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Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives

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Abstract

We previously proposed that extractives in highly durable heartwood may protect wood against fungal colonization and subsequent degradation by dual mechanisms: the extractives have some fungicidal activity and are also free radical scavengers (antioxidants). In short-term laboratory decay tests using two different wood species and decay fungi, the antioxidant 2,6-dimethyl-di-*tert*-butyl-4-methylphenol (BHT) alone had little or no preservative effect. In contrast, the combination of BHT with different organic commercial biocides always showed an increase in efficacy compared to the organic biocide alone. Consequently, we conclude that the combination of a commercial antioxidant and biocide is synergistic. This implies that extractives may protect wood by more than simply being fungicidal. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Antioxidants; Extractives; Heartwood; Natural durability; Wood decaying fungi

1. Introduction

Wood and lumber products are degraded by many organisms, principally fungi and insects. Consequently, lumber should be treated with biocides when wood will be wetted frequently or placed in ground contact. The majority of gymnosperm lumber manufactured in the US is produced in the southeastern region and over half of all southern yellow pine (SYP) dimension stock is treated in some manner, principally with a preparation referred to as chromated copper arsenate (CCA). The environmental hazards associated with the use of both chromium and arsenic salts will likely adversely limit its future use. Indeed, its deployment has already been greatly reduced in the Hawaiian Islands, and its use in above-ground applications is banned in Denmark, Sweden, Germany and other countries. Consequently, a need exists for developing alternative, more environmentally-benign wood preservatives.

One approach in developing new wood preservatives is to understand why the heartwood of certain tree species has considerable natural resistance against insect attack and fungal colonization and degradation. Consequently, we have studied the role which extractives, particularly stilbenes (Schultz et al., 1997), play in the natural durability of some angiosperm heartwoods (Schultz et al., 1995). Surprisingly, we found that extractives in highly durable heartwood have very poor fungicidal activities compared to commercial biocides. In further work (Schultz et al., 1998), we hypothesized that extractives may protect heartwood against fungal colonization by a dual function: the extractives posses both fungicidal activity as well as being excellent free radical scavengers (antioxidants). This dual defense hypothesis was based on the fact that both white- and brown-rot fungi are believed to use some type of free radical species in order to initially disrupt cell walls (Backa et al., 1992, 1993; Tanaka et al., 1999; Lu et al., 1994) i.e. to increase the pore size so that the relatively large extracellular fun-

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gal enzymes can penetrate into the cell walls (Backa et al., 1993; Flournoy et al., 1993); however, the phenolic extractives in heartwood are excellent antioxidants (Larson, 1988; Cooper-Driver and Bhattacharya, 1998). Thus, although the mechanisms/enzymes by which brown- and white-rot fungi degrade wood differ, the initial step is presumed to involve some type of free radical which perturbs the cell wall (Backa et al., 1992, 1993; Tanaka et al., 1999; Lu et al., 1994), and it is this early step in the decay process which may be inhibited by the antioxidant properties of the heartwood extractives.

In preliminary tests (Schultz et al., 1998; Schultz and Nicholas, 1998), we found that the combination of the antioxidant BHT (2,6-di-*tert*-butyl-4-methylphenol), which has no fungicidal properties on its own, with two organic biocides, which were non-phenolic and thus should have no antioxidant properties, showed enhanced (i.e., synergistic) efficacy against two white-rot fungi as compared to the biocide alone. In these studies, we used a commercial antioxidant, rather than a natural antioxidant isolated from woody plants which might have both fungicidal and antioxidant properties. Decay levels were measured by either mass (Schultz and Nicholas, 1998) or strength loss (Schultz et al., 1998). Strength loss has the advantages of reducing the test time and is more indicative of incipient decay, but has much greater variability due to the wide diversity in wood strength.

The objective of this work was to further test the above hypothesis using six commercial organic biocides in two different wood-containing laboratory decay tests using both white- and brown-rot fungi and two wood species. The biocides selected for this study are known to protect wood (Nicholas and Schultz, 1995), but contain no phenolic or aromatic amine groups and thus should have no antioxidant properties.

