Source of Increased Decay Resistance in Sodium Hydroxide- and Ammonia-Treated Wood

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ABSTRACT

In tests that simulated aboveground exposure to promote low decay by brown rot, the increase in decay resistance in wood treated with ammonia or sodium hydroxide and heat was not attributed to destruction of the thiamine in the wood. The pH and the ammoniacal nitrogen content of the wood were found to affect decay resistance. When the pH of sodium hydroxide-treated southern pine (*Pinus* sp.) and sweetgum (*Liquidambar styraciflua* L.) was lowered by leaching in acid, decay by *Poria monticola* occurred. When ammonia-treated southern pine was leached in acid to lower ammoniacal nitrogen content, the wood was readily decayed by *P. monticola*.

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The decay resistance of wood in aboveground exposures produced by alkaline treatment followed by heat has been attributed to destruction of thiamine, a nutritional requirement of most wood-rotting Basidiomycetes (1, 4, 11). Preliminary evidence indicates, however, that other factors may increase decay resistance (5, 7, 12). The objectives here were to determine whether destruction of thiamine is a principal source of increased decay resistance in wood treated with ammonia or sodium hydroxide and heat, and if not, to find other explanations for resistance.

MATERIALS AND METHODS.—Southern pine (*Pinus* sp.), Douglas fir (*Pseudotsuga menziesii* [Mirb.] Franco), and sweetgum (*Liquidambar styraciflua* L.) sapwood blocks were cut 2.5 by 1.3 by 0.9 cm with the small dimension in the grain direction; they were treated with solutions of 1% NH_3 or NaOH, except where stated otherwise, by subjecting the blocks to a vacuum (72 cm of Hg) for 20 min, then flooding with the treating solution. After 20 min, they were removed from the treating solution and steam heated in test tubes sealed with aluminum foil for 1 hr at 100 C. After an air-drying at room temperature for at least 24 hr, blocks treated with NaOH were leached in distilled water until the pH of the leach water was near neutral.

Before inoculation, blocks were conditioned to equilibrium moisture content at 27 C and 70% relative humidity; they were then weighed and steam-sterilized at 100 C for 20 min. Decay resistance was determined by a previously described method designed to eliminate the influence of extraneous nutrients (5). After incubation, each test block was removed from the test bottle, dried to equilibrium moisture content at 27 C and 70% relative humidity, and weighed. The loss in weight of each block indicated the extent of fungus decay.

Unless stated otherwise, the following basal medium was used in fungal growth studies: 2 g NH_4NO_3, 1.5 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 10 g glucose, 0.57 mg H_3BO_4, 0.036 mg $MnCl_2 \cdot 4H_2O$, 0.31 mg $ZnSO_4 \cdot 7H_2O$, 0.039 mg $CuSO_4 \cdot 5H_2O$, 0.018 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.051 mg $FeSO_4 \cdot 7H_2O$, and 1,000 ml distilled water. Culture medium was autoclaved at 121 C for 15 min prior to inoculation. I estimated fungal growth by weighing mycelial mats that had been separated from cultures by vacuum filtration through Whatman glass fiber filter paper and oven-dried at 40 C for 24 hr.

Poria monticola (Murr.) (Madison 698) was used most frequently because extensive testing showed that this fungus failed to attack NH_3- and NaOH-treated wood under the previously described decay conditions (5).

Concentration of thiamine in wood and effect on fungus growth.—Thiamine has been detected in bark (2), but its presence in xylem has not been confirmed. Therefore, samples of untreated southern pine and Douglas-fir sapwood, ground to a fine powder in a Wiley mill, were analyzed by the thiochrome method (Wisconsin Alumni Research Foundation, Madison, Wis.) to establish the level of thiamine in wood prior to alkali treatment.

To determine the effect of thiamine concentration on fungus growth, *Poria monticola* was transferred four successive times on thiamine-free basal medium with Difco purified agar, after which the fungus was transferred to 250-ml Erlenmeyer flasks with 100 ml of basal medium and thiamine at 0, 0.00001, 0.0001, 0.001, 0.01, 0.10, and 1.0 μg/ml. After 4 weeks on a reciprocating shaker at 23 C, growth was estimated by weighing mycelial mats.

