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Protection of *Ochroma pyramidale* from fungal decay with N,N-naphtaloylhydroxylamine

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Protection of Ochroma pyramidale from fungal decay with N,N-napthaloylhyroxylamine

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Fungal decay of wood in service results in billions of dollars (U.S.) in losses annually. Recent environmental restrictions, both U.S. and international, are limiting and eliminating the use of broad-spectrum, heavy metal biocides for wood preservation. Restrictions result primarily from problems with disposal. New wood preservatives need to be developed and tested which specifically target key elements in the sequence of fungal decay mechanisms. Our laboratory has been experimenting with chemicals which inhibit pectin hydrolysis during incipient brown-rot and white-rot decay in southern pine sapwood (Inter. Biodeter. Biodegrad. 39:103). In the present paper these results are extended to include the tropical hardwood Ochroma pyramidale (balsa). Balsa blocks (24xl8x12mm) were exposed to two brown-rot fungi and one white-rot fungus in ASTM soil block tests for 10 weeks. CCA (6.4 km/m^3) was compared with the calcium binding agent N,N-napthaloylhydroxylamine (NHA; 1.6, 3.2 & 6.4 km/m^3) in leached and unleached blocks. CCA protected balsa with minimal weight loss (> 7.4%) with no leaching effects. NHA (6.4 km/m^3) protected balsa (0.3-1.29) weight loss but leaching raised the weight losses to 25% with the brown-rot fungus Tyromyces palustris. We conclude that NHA can protect balsa against G. trabeum and T. versicolor with comparable efficiency to CCA (leached and unleached) but not T. palustris.

INTRODUCTION

Balsa trees are widely distributed in tropical America, throughout the West Indies, and from southern Mexico, through Central America and into Venezuela, Columbia, Brazil, Ecuador, Peru and Bolivia. Plantation balsa is grown primarily in Ecuador by Baltek Corporation (Northvale, NJ). Balsa trees grow very rapidly. Six to ten years growth yields a tree between 60-90 ft. tall and a butt diameter of 12-45 inches. The wood is one of the lightest commercial species (7.8 to 13 pounds cubic ft.). Often annual rings are absent because the tree does not have a period of rest, hence no evidence of seasonal growth. The durability of the wood is low (5 on a scale of 1-5 where 5 is the least durable). The wood is perishable and vulnerable to decay and dry-woodtermite attack (Chudnoff 1984).

Due to the fact that end-grain balsa panels are predominantly used as a core material for structural sandwich applications, kiln-dried balsa is generally unaffected by wood decay fungi or insect attack. Only damage or machined holes in the outer skin material permits the moisture conditions necessary for support of fungal growth. Balsa Ecuador, Inc. did not find one single case of fungus attack or deterioration in any sandwich structure which used kiln dried sandwich core (Kohn 1958).

In a series of recent papers, our laboratory has been investigating the ability of calcium precipitating agents to inhibit wood decay and termite damage in southern pine (Green et al., 1996, 1997a and 1997b). We have had the best success with N,N-napthaloylhydroxylamine (NHA) although ruthenium red also resulted in low weight losses. Because of the perishable durability classification of balsa (Purslow1976) and the wide differences between it and southern pine, the objective of this study was to evaluate the ability of NHA to protect balsa from brown-rot and white-rot decay in soil block tests. Unleached balsa was well protected by NHA from all decay fungi tested, but leached blocks were susceptible to brown-rot decay by Tyromyces palustris.

MATERIALS AND METHODS

Fungi

Two brown-rot fungi were used in soil-block tests: Gloeophyllum trabeum (Pers:Fr.) Murr. (MAD-617), and Tyromyces palustris (Berk et. Curt) Murr. (TYP-6137); and one white-rot fungus Trametes versicolor (L:Fr.) Quel (MAD-497). All fungal isolates were maintained on 2% malt-extract agar tubes at 4°C for the duration of the study.

Wood and treatment of blocks

End-grain baler panels 1.9 cm thick were obtained from Baltek, Inc. (Northvale, NJ). Balsa blocks were steam sterilized (100°C) for 30 minutes and impregnatesd with sterile aqueous solutions of NHA (Avo ado Chemicals) (0.1, 0.2 & 0.4 pcf/l.6, 3.2 & 6.4 km/m^3) and CCA (6.4 km/m^5). Four blocks per group were treated. Leachability of NHA and CCA was determined by the standard method of the American Wood Preserver's Association (AWPAE-11-87) using 4 blocks per treatment group.