2. Results and discussion

Table 1 gives the average strength loss experienced for wood samples treated with 2 or 5% BHT, and control (untreated) samples, then exposed to *Gloeophyllum trabeum or Trametes versicolor* in the soil- and agar-block tests (Archer et al., 1995), respectively. BHT alone provided no protection against *G. trabeum* and essentially no protection against *T. versicolor*. These results, which showed that an antioxidant alone imparts no protection, are consistent with our (Schultz et al., 1998; Schultz and Nicholas, 1998) and other researchers (Highley, 1982; Green et al., 1997; Green and Kuster, 1999) earlier results.

The combination of the biocide propiconazole at four different retentions, either alone or with 5% BHT, was examined using SYP sapwood and *G. trabeum* in the soil-block test (Table 2). The average strength loss of samples treated with 5% BHT was the same as the untreated control sets, again indicating that BHT alone provides no protection. Comparison of samples treated with propiconazole alone, versus samples treated to the same biocide retention but with 5% BHT co-added, showed that the addition of BHT increased the biocide's efficacy in this short-term laboratory test. Since BHT alone had no effect, we concluded that the combination of an antioxidant and biocide gave enhanced (i.e., synergistic) efficacy.

In a final study, six organic biocides were selected from a recent list (Nicholas and Schultz, 1995) of potential wood preservatives. Wood samples were treated to four retentions, with or without 5% BHT co-added, and tested using either the SYP/soil-block test with *G. trabeum* or the aspen/agar-block test with *T. versicolor*, with the average strength loss measured (Fig. 1). The addition of 5% BHT increased the biocide's efficacy in all cases.

Based on the above data (Table 2 and Fig. 1) and our earlier results (Schultz et al., 1998; Schultz and Nicholas, 1998), we concluded that the addition of the commercial antioxidant BHT to an organic biocide increased the biocide's efficacy in protecting wood against brown- or white-rot fungi in short-term laboratory decay tests. Furthermore, these results are consistent with our hypothesis that heartwood extractives may protect wood against fungal colonization and sub-

Table 1
Average % strength loss for sapwood samples treated with 2 or 5% of the antioxidant BHT after exposure to *G. trabeum* or *T. versicolor*[a]

Treatment	*G. trabeum*/SYP samples		*T. versicolor*/aspen samples	
	BHT retention (kg m^{-3})	Average % strength loss	BHT retention (kg m^{-3})	Average % strength loss
2% BHT	10.2	96.6 (0.8)	9.6	83.8 (8.4)
5% BHT	25.6	93.3 (1.4)	24.1	76.9 (18.1)
Control set 1	0	96.5 (0.7)	0	88.0 (6.8)
Control set 2	0	96.3 (0.09)	0	95.6 (3.2)

[a] The results are an average of five replicates, and the standard deviations are in parentheses.

sequent degradation by having both fungicidal and antioxidant properties. It is also possible that other non-biocidal properties of extractives could contribute to natural durability. Thus, it appears that natural durability is relatively complex (Schultz and Nicholas, 2000) and due to more than just the bioactivity of the heartwood extractives.

In this study, relatively high levels of the antioxidant BHT was used relative to the biocide levels. However, treatment with 5% BHT gives only about 20% of the levels of phenolic (antioxidant) groups found in the highly durable heartwood of osage orange (Schultz and Nicholas, 2000) (*Maclura pomifera* (Raf.) Schneid.). For practical applications, the antioxidant levels needed for an effective wood preservative system is probably highly dependent on both the type and amount of antioxidant and fungicide. Also, while this study found that BHT alone provides no protection under the test conditions, we earlier found (Schultz and Nicholas, 2000) that BHT alone will provide modest protection for short (2- or 3-week) incubation times. This is undoubtedly due to the artificial and ideal fungal conditions of this laboratory test which exposes wood samples to a large number of free radicals in a short time (Backa et al., 1992, 1993), and thus, rapidly depletes the antioxidant. Until depleted, however, the BHT may prevent the fungal-generated radicals from perturbing the cell wall. Under actual field exposure conditions, especially above-ground, it is likely that antioxidants may be effective for longer periods since wood would be exposed to lower levels of free radicals.