Growth of Poria monticola with NH_3-treated wood or extracts from NH_3-treated wood.—The purpose was to determine if NH_3-treated pine, as well as extracts from the treated wood, could replace thiamine to promote fungus growth in a liquid culture media with all the essential nutrients except thiamine.

Ammonia-treated and untreated pine blocks were ground to a fine powder in a Wiley mill. I prepared water extracts from the powder by heating 2 g of powder in 100 ml of distilled water acidified with 0.2 ml concentrated HCl at 100 C for 1 hr. Twenty ml of the extract were added to 80 ml of basal medium. Flasks of the basal medium with 1.0 μg/ml of thiamine and without thiamine served as controls. Inoculum was grown on filter paper over a nutrient medium without thiamine as previously described (5). A 5-mm square piece of filter paper with *P. monticola* mycelium attached was placed in each flask. The flasks were then placed on a shaker. After 3 weeks, mycelial weights were determined.

In place of the water extracts, 2.5 × 7.5 × 0.15 cm veneer strips of treated and untreated southern pine were placed in 250-ml Erlenmeyer flasks with 50 ml of basal medium with thiamine (1.0 μg/liter) and without thiamine. The strips were placed on end so that two-thirds of their length was exposed above the solution. Mycelial weights were determined after 3 weeks of growth in shake culture.

Decay resistance of ammonia-treated blocks supplemented with thiamine.—Sterile NH_3-treated southern pine blocks were impregnated with sterile thiamine-hydrochloride solutions to give concentrations in blocks ranging from 0.0006 μg to 0.6 μg/g of wood substance. Thiamine-pyrophosphate and yeast extract were also added to blocks. Blocks were tested for decay resistance with *Poria monticola* and *Lentinus lepideus* (Fr.) (Madison 534). In addition, NH_3-treated pine blocks were exposed to fungus attack over purified agar to which thiamine-hydrochloride was added (1.0 μg/liter). The blocks were placed on glass rods over the agar and were inoculated with *P. monticola* on filter paper.

pH of alkaline-treated and untreated wood.—Pine blocks were treated under vacuum with 1, 5, and 10% aqueous NH_3, and 1 and 5% NaOH. After treatment, the blocks were heated as described. After air-drying for at least 24 hr, NaOH-treated blocks were leached until the pH of the leach water was near neutral. NH_3-treated blocks were not leached, but were air-dried for 7 days to allow the NH_3 to escape. Blocks were ground to a fine powder in a Wiley mill, the powder was suspended in distilled water, and the pH was measured with a glass electrode pH meter after 5 min.

Resistance of acidified NaOH-treated blocks to brown-rot attack.—Heated NaOH-treated pine and sweetgum blocks were acidified by a soaking in 0.01 N HCl for 24 hr, followed by a 24-hr soaking in distilled water. Blocks were tested for decay resistance with *Poria monticola*.

Growth of brown- and white-rot fungi at different pH values.—The pH tolerance of two white-rot fungi, *Polyporus versicolor* (L. ex Fr.) (Madison 697) and *Peniophora* "G" (ME-461), and two brown-rot fungi, *Poria monticola* and *Lentinus lepideus*, were compared. The fungi were grown in media of varying pH prepared by a mixing of 50 ml of 2% malt extract with 50 ml of the following buffers: 0.1 M Na_2HPO_4-citric acid for pH 4 and 5, 0.1 M Na_2HPO_4-KH_2PO_4 for pH 6 and 7, and 0.1 M Tris [tris(hydroxymethyl)amino methane] HCl for pH 8 and 9. After autoclaving, the pH values were checked and adjusted when necessary. After 16 days on a shaker, mycelial weights were determined.