Wood and test conditions

Square blocks (19 mm^2) were exposed to decay fungi for 10 weeks in standard American Wood Preserver's Association (AWPA) soil-block tests (ASTM, D2017). Following incubation, test blocks were removed from bottles, oven dried at 80°C and weighed. Weight loss was used as an estimate of decay inhibition.

Gas permeability

Balsa cores (64 mm diameter) were bored from end-grain panels and permeability was estimated by the method of Milota et al.,(1995).

RESULTS

Table 1 shows the results of the soil block tests. The brown-rot fungi G. trabeum and T. palustris produced 62% and 42% weight loss, respectively, in controls and the white-rot fungus T. versicolor produced 44% weight lose. Unleached NHA blocks treated to a retention of 3.2 or 6.4 km/m^3 and CCA (0.4 pcf) had comparable decay resistance to all three fungi. NHA treated blocks treated to 1.6 km/m^3 retention were also resistant to decay by G. trabeum and T. versicolor, but not T. palustris. Weight losses by all 3 fungi were

increased in leached blocks of NHA treated to 1.6 and 3.2 km/m^3. However, at 6.4 km/m^3 leached NHA blocks were resistant to decay by T. versicolor and G. trabeum but not T. palustris.

Table 2 shows the partial results of a survey of 17 brown-rot fungi and 8 white-rot fungi for polygalacturonase activity and oxalic acid production (Green and Clausen 1998). T. palustris (TYP-6137) had higher enzymatic activity and higher oxalic acid production than G. trabeum or T. versicolor. However, gas permeability of southern pine cores after 3 week6 of liquid culture for T. palustris was unremarkably low (2.78 darcys),

The mean gas permeability of untreated, control balsa wood cores was estimated to be 31.80 (n=6) or over thirty times that of southern pine.

DISCUSSION

This study represents our first attempt to protect a tropical hardwood (balsa) from wood decay using a calcium precipitating agent (NHA). Previous papers by these authors have shown that NHA has the ability to protect southern pine from weight loss by brown-rot and white-rot decay fungi at treatment levels of 0.59-1.01 (Green et al., 1996; Green et al., 1997). In this study it was determined mathamatically that 0.5 and 1.0% NHA treatments corresponded to retention levels of 1.6 and 3.2 km/m^3, respectively. From Table 1, it is clear that although NHA provides good protection in low durability balsa, the two lower treatments of NHA were not as succesful at protecting balsa as the same treatments were at protecting pine. The most noticeable disparity is in the leached balsa blocks which ranged in weight loss from 19-44% at 1.6 km/m^3 retention of NHA. In southern pine, these same treatments resulted in weight losses between 9-209 (leached). Generally, weight losses are double in leached balsa over southern pine. This is partly explained by the very high porosity of balsa (32 darcies) which is higher in untreated control balsa than after 509 weight loss in southern pine, which gives large surface areas of the balsa wood access to the decay fungi (Green et al., 1996). It is equally clear that retention of NHA in the balsa wood blocks after aqueous leaching for 2 weeks is low--and probably indicates low calcium in balsa wood. Examination of Table 1 shows that only about 50% of the NHA was retained after leaching. (Attempts to determine the calcium levels in balsa by ISP spectroscopy were unsuccessful).

One additional factor to take into account in this study is the aggressive nature of the brown-rot decay fungus T. palustris (TYP-6137) which was acquired from the Wood Research Institute of Kyoto University. Both NHA and CCA showed reduced protection from decay, with the exception of NHA (6.4 km/m^3) unleached (Table 1). This isolate has been extensively studied for its production of oxalic acid by Akamatsu, Shimada and Takahashi for more than 5 years, Although we have as yet only preliminary data on TYP-6137 on NHA treated southern pine--its close relative F. palustria (L-15755) showed 11% weight loss in southern pine treated with 1% NHA. Both fungi have been shown to produce polygalacturonase and high oxalic acid when compared with other brown-rot fungi (Table 2 and Shimada et al., 1994 & 1996; Akamatsu et al., 1994). It has been our understanding for some time that oxalic acid is a key component of the mechanism of brown-rot decay (Green et al., 1991). The

impetus behind the use of calcium precipitating agents as wood preservatives was derived from the observation that oxalic acid acts am a chelating agent to remove the calcium from pectin in wood during incipient decay (Green et at., 1995).