Younger heartwood is usually more decay resistant than aged heartwood (Scheffer and Cowling, 1966), reportedly due to a slow degradation of the extractives which lowers their toxicity. Based on our results, we suggest that this loss in durability may be partly due to the extractives simply undergoing slow autoxidation over time and thus having a lessened antioxidant capacity.

This hypothesis needs to be evaluated by further laboratory tests with other fungi, wood species and antioxidants. We are also interested in investigating this hypothesis using natural extractives, provided that an experimental method which separates out the fungicidal and antioxidant influences (and other possible properties) can be developed. Finally, we need to point out that we believe natural durability is extremely complex and additional factors, besides this dual fungicidal/antioxidant action, may be involved.

The long-term goal of this basic research project was to develop more environmentally-benign wood preservatives. We were thus pleased that an applied idea may be developed from this basic project (Schultz and Nicholas, 1998, 1999a,b). To evaluate this, we have recently installed a few preliminary long-term outdoor exposure samples treated with biocide/antioxidant mixtures, and will report on these samples in the future.

3. Experimental

Sapwood portions of kiln-dried, defect-free SYP (*Pinus* spp.) or aspen (*Populus* spp.) lumber were cut into 19×19 mm ($r \times t$) sticks, with growth rings oriented parallel to one edge. Wafers, 5 mm thick (l), were cut sequently from the sticks and numbered. Ten sequential wafers were used for each biocide/treatment level, with the even-numbered samples serving as controls (treated with the biocide and then sterilized in an autoclave, but not exposed to the fungus) and the matched, odd-numbered samples exposed to the fungus. Wood wafers were treated using a full-cell process

Table 2
Average % strength loss for SYP sapwood samples treated with the biocide propiconazole (Prop.) and exposed to the brown-rot fungus *G. trabeum*[a]

Treatment	Average retention (kg m^{-3})		Average % strength loss
	Propiconazole	BHT	
Control set 1	0	0	96.7 (0.5)
Control set 2	0	0	98.3 (2.1)
5% BHT	0	28.8	97.3 (1.9)
0.01% Prop.	0.1	0	95.5 (0.8)
0.03% Prop.	0.2	0	93.9 (1.5)
0.06% Prop.	0.3	0	69.9 (16.8)
0.12% Prop.	0.7	0	30.8 (6.7)
0.01% Prop. + 5% BHT	0.1	28.6	39.1 (2.2)
0.03% Prop. + 5% BHT	0.2	29.1	41.1 (2.8)
0.06% Prop. + 5% BHT	0.3	28.6	30.6 (6.2)
0.12% Prop. + 5% BHT	0.7	29.1	12.7 (5.1)

[a] Results are an average of five replicates, and the standard deviations are in parentheses.

