Termite-Resistant Heartwood: The Effect of the Non-Biocidal Antioxidant Properties of the Extractives (Isoptera: Rhinotermitidae)

by

Kevin W. Ragon, Darrel D. Nicholas & Tor P. Schultz*

ABSTRACT

Due to a need to develop more environmentally benign methods to control termites, many researchers have studied extractives isolated from naturally resistant heartwood and some plants. While it has long been recognized that extractives affect termite feeding, the relationship between total extractives, extractive types and termite resistance remains unclear. Generally, researchers have proposed that the extractives are toxic and/or repel termites. We recently hypothesized that both the toxicity and antioxidant properties of extractives affect termites. In this paper we report on continued studies with a benign antioxidant, butylated hydroxytoluene (BHT). Use of an artificial and non-toxic antioxidant allowed us to definitively test our hypothesis without the use of natural extractives where the test results would be confounded by both toxicity and antioxidant properties. Southern pine (*Pinus* spp.) [SP] blocks were treated with 0, 1, 2, 4, and 6% BHT, with these blocks exposed to two termite species using a no-choice test. With *Reticulitermes flavipes* Kollar (Eastern subterranean termite), a 2% treatment resulted in 0.9% mass loss, only slight attack and 100% termite mortality. BHT also affected *Coptotermes formosanus* Shiraki (Formosan subterranean termite), with a 4% treatment resulting in only 3.3% mass loss, no attack on any wafers and 100% termite mortality. An initial field test at Viance's Hilo Formosan Termite Test Site using SP blocks treated with 0, 1, 2 and 3% BHT showed that treatment with 2 and 3% BHT greatly reduced Formosan degradation after six months of exposure. We conclude that treating wood with the non-toxic and artificial antioxidant BHT at a sufficient level reduces subterranean termite attack and subsequent damage. Furthermore, this study suggests that the well-known

Forest Products Department/FWRC, Mississippi State University, MS 39762-9820 USA
*Corresponding Author: tschultz@cfr.msstate.edu.

antioxidant properties of extractives in termite resistant heartwood, along with the extractives' inherent toxicity, affect subterranean termites.

Keywords: Antioxidants, butylated hydroxytoluene (BHT), extractives, heartwood, natural resistance, termites

INTRODUCTION

Subterranean termites cause extensive damage to wood and cellulosic products in temperate and tropical climates. Protection of wood products and structures against termite attack employs various bioactive compounds. However, many of these compounds negatively impact other non-target organisms, persist in the environment, and may migrate into groundwater. Therefore, in the past decade many termite bioactive compounds have been voluntarily withdrawn by manufacturers, which has accelerated the need for green, non-biocidal termite control technologies.

It is well known that extractives in termite-resistant woods and other plants affect subterranean termites. The exact mechanism(s) by which extractives repel, deter or kill termites is unclear, but some researchers have assumed that extractives have some termite toxicity and/or repellency properties. The activity of most natural extractives against termites is generally low, however, relative to commercial insecticides.

In reviewing studies on the effect of extractives on termites, we were struck by the fact that many studies have separately shown that the same class of extractives often has both termiticide and free radical scavenging (antioxidant) properties. This includes flavonoids (Morimoto *et al.* 2006, Doi *et al.* 2002, Chen *et al.* 2004, Dietrichs & Hausen 1971, Reyes-Chiolpa *et al.* 1995, Rie *et al.* 2005), tannins (Fava *et al.* 2006, Oszmianski *et al.* 2006), quinones (Arndt 1968a and 1968b, Ganapaty *et al.* 2004, Dietrichs & Hausen 1971, Carter *et al.* 1978, Sizova & Popova 2006), stilbenes in general (Morimoto *et al.* 2006, Torres *et al.* 2003) and the stilbene chlorophorin (Arndt 1968, Minn *et al.* 1996). [For various articles on extractives with antioxidant properties, see chapters in Ho *et al.* 1992.]

That extractives often have antioxidant properties and affect termites may be due to: 1) coincidence, 2) evolutionary design, or 3) unknown factors. As wood preservationists our research into natural fungal durability (Schultz

& Nicholas 2000, 2002) has made us aware of nature's many effective and elegant solutions; thus, we favored the second possibility.