Effect of various nutrients on decay resistance of NH_3-treated pine.—I supplied nutrients to *P. monticola* or to *L. lepideus* by adding them to the blocks or to the agar. Blocks were placed in a vacuum desiccator, a vacuum was drawn for 20 min, and the nutrient solutions were added. After a soaking for 20 min, blocks were steam-sterilized at 100 C for 30 min. Blocks were put in 225-cc French-square bottles, and the inoculum (the fungus on filter paper) was placed on the block.

Twenty-five ml of 1.5% purified agar with the various nutrients were placed into 225-cc French-square bottles. Blocks were placed in bottles on glass rods and inoculated with the fungus on filter paper. Nutrient solutions used were 1% glucose, 2% malt extract, vitamin-free casein hydrolysate, the basal minerals, 0.1 μg/liter thiamine-HCl, and various combinations of these.

Ammoniacal nitrogen retained by wood treated with NH_3 and the effect of its removal on decay resistance.—The purpose of this experiment was to determine whether a significant amount of ammoniacal nitrogen was retained by wood after treatment and, if so, whether removing the ammoniacal nitrogen by acid-leaching would render the wood more susceptible to brown-rot attack.

The amount of ammoniacal nitrogen in acid-leached and unleached pine treated with 1, 5, and

10% NH_3 was determined, as was the amount of ammoniacal nitrogen in the leachate. Both heated and unheated NH_3-treated blocks were used. Blocks were air-dried for 7 days after treatment before ammoniacal nitrogen was determined. Five blocks were placed in 150 ml of 0.1 N H_2SO_4 for 24 hr on a shaker. Ammoniacal nitrogen was determined with Nessler's reagent (6).

Both NH_3-treated and nontreated pine blocks leached in 0.01 N HCl for 24 hr followed by 24 hr leaching in water were tested for decay resistance to *P. monticola*.

RESULTS.—Thiamine was not detected in either the southern pine or the Douglas-fir sapwood by chemical analysis (0.1 μg/g wood can be detected), and growth of *Poria monticola* declined markedly at concentrations <0.1 μg of thiamine/ml of culture media (Table 1); thus, the natural, or untreated, wood contained less thiamine than that required for optimal fungal development in a synthetic culture medium.

Growth of *P. monticola* was as abundant with only the water extracts from both the treated and the untreated pine in the basal medium as it was when the basal medium contained thiamine (Table 2). It is interesting that the thiamine concentrations in the culture with the wood extracts are probably well below those necessary for optimal fungus growth. Assuming 100% removal of thiamine from wood during extraction and its concentration to be 0.1 μg/g of plant material (analysis showed the concentration of thiamine to be actually less than this), the maximum amount of thiamine in the culture medium would be 0.0004 μg/ml. It was found that growth of *P. monticola* was reduced at a thiamine concentration of 0.01 μg/ml, and at 0.001 μg/ml, growth was similar to that of the controls with no thiamine (Table 1). Both NH_3-treated and untreated veneer strips of southern pine also promoted similar fungal growth when thiamine was omitted from the culture medium (Table 3). Thiamine added to cultures with the wood strips further stimulated growth of *P. monticola*, which is additional evidence that normal wood contains insufficient thiamine for optimal fungal growth.

Final evidence that the increased resistance to brown rot by alkaline treatment and heat is not solely due to thiamine destruction was obtained from decay tests of NH_3-treated wood supplemented with thiamine. Thiamine-hydrochloride added to treated blocks in various concentrations did not permit attack by *P. monticola* or *L. lepideus*. Likewise, thiamine-pyrophosphate and yeast extract (high in B-complex vitamins) did not alter the resistance of treated blocks to attack by the fungi. Addition of thiamine-hydrochloride to agar rather than directly to the test block also did not permit treated blocks to be attacked.

The pH of untreated southern pine was 4.7. The pH values of the pine treated with the different concentrations of ammonia were all near 6.0, and most wood-decaying fungi grow well at pH values of ca. 6.0 (9). The pH values of the NaOH-treated wood, however, were about 8.0, which could be inhibitory to some fungi. This was found when the pH tolerances of two brown-rot and two white-rot fungi were compared (Table 4). The brown-rot fungi did not grow at pH >7 and above, whereas the white-rot fungi grew at pH >7 to 9. This probably explains why the brown-rot fungi tested cannot attack NaOH-treated wood exposed under low-decaying-promoting conditions, whereas the more pH-tolerant white-rot fungi can. The lowering of the pH of NaOH-treated pine by a soaking in acid permitted brown-rot attack in the NaOH-treated wood similar to that in untreated controls (Table 5).