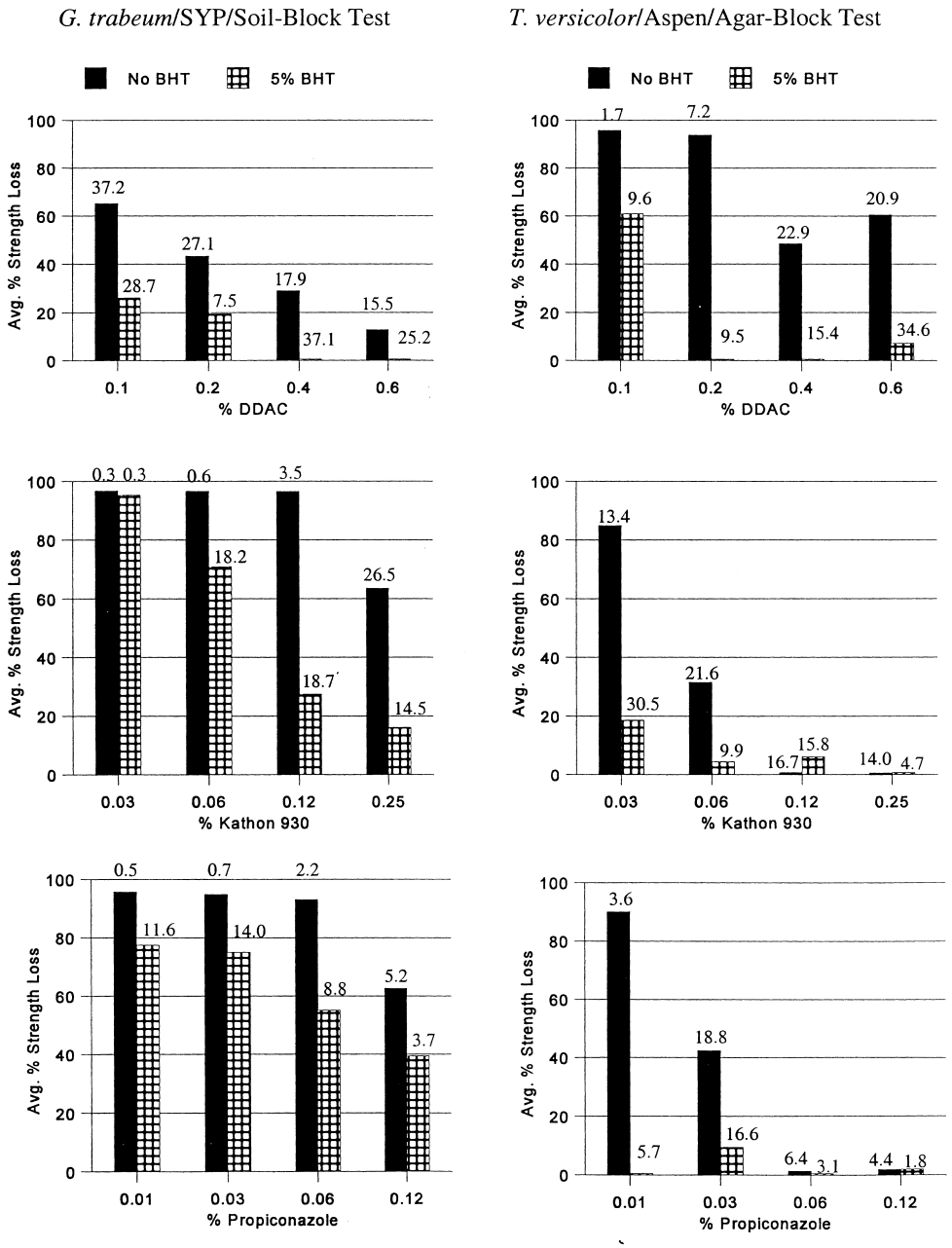

Fig. 1. Average % strength loss of five replicates for sapwood samples treated with four levels of organic biocides, both with and without 5% BHT. The SYP samples were exposed to *G. trabeum* (left column) and the aspen samples to *T. versicolor* (right column). The numbers above the bars are the standard deviations.

(15 min vacuum at 28 in. Hg, followed by 100 psig pressure for 30 min) in a mini-treating cylinder. Sample sets were treated with four retention levels of the biocide alone, both with and without 5% BHT co-added.

Toluene was used as the solvent to prepare all formulations. The control samples were untreated. The commercial biocides were all organic compounds which have shown promise as wood preservatives (Nicholas and Schultz, 1995) and are: propiconazole [(2*RS*,4*RS*)-2-(2,4-dichlorophenyl)-2-[1-1*H*-(1,2,4-triazole)methyl]-4-propyl-1,3-dioxolane]; DDAC [didecyldimethylammonium chloride]; Kathon 930™ [4,5-dichloro-2-*n*-octyl-4-isothiazolin-3-one]; tebuconazole [(3*RS*)-5-(4-chlorophenyl)-2,2-dimethylethyl-3-1*H*-[1,2,4-triazole]methyl)-3-pentanol]; chlorothalonil [2,4,5,6-tetrachloroisophthalonitrile]; and IPBC [3-iodo-2-propynyl butyl carbamate]. BHT, obtained from Aldrich, was chosen as the antioxidant based on the assumption that it would have little or no biologi-