In summary, balsa can be considered a wood of high permeability and low natural durability with little resistance to fungal decay. Balsa wood treated with aqueous NHA at 3.2 and 6.4 km/m^5 retentions and not leached was adequately protected from all three decay fungi tested. Leaching reduced the effectiveness of NHA treated to 1.6 and 3.2 km/m^3 against all three fungi. However, NHA leached blocks were protected from decay by G. trabeum and T. versicolor but not T. palustris. These results indicate that the calcium precipitating agent NHA can protect balsa against brown-rot and white-rot decay, but that the minimum inhibitory concentration (MIC) to protect against T. palustris is higher than the other two fungi.

ACKNOWLEDGEMENTS

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Table 1. Mean percent weight loss of NHA and CCA treated Balsa in 10 week soil-block tests

Fungus	TREATMENT None	NHA/NHA-L[1] (0.1 pcf)	NHA/NHA-L (0.2 pcf)	NHA/NHA-L (0.4 pcf)	CCA/CCA-L (0.4 pcf)
Gloeophyllum trabeum (MAD-617)	61.6[2] ±9.3	2.2±1.2/ 18.7±12.4	1.5±1.1/ 10.9±12.4	0.3±0.31 0.6±1.0	2.3±0.7/ 2.7±0.9
Tyromyces palustrs[1] (TYP 6137)	42.2± 10.6	38.8±6.7/ 43.8±3.6	5.3±6.1/ 49.0±5.8	1.2±0.31 25.0±8.6	6.8±6.0/ 7.4±4.6
Trametes versicolor (MAD-697)	43.5± 1.8	5.3±3.2/ 30.4±10.0	2.5±1.7/ 6.3±3.0	1.2±0.4/ 0.9±0.6	0.1±0.1/ 0.5±0.0

1. L = leached 2 weeks in H_20

2. Mean ± S.D. (N=4)

Table 2. Survey of polygalacturonase activity, and oxalic acid production in liquid cultures of representative brown and white rot fungi and gas permeabilities of Southern pine wood cores exposed to select fungi.

	Cup-plate Assay (mm)[1]	Viscosimetry (NR)[2]	Oxalic acid (uM/mg mycelium)	Gas Permeability (Darcys)
Brown – rot Isolates:				
Coniophora puteana (Schum.:Fr.) Karst. (Mad-515)	9	11.1	0.69	1.58
Postia placenta (Fr.) Lars. & Lomb. (Mad-698)	12	26.9	0.30	3.30
Gloeophyllum trabeum (Pers.:Fr.) Murrill (Mad-617)	10	27.0	0.20	5.03
Postia placenta (Fr.) Lars. & Lomb. (ME-20)	12	24.9	0.16	2.03
Antrodia carbonica (Overh.) Gilbn. & Ryv. (HHB-5104)	8	10.1	1.15	2.66
Fomitopsis palustris (Berk. & Curt.) Gilb & Ryv.(L-15755)	10	8.6	4.70	4.08
Fomitopsis pinicola (Fr.) Karst. (105877)	10	6.4	4.77	4.25
Tyromyces palustris (Berk. et. Curt.) Murr. (TYP-6137)	20	N.D.	4.00	2.78
White-rot Isolates:				
Trametes versicolor (L.:Fr.) Pil. (Mad-697)	9	0	0	13.63
Irpex lacteus (Fr.:Fr.) Fr. (HHB-7328)	11	4.8	0	18.76
Phanerochaete chrysosporium Burds. (ME-461)	4	9.4	0	22.18
Mold Isolates:				
Aspergillus niger	2	5.6	ND	ND
Trichoderma harzianum ATCC 20476	10	0	ND	ND
Pos. Control[3]	13	22.6		0.64

[1] Well diameter (5mm) was subtracted from each total precipitin measurement.
[2] Viscometric data are expressed as $10,000/t_{50}$ per ml enzyme solution, where t_{50} equals the time for relative viscosity of the solution to be reduced by 50% at 28°C.
[3] Positive control equals 0.9 units pectinase activity/ml.
[4] From Green & Clausen (1998)
ND=not determined