 1985; Tsunoda 1989). While freshly sawn sapwood is preferable for testing, such materials are sometimes difficult to obtain in a timely manner or with assurance that the material has not been exposed to anti-stain chemicals or fungal attack. Careful collection and frozen storage of large volumes of sapwood is an ideal method for providing uniform test material over time. Freezing might redistribute nutrients and alter wood-water relationships, thus altering susceptibility to stain; however, there are few data to support this premise.

In this study, we evaluated the susceptibility

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of ponderosa pine sapwood to microbial attack when freshly sawn, frozen and thawed, or oven-dried and rewetted. We also examined the ability of biological protection agents to protect wood prepared by these methods.

#### MATERIALS AND METHODS

Freshly sawn sapwood boards of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) were obtained from a mill near Prineville, Oregon. Logs at the mill were generally less than 2 weeks old. The wood was cut into defect-free wafers, 5 mm × 14 mm × 30 mm long, and divided equally into three groups. One group was oven-dried for 24 h at 54 C and rewetted by soaking in water for 3 h at room temperature. Another group was frozen for 24 h (−5 C) and thawed before use. The third group was used freshly sawn. In each group, one-third of the wafers was then sterilized by steaming for 10 min at 100 C, one-third was sterilized by exposure to 2.5 Mrads of ionizing radiation for 6 h, and one-third was left unsterile.

The wafers were dipped for 30 sec into either sterile nutrient broth or nutrient broth inoculated 48 h earlier with a bioprotectant—either *Bacillus subtilis* Cohn (Isolate 733A, Forintek Canada Corp., Ottawa, Canada) or *Pseudomonas putida* Migula (Isolate A-12, J. Loper, USDA-ARS, Corvallis, OR). After drip-drying on glass rods for 5 min, they were sprayed with a suspension of spores and mycelial fragments of common mold and stain fungi: *Aspergillus niger* van Tieghem, *Alternaria alternata* (Fr:Fr) Keissl, *Bispora betulina* (Corda) S.J. Hughes, *Phialophora fastigiata* Lageberg and Melin, and *Phialocephala dimorphospora* Kendrick. The suspension was prepared by flooding petri dishes containing actively growing colonies and rubbing spores and hyphal fragments from the agar with a rubber rod. The suspensions from each isolate were combined for application to the wood.

The wafers were then placed on glass rods on top of five layers of moistened filter paper in a glass petri dish. Four wafers dipped in either the sterile medium or a medium inoc-

ulated with bioprotectant were included in each dish, along with an untreated control. Each combination of preconditioning and bioprotectant was replicated on three dishes, which were incubated for 10 weeks at 23 C. Degree of stain and mold on each wafer was then assessed visually on a scale from 0 (no stain) to 100 (completely discolored). The values for each treatment group were averaged, and an analysis of variance was performed (SAS for Microcomputers 1987). Interactions among the preparation methods limited the possible statistical inferences.

#### RESULTS AND DISCUSSION

Sterile wafers that had not been treated with a bioprotectant did not differ markedly in degree of stain; thus, wood conditioning before treatment did not appear to affect the outcome of the trial adversely. Staining of unsterile wafers was substantially less on fresh wafers than on those that had been either frozen and thawed or oven-dried and rewetted (Table 1). *Aspergillus niger*, a common mold, was the most abundant colonizer; however, *Penicillium* spp. were more abundant on the fresh, unsterile treatments and may have reduced colonization by more significant wood stainers.

The use of heat-sterilized wood is a standard in many laboratory trials because the practice minimizes variation and simplifies interpretation of the results (Chapman 1933). Steaming can, however, redistribute or solubilize sugars, increasing susceptibility to staining. Substitution of irradiation might minimize the effects of wood modification on degree of staining. In the absence of bioprotectants, however, staining of irradiated samples apparently differed little from that in steamed samples (Table 1). These results suggest that irradiation significantly modified the wood and rendered it more susceptible to attack; in previous studies, however, irradiation at the dosages employed in our study did not alter decay resistance of a number of wood species, including ponderosa pine (Kenega and Cowling 1959; Scheffer 1963). Irradiated specimens exposed

TABLE 1. Degree of staining of sterilized or nonsterilized ponderosa pine sapwood wafers exposed to combinations of stain fungi and bioprotectants over 10 weeks at 23°.

Wood pretreatment <sup>1</sup>	Sterilization method	Bioprotectant treatment		
		None	<i>Bacillus subtilis</i>	<i>Pseudomonas putida</i>
Fresh	none	39.3 (23.1)	23.3 (16.8)	17.7 (14.9)
	steaming	92.1 (4.3)	89.3 (2.7)	35.5 (29.1)
	irradiation	96.7 (4.9)	86.0 (5.1)	98.7 (3.5)
Frozen	none	90.7 (8.8)	73.6 (7.4)	28.0 (29.9)
	steaming	99.3 (2.6)	85.3 (5.2)	68.0 (33.0)
	irradiation	94.1 (5.1)	88.0 (4.1)	98.0 (5.6)
Oven-dried	none	87.3 (5.9)	89.3 (2.6)	81.3 (3.5)
	steaming	83.3 (7.2)	80.7 (9.6)	90.7 (8.0)
	irradiation	82.1 (5.8)	80.7 (12.2)	76.0 (6.3)

<sup>1</sup> Values represent mean ( $\pm$  standard deviation) of 12 samples. Staining scale based on visual assessment: 0 = no stain, 100 = complete discoloration.

<sup>2</sup> Samples were either freshly sawn; frozen for 24 hours, then thawed; or oven-dried and rewetted.

to bioprotectants produced more variable results, depending on the test organism and the preconditioning method employed.

Treatment with a potential bioprotectant had little or no effect on overall degree of staining of oven-dried and rewetted wafers, regardless of whether or not wafers were sterilized. Drying may have permanently redistributed nutrients to the extent that the bioprotectants were less effective. In most bioprotectant trials, samples have been freshly collected or frozen, rather than oven-dried, and our results confirm the need for such material.

Application of bioprotectants to fresh or frozen wafers produced less consistent results. Neither bioprotectant significantly affected staining of irradiated wafers, and *B. subtilis* did not influence staining of steamed wafers; however, staining of steamed wafers was markedly less in the presence of *P. putida*. Both bioprotectants were associated with minimal staining of unsterilized wood; but staining differed little from that on unsterile wafers treated with sterile nutrient broth before inoculation with fungi.

Our results indicate that drying or sterilizing wood can significantly alter the results of screening trials of biological agents as stain preventives. Elimination of potentially beneficial or competitive agents from the wood is particularly important in affecting the outcome of the trial. While these organisms do not ad-

versely affect the degree of staining on specimens not treated with bioprotectants, they can significantly alter the ecology of the wood, either favoring the bioprotectant or outcompeting it for nutrients or substrate. The use of fresh or frozen unsterile wood coupons for evaluating potential bioprotectants must therefore be considered essential for producing reliable results in laboratory trials of staining.

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