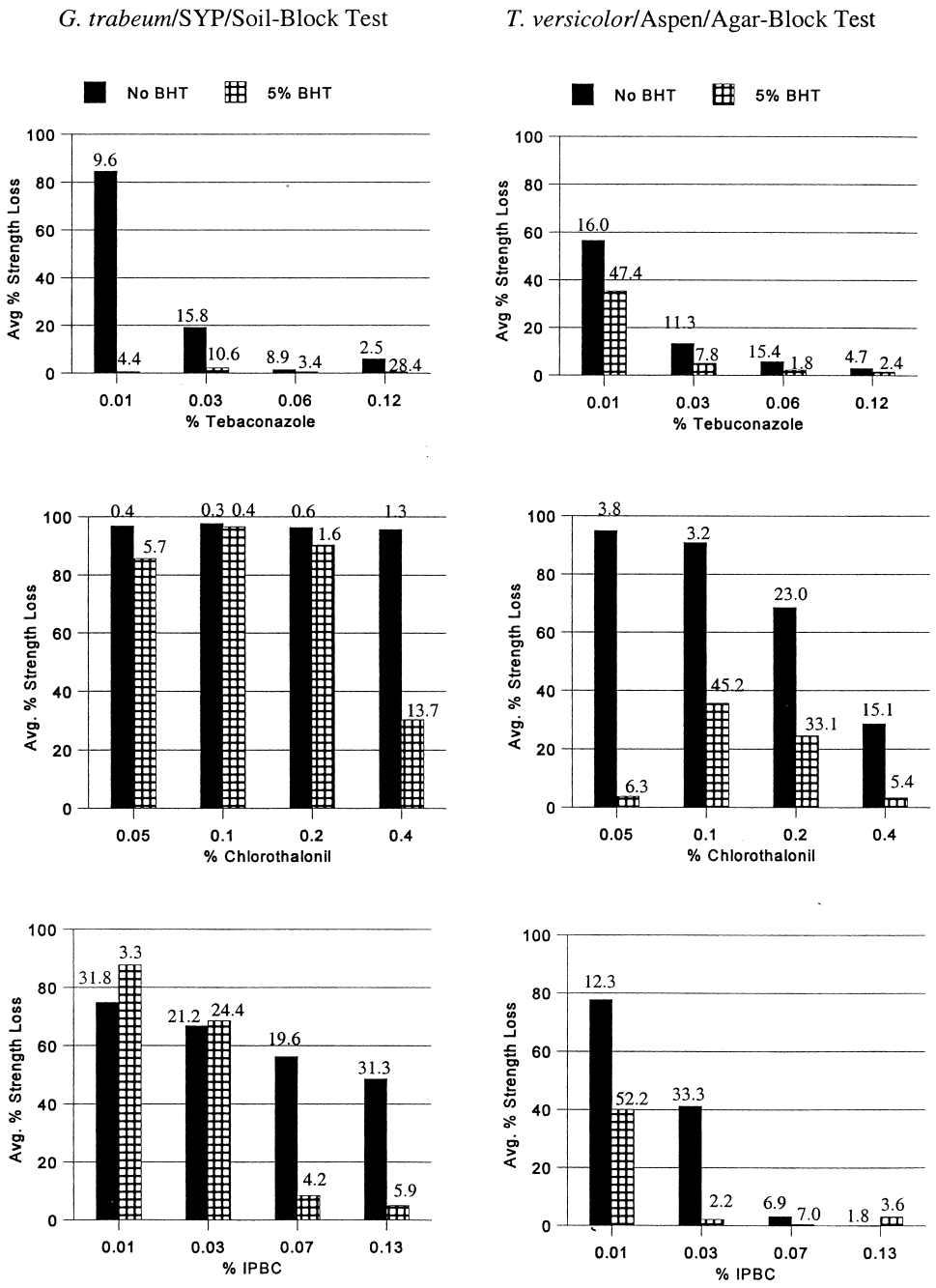

Fig. 1 (*continued*).