NH_3-treated pine blocks impregnated with various nutrients were not attacked by the brown-rot fungi,

TABLE 1. Effect of thiamine concentration on growth of *Poria monticola* in liquid shake culture

Thiamine concentration (μg/ml)	Growth response (mycelial dry weight)[a] (mg)
0.0	35
0.00001	11
0.0001	24
0.001	50
0.01	92
0.10	138
1.0	137

[a] Average weight for seven flasks/treatment after 4 weeks.

TABLE 2. Effect of water extracts from ammonia-treated and untreated southern pine on growth of *Poria monticola*

Medium	Mycelial dry weight per medium[a] (mg)
Basal (B)	10
B + extract from treated pine	81
B + extract from untreated pine	95
B + thiamine-hydrochloride	70

[a] Average weight for five flasks/treatment after 3 weeks.

TABLE 3. Effect of ammonia-treated and untreated southern pine wood on growth of *Poria monticola*

	Mycelial dry weight in medium containing[a]		
Medium	No wood (mg)	NH_3-treated pine (mg)	Untreated pine (mg)
Basal	4	65	67
Basal + thiamine	64	117	107

[a] Average weight for three flasks/treatment after 3 weeks.

TABLE 4. Effect of pH on growth of white- and brown-rot fungi in buffered medium

Fungus	Mycelial dry weight at pH[a] 4 (mg)	5 (mg)	6 (mg)	7 (mg)	8 (mg)	9 (mg)
White rot						
Polyporus versicolor	115	108	156	87	67	43
Peniophora "G"	304	161	252	92	61	27
Brown rot						
Poria monticola	91	187	135	0	0	0
Lentinus lepideus	31	30	41	0	0	0

[a] Average weight for five flasks/treatment after 16 days.

TABLE 5. Effect of leaching in acid on decay by *Poria monticola* in NaOH-treated southern pine and sweetgum[a]

Treatment	% Weight loss in: Unleached blocks: Southern pine	Unleached blocks: Sweetgum	Blocks leached in 0.01 N HCl: Southern pine	Blocks leached in 0.01 N HCl: Sweetgum
1% NaOH + heat[b]	0	0	25	28
Untreated control	22	21	21	24

[a] Average weight loss for six blocks/treatment after 8 weeks.
[b] Blocks heated at 100 C for 1 hr.

P. monticola and *L. lepideus*. However, treated blocks exposed to fungus attack over agar to which glucose or malt was incorporated were attacked as severely as were untreated control blocks. The different results obtained by the two methods of supplying nutrients may be due to differences in inoculum strength. The fungi grew profusely over the agar with glucose or malt; thus, the blocks were exposed to a vigorously growing fungus inoculum. Conversely, fungi did not colonize blocks impregnated with nutrient solution and inoculated directly. It was found earlier that when test conditions promoted high decay, as in the soil-block test, alkaline-treated wood was very susceptible to decay (T. L. Highley, *unpublished data*).

The ammoniacal nitrogen level in blocks treated with 1% NH_3 and heated was almost 3 times that of untreated blocks (Table 6). Heated wood retained more NH_3 than unheated, and retention was greater with the higher concentrations of treating solutions. Considerable amounts of ammoniacal nitrogen were removed by leaching in the acid. Weight losses of treated pine blocks leached in the acid to lower the ammoniacal nitrogen content and decayed by *P. monticola* were similar to the untreated controls (Table 7).

DISCUSSION.—Improved resistance of NaOH- or NH_3-treated wood to brown rot in decay tests to simulate aboveground decay conditions could not be attributed to destruction of thiamine or other nutrients in wood. Based on tests with synthetic culture medium, the amount of thiamine in normal wood is not sufficient to support optimum fungus growth.