Antioxidants may interfere with lignocellulosic digestion by the termites and their symbiotic microbes. For example, antioxidants might obstruct the multiple single-electron cyclic redox mechanisms involved in the complex conversion of the woody polysaccharides into acetate, which is the calorie energy source for termites, and the methane by-product (Abe *et al.* 2000, Kappler & Brune 2002, Ohkuma 2003). Based on this and the excellent antioxidant properties of many extractives we (Schultz *et al.* 2008) recently hypothesized that subterranean termites have evolved the ability to detect heartwood, which contains extractives with a certain level of antioxidant activity. Consequently, termites will avoid wood that contains some minimal level or more of antioxidant compounds, whether the antioxidants are natural extractives or man-made, and toxic or benign.

Thus, it may not be solely the amount and toxicity of extractives in a heartwood sample that gives heartwood some termite resistance, but the extractives' dual toxicity and antioxidant properties. Extractives may have other properties, in addition to toxicity and antioxidant activity, that could also affect termites, or two or more factors could act together, perhaps synergistically.

In preliminary studies we conducted a few laboratory American Wood Preservers' Association (AWPA) Standard E-1 choice tests with *R. flavipes*, employing two SP sapwood wafers in each container, with one wafer treated with 1 or 3% BHT and the other wafer untreated (Schultz *et al.* 2008). We chose the commercial antioxidant butylated hydroxytoluene (BHT) because it is non-toxic. [BHT is so benign that it is classified as a GRAS (Generally Recognized as Safe) compound by the US Food and Drug Administration (FDA) and is added to human foodstuffs.] By employing a non-toxic and artificial antioxidant we could avoid the dual toxicity/antioxidant properties that most heartwood extractives have. Furthermore, since BHT has only been made for 50 or so years it is unlikely that termites have evolved to avoid it in such a relatively short period.

In the preliminary study we found that 1- and 3% BHT-treated wood had no termite damage, and further that all termites exposed to jars with a BHT-treated wafer had 100% mortality (Schultz *et al.* 2008). This preliminary study consisted of only a small number of samples and two BHT treatment levels, however. Furthermore, only one termite species was examined, and the termites employed had been stored in a container for a few months prior to the experiment which may have affected their vitality.

The purpose of this study was to further test our antioxidant hypothesis by laboratory no-choice experiments with SP sapwood wafers treated with a greater range of BHT levels and employing two termite species. In addition, an initial field test was conducted with BHT-treated spruce pine (*Pinus glabra* Walt.) blocks exposed to *C. formosanus*.

METHODS AND MATERIALS

Laboratory No-choice Tests

SP wafers, 25 x 25 x 6 mm (t x r x l), were treated with 1, 2, 4, and 6% BHT dissolved in isopropanol using a vacuum/atmospheric pressure method. The retentions were calculated based on initial and final weight, with the control samples treated using only the solvent. The samples were air-dried for 21 days, then used in the standard AWPA Standard E-1 no-choice termite test with five replicate samples at each treatment level and each termite species, *R. flavipes* or *C. formosanus*. After 28 days of exposure to the termites the wafers were visually rated based on the AWPA scale where a 10 is no attack; 9.5 trace, surface nibbles; 9 slight attack, up to 3% of cross section area affected, etc. down to a 0 or failure. The samples were then air-dried and the mass loss and termite mortality determined.

Field Exposure Efficacy Against *C. formosanus*

Spruce pine quartersawn sapwood samples, 1.9 x 3.8 x 19.3 cm, were treated by a full cell process (-95 kPa vacuum for 20 minutes, then 850 kPa for 30 min.) using 0, 1, 2, or 3% BHT dissolved in toluene, with four or five replicate samples per treatment. The BHT retentions were determined by the pre- and post-treatment weights. The samples were then air-dried and shipped to Viance's Formosan Hilo Termite site and placed on a modified ground-proximity test in July 2007. Samples were rated in January 2008 using the AWPA Standard E-7 scale where a 10 is no attack; 9.5 trace, surface nibbles; 9 slight attack, up to 3% of cross section area affected, etc. down to a 0 or failure.

Table 1. Average mass loss, ratings, and mortality results from the AWPA Standard E-1 no choice termite test with *R. flavipes*, based on the average of five southern pine wafers treated with the artificial and benign antioxidant BHT.