cal activity. The SYP samples were evaluated using the soil-block test (Archer et al., 1995) with the brown-rot fungus *G. trabeum* (ATCC 11539) and the aspen samples tested using the agar-block test (Archer et al., 1995) with the white-rot fungus *T. versicolor* (ATCC 12679). Lab wood decay data often do not match results run at a latter time or at another lab (deGrout and Evans, 1999; Leithoff et al., 1999; Smith and Gjovik, 1972) due to differences in the wood, soil, fungal activity, etc. Consequently, matched sets (samples with and without BHT using the same biocide) were run at the same time so that experimental variables would be as similar as possible. After a 4-week incubation period, the samples were saturated with water. The compression strength of the exposed and unexposed matched samples were then measured and used to determine compression strength loss (Nicholas and Jin, 1996) in the radial direction at 5% compression strain. The average strength loss of five replicate samples was used as a measure of decay. Due to the wide variabil-

ity in strength typically seen with wood, small negative numbers were sometimes observed and assumed to be 0.

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References

Archer, K., Nicholas, D.D., Schultz, T.P., 1995. Forest Products J. 45, 86, and references therein.

Backa, S., Gierer, J., Reitberger, T., Nilsson, T., 1992. Holzforschung 46, 61.

Backa, S., Gierer, J., Reitberger, T., Nilsson, T., 1993. Holzforschung 47, 181.

Cooper-Driver, G.A., Bhattacharya, M., 1998. Phytochemistry 49, 1165, and references therein.

deGrout, R.C., Evans, J., 1999. Forest Products J. 49(9), 59, and references therein.

Flournoy, D.S., Paul, J.A., Kirk, T.K., Highley, T.L., 1993. Holzforschung 47, 297.

Green, F., Kuster, T.A., 1999. International Research Group Paper IRG/WP-10321.

Green, F., Kuster, T.A., Highley, T.L., 1997. International Research Group Paper IRG/WP-10203.

Highley, T.L., 1982. Material und Organismen 17, 205.

Larson, R.A., 1988. Phytochemistry, 27, 969, and references therein.

Leithoff, H., Deek, R.-D., Borck, V., Göttsche, R., Kirk, H., Grinda, M., 1999. International Research Group Paper IRG/WP-20176.

Lu, J., Goodell, B., Liu, J., Enoki, A., Jellison, J., Tanaka, H., Fekete, F., 1994. International Research Group Paper IRG/WP-10086.

Nicholas, D.D., Jin, Z., 1996. International Research Group Paper IRG/WP-20083.

Nicholas, D.D., Schultz, T.P., 1995. Wood preservation in the 90s and beyond. Forest Products Society, Madison, WI pp. 169.

Scheffer, T.C., Cowling, E.B., 1966. Ann. Review of Phytopath. 4, 147.

Schultz, T.P., Harms, W.B., Fisher, T.H., McMurtrey, K.D., Minn, J., Nicholas, D.D., 1995. Holzforschung 49, 29, and references therein.

Schultz, T.P., Nicholas, D.D., 1998. U.S. Patent, 5,730,907.

Schultz, T.P., Nicholas, D.D., 2000. Lignin: historical, biological, and materials perspectives. American Chemical Society, Washington DC. ACS Symp. Ser. 742 (Chapter 8).

Schultz, T.P., Nicholas, D.D., 1999a. Enhanced wood preservative composition. U.S. Patent, 5,944,884.

Schultz, T.P., Nicholas, D.D., 1999b. Enhanced wood preservative composition. U.S. Patent App., Ser. 09/336,334.

Schultz, T.P., Nicholas, D.D., Fisher, T.H., 1997. Recent Res. Dev. in Agric. Food Chem. 1, 289, and references therein.

Schultz, T.P., Nicholas, D.D., Minn, J., McMurtrey, K.D., Fisher, T.H., 1998. International Research Group Paper IRG/WP 98-30172.

Smith, R.S., Gjovik, L.R., 1972. Wood and fiber 4, 170, and references therein.

Tanaka, H., Itakura, S., Enoki, A., 1999. Holzforschung 53, 21.