A number of reasons could account for thiamine-requiring fungi attacking thiamine-deficient wood: (i) The thiamine requirement is eliminated by provision in wood of a precursor or of a metabolite for the synthesis of which the vitamin is essential (3); (ii) other growth-promoting substances in wood may replace thiamine in the metabolic processes of the fungus [Jennison & Henderson (8) found that many wood-decaying Basiodiomycetes can substitute biotin for thiamine, suggesting that fungi have more than one metabolic pathway]; (iii) vitamin requirements vary with the composition of the medium (3); a thiamine requirement on a rather simple synthetic medium may not necessarily imply a thiamine requirement on a complex subtrate such as wood; and (iv) sources of thiamine outside of wood are utilized.

The inability of the brown-rot fungi to decay NaOH-treated blocks under low-decaying-promoting conditions is apparently due to the increased pH of wood. The pH is not sufficiently lowered by leaching

TABLE 6. Ammoniacal nitrogen in acid-leached and unleached ammonia-treated pine

NH_3 treatment level	NH_3 nitrogen in unleached pine[a] (mg/g)	NH_3 nitrogen in 0.1 N H_2SO_4-leached pine[a] (mg/g)
Heated[b]		
1%	3.3	1.6
5%	4.9	2.6
10%	6.4	2.7
Unheated		
1%	1.5	0.8
5%	2.9	1.2
10%	2.7	1.3
Control	1.3	0.8

[a] Five blocks/150 ml 0.1 N H_2SO_4 leached on a shaker for 24 hr.
[b] Blocks heated at 100 C for 1 hr.

TABLE 7. Effect of leaching in acid on decay by *Poria monticola* in ammonia-treated pine

Treatment	% Weight loss in:[a] Unleached blocks	Blocks leached in 0.01 N HCl
1% NH_3 + heat[b]	0	20
Untreated control	23	21

[a] Average weight loss for 10 blocks/treatment after 10 weeks.
[b] Blocks heated at 100 C for 1 hr.

in water to permit establishment of a fungus such as *Poria monticola,* which is very sensitive to an alkaline medium. Lowering the pH of NaOH-treated wood by leaching in acid made it possible for *P. monticola* to decay treated blocks as readily as untreated blocks. The white-rot fungi, *Polyporus versicolor* and *Peniophora* "G", not as sensitive to alkaline conditions, readily decayed NaOH-treated wood under low-decay conditions without the blocks being leached (T. L. Highley, *unpublished data*). When the brown rotter grew abundantly as in the soil-block test, resistance of NaOH-treated wood was overcome, presumably because the acids produced by the fungus are sufficient to neutralize the alkali. Thus, when the fungus becomes established on a block, it can spread.

Improved resistance of NH_3-treated wood exposed to brown-rot attack with low-decay conditions is associated with the higher ammoniacal nitrogen content in the wood after treatment. NH_3-treated blocks heated after treatment retained more ammoniacal nitrogen than did unheated blocks; this probably accounts for the greater resistance of heated blocks. The lowering of the ammoniacal nitrogen of treated wood by leaching in acid permitted as much decay by the brown-rot fungus *P. monticola* in NH_3-treated wood as in untreated controls.

It was not established as to how the increased ammoniacal nitrogen suppressed brown-rot growth. Leal et al. (10) cite many instances of the deleterious effects of NH_3 on fungus growth. Formation of toxic ammonia compounds with wood constituents is another possibility. Although the NH_3 treatment did not greatly raise the pH of the wood after air drying, NH_3 combined as NH_4 + salt could buffer the wood on the unfavorable side of the growth optimum and act as a supplier of basic NH_4 + ions. The NH_3-treated wood, like NaOH-treated wood, is susceptible to brown-rot attack when exposed to a high-decay condition (T. L. Highley, *unpublished data*). Evidently, a vigorously growing fungus can overcome the inhibiting effects of the ammoniacal nitrogen and, as with the NaOH-treated wood, when the fungus becomes established in a block, it can easily spread.

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