Treatment, % BHT	Avg. Retention, kg/m^3	Avg. % Mass Loss	Average Rating[1]	Avg. % Mortality
Control	0	27.8	0.8	<10
1	3.4	21.8	4	17
2	6.9	0.9	9	100
4	14.1	1.1	10	100
6	21.8	1.3	10	100

[1]Samples in this and the below tests were rated using the AWPA Standards E-1 and E-7, where a 10 is no attack; 9.5 trace, surface nibbles; 9 slight attack, up to 3% of cross section area affected, etc. down to a 0 or failure.

Table 2. Results from the AWPA Standard E-1 no-choice termite test with *C. formosanus*, based on the average of five southern pine wafers treated with BHT.

Treatment, % BHT	Avg. Retention, kg/m^3	Avg. % Mass Loss	Average Ratings	Avg. % Mortality
Control	0	32.1	0.8	<10
1	3.5	21.9	4	17
2	6.7	6.7	6	65
4	13.9	3.3	10	100
6	21.9	4.1	10	100

RESULTS AND DISCUSSION

AWPA No-choice Laboratory Tests

Table 1 shows the average results from the no-choice test with *R. flavipes*. Wafers treated with only the solvent had a 27.8% mass loss, with one wafer rated a 4 and the rest 0, and termite mortality of less than 10%. In contrast, a 2% BHT treatment reduced the average mass loss to 0.9%, with only one wafer having minimal termite attack and 100% termite mortality. The higher treatments, 4 and 6%, resulted in no wafer degradation and 100% termite mortality.

These results are similar to our preliminary study with the same termite species, except that treatment levels of 2% or greater were necessary while in our earlier study a 1% treatment was effective (Schultz *et al.* 2008). This could possibly be due to the three months that the termites were stored prior to starting the preliminary study, which could have affected termite vitality.

C. formosanus results are given in Table 2. The control wafers had an average mass loss of 32.1% with four of the five wafers rated as 0 and only one a 4, and termite mortality was less than 10%. For the BHT-treated wafers, the Formosan termites were affected, but higher levels were required than

Table 3. Average termite attack on spruce pine samples treated with BHT and then exposed to Formosan termites for six months at Viance's Hilo, HI Formosan Termite site in a modified ground-proximity test. Each value is the average of five replicates, with a "10" showing no attack; 9.5 slight; etc., down to a 0 for failure.

Treatment, % BHT	Average Retention, kg/m^3	Number of Replicates	Average Rating	Range of Ratings
0	0	5	6.0	4 - 7
1	3.1	4	7.5	6 - 9
2	6.3	5	8.9	7 - 9.5
3	9.8	4	8.9	8 – 9.5

observed with Eastern subterranean termites. For example, a 2% BHT treatment resulted in 100% mortality, an average 9 rating and about 1% mass loss with *R. flavipes*. The same treatment with *C. formosanus* resulted in only 65% mortality, an average 6.0 rating and 6.7% mass loss. Wafers treated with 4 or 6% BHT all had 100% *C. formosanus* mortality, no wafer degradation, and less than 5% mass loss.

Field Exposure Efficacy Against *C. formosanus*

The average and range of Formosan termite ratings on the spruce pine samples treated with BHT and exposed for six months are shown in Table 3. Samples not treated with BHT had an average 6.0 rating, and 1% BHT-treated samples had a 7.5 rating. Samples treated with 2 or 3% BHT performed better with 8.9 ratings. These short duration field results are similar to the laboratory no choice results discussed above, where treatment with 2% BHT greatly reduced termite feeding.

A literature search found only one prior article that indirectly supports our results that antioxidants affect termite behavior. Grace (1990) showed that adding BHT to *Gloeophyllum trabeum* fungal extracts slightly decreased subterranean termite trail-following activity, and the more active analogue BHA totally inhibited the activity. However, trail-following is not necessarily related to repellency, so these results should not be considered as direct confirmation.

We are continuing field studies, examining additional BHT-treated wood samples and the effect of termite migration through antioxidant-treated soil, and hope to present additional results shortly.

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REFERENCES

Abe, T., D.E. Bignell & M. Higaski 2000. Termites: Evolution, Sociality, Symbioses, Ecology. Kluwer Academic Publishers, Boston, US.

Arndt, A. 1968. Determination of the biological activity of small quantities of wood extractives against the subterranean termite, *Reticulitermes*. Holzforschung 22:104-109.

Carter, F. L., A.M. Farlo & J.B. Stanley. 1978. Termiticidal components of wood extracts: 7-Methyljuglone from *Diospyros virginiana*. Journal of Agricultural and Food Chemistry 26:869-873.

Chen K., W. Ohmura, S. Dio & M. Aoyama 2004. Termite feeding deterrent from Japanese larch wood. Resource Technology 95:129-134.

Dietrichs, H.H. & B.M. Hausen. 1971. Effective substances from Dalbergia species. Holzforschung 25:183-187.

Doi, S., M. Aoyama, S. Ohara, & W. Omura 2002. Insect repellent containing flavonoids and extraction of them from plants. Patent Application JP 2001-169175, also see Chemical Abstracts CAN 136:179318.

Fava, F., M. Monteiro de Barros, E. Stumpp & F. Ramao Marceli Jr. 2006. Aqueous extract to repel or exterminate termites. Patent Application WO 2005-BR173 20050824.

Ganapaty, S., P. S. Thomas, S. Fotso and H. Laatsch 2004. Antitermitic quinones from *Diospyros sylvatica*. Phytochemistry 65:1265-1271.

Grace, K.J., B. Goodell, W.E. Jones, V. Chandhoke & J. Jellison. 1992. Evidence for inhibition of termite (Isoptera: Rhinotermitidae) feeding by extracellular metabolites of a wood decay fungus. Proceedings, Hawaiian Entomological Society 31:249-252.

Ho, C-T., C.Y. Lee & M-T Haung. 1992. Phenolic Compounds in Food and Their Effects on Health. II. Antioxidants and Cancer Prevention. ACS Symp. Series 507, American Chemical Society, Washington, DC.

Kappler, A. & A. Brune 2002. Dynamics of redox potential and changes in redox state of iron and humic acids during gut passage in soil-feeding termites (*Cubitermes* spp.) Soil Biology & Biochemistry 34:221-227. CAN 136:291939.

Minn, J., W.D. Daly, I.I. Negulescu, K.D. McMurtrey & T.P. Schultz. 1996. Antioxidant properties of the hydrophobic stilbenol chlorophorin. Journal of Agricultural and Food Chemistry 44:2946-2947.

Moriminto, M., Y. Ohta, T. Ihara, K. Arima, H. Fukumoto, W. Chavasiri & K. Komai 2006. Insect antifeedants in Thailand plant extractives against the common cutwork, *Spodoptera litura*, and a subterranean termite, *Reticulitermes speratus*. Japan Journal of Environmental Entomology and Zoology 17:1-7.

Ohkuma, M. 2003. Termite symbiotic systems: efficient bio-recycling of lignocellulose. Applied Microbiology and Biotechnology 61:1-9.

Oszminanski, J., A. Wojdylo, *et al.* 2006. Antioxidant tannins from Rosaceae plant roots. Polish Food Chemistry 100:579-583.

Reyes-Chilpa, R., N. Viveros-Rodriguez, *et al.* 1995. Antitermitic activity of *Lonchocarpus castilloi* flavonoids and heartwood extracts. Journal of Chemical Ecology 21:455-463.

Rie, M., *et al* 2005. Comparison of antifungal and antioxidant activities of *Acacia mangium* and *A. auriculiformis* heartwood extracts. Journal of Chemical Ecology 31:789-804.

Schultz, T.P. & D.D. Nicholas 2000. Naturally durable heartwood: Evidence for a proposed dual defensive function of the extractives. Phytochemistry 54:47-52.

Schultz, T.P. & D.D. Nicholas 2002. Development of environmentally-benign wood preservatives based on the combination of organic biocides with antioxidants and metal chelators. Phytochemistry 61:555-560.

Schultz, T.P., K.W. Ragon & D.D. Nicholas 2008. A hypothesis on a second non-biocidal property of wood extractives, in addition to toxicity, that affects termite behavior and mortality. International Research Paper on Wood Preservation, Stockholm, Sweden (in press).

Sizova, N.V., & I.Y. Popova 2006. Content of antioxidants in plant extracts obtained by supercritical extraction. Pharmaceutical Chemistry Journal 40:206-210.

Torres, P., J.G. Avila, R.D.V. Alfonso, A.M. Garcia, J.C. Marin, E. Avanda & C.L. Cespedes 2003. Antioxidant and insect growth regulatory activities of stilbenes and extracts from *Yucca periculosa*. Phytochemistry 64:463-473.