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A hypothesis on a second non-biocidal property of wood extractives, in addition to toxicity, that affects termite behavior and mortality

Tor P. Schultz, Kevin Ragon and Darrel D. Nicholas

Forest Products Laboratory
Box 9820
Forest and Wildlife Research Center
Mississippi State University
Mississippi State, MS 39762 USA

tschultz@cfr.msstate.edu

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IRG Secretariat
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A HYPOTHESIS ON A SECOND NON-BIOCIDAL PROPERTY OF WOOD EXTRACTIVES, IN ADDITION TO TOXICITY, THAT AFFECTS TERMITE BEHAVIOR AND MORTALITY

Tor P. Schultz, Kevin Ragon and Darrel D. Nicholas

Forest Products Laboratory
Box 9820
Forest and Wildlife Research Center
Mississippi State University
Mississippi State, MS 39762 USA

ABSTRACT

While it is has long been recognized that heartwood extractives affect termite behavior, the exact cause and 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 propose, based on the well known fact that many extractives have excellent antioxidant properties in addition to some termite toxicity, that: 1) the antioxidant properties of wood extractives interfere with the termite and symbiotic microbial digestion of lignocellulose; 2) thus, over time termites have learned to recognize and avoid wood with high levels of antioxidants; and 3) consequently, termites will also avoid wood treated with artificial and nontoxic antioxidants. Use of an artificial and nontoxic antioxidant will allow one to definitively test our hypothesis, while tests with natural extractives would be confounded by natural extractives often having both toxicity and antioxidant properties.

We conducted some initial tests employing a man-made and benign antioxidant, BHT. In outdoor field trials, ground-contact stakes treated with only the antioxidant had less termite degradation than the control stakes at up to three years of exposure. An initial termite choice experiment using *Reticulitermes flavipes* Kollar (Eastern subterranean termite) was run with an untreated and 1- or 3%-BHT treated wafer in each jar, along with control jars that contained two untreated wafers. No termite feeding occurred on any of the BHT-treated wafers, indicting that the non-biocidal antioxidant was a strong feeding repellent. Furthermore, while only about 2% termite mortality occurred in the jars that contained two untreated wafers, in every jar that had an untreated wafer and a second BHT-treated wafer 100% mortality occurred. Recent no-choice laboratory tests with two subterranean termite species, *R. flavipes* and *Coptotermes formosanus* Shiraki (Formosan subterranean termite), and an exterior test with BHT-treated wood with Formosan termites, confirmed our initial results.

Keywords: Antioxidant, butylated hydroxytoluene (BHT), heartwood, extractives, non-biocidal termite control, repellent, soil barrier treatment, termites, wood protection

INTRODUCTION

The degradation of wooden components in homes and other cellulosic products by termites is estimated to cost $6 billion annually in damage, repair and control in the U.S. market. Protection of wood products and structures against subterranean 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 termiticides have been voluntarily withdrawn by manufacturers, which has accelerated the need for green, non-biocidal termite control technologies.

It is well known that the extractives in many woods and other plants affect termites. However, the exact mechanism by which extractives repel, deter or kill termites is unclear. For example, a recent study showed that while the methanol-soluble extractive content in the heartwood of two tree species was positively correlated with termite resistance, "Variations in extractive components ….. explained relatively little of the variation in termite and fungal resistance." (Taylor et al. 2006). Generally, most researchers have assumed that extractives have some termite toxicity and/or repellency properties.

For many years we have studied how heartwood extractives protect wood against decay fungi (Schultz et al. 1995, Schultz and Nicholas 2000 and 2002, Schultz et al. 2005, and references therein). Based on these basic studies we proposed that extractives generally have minimal fungicidal activity but often have excellent antioxidant and metal chelator properties, and that these latter properties help prevent wood decay. While our proposed mechanism was based on the fundamental mechanisms by which decay fungi degrade wood, in outdoor ground-contact studies we noticed that stakes treated with an antioxidant/biocide combination or stakes treated with only an antioxidant not only had less decay, as expected, but also less termite degradation than stakes treated with only the biocide or untreated control stakes.

The purpose of this article is to: 1) briefly discuss earlier field results with biocide/antioxidant treated stakes that led us to initiate this termite study; 2) propose a hypothesis; 3) review prior literature that supports our hypothesis; 4) discuss the results of an initial termite choice test that suggests that a man-made, non-biocidal antioxidant is a strong feeding deterrent; 5) discuss some possible difficulties in testing our hypothesis with natural extractives; and 6) briefly discuss how our basic inquiries might have some practical applications.

PRIOR WORK

A number of years ago we proposed, and subsequently verified, that the combination of antioxidants with organic biocides was more effective at protecting wood against fungal degradation than a biocide alone (Schultz and Nicholas 2000 and 2002, Schultz et al. 2005, and references therein). While our hypothesis was developed to explain the role of heartwood extractives in protecting wood against decay fungi, wood stakes treated with biocide/antioxidant combinations and placed in ground contact at two locations where multiple species of subterranean termites native to Mississippi

(*Reticulitermes spp*.) have historically been active (Dorman Lake, MS, and Saucier, MS) were observed to have less termite degradation than stakes treated with the same retention of only the biocide (Schultz et al. 2005). We later found that the antioxidant employed, butylated hydroxytoluene or BHT, apparently also helped protect the organic biocide, chlorothalonil, against biodegradation (Schultz et al. 2006). Thus, stakes co-treated with BHT and chlorothalonil had more biocide remaining after 52 months of ground contact exposure than stakes treated with only the biocide, which was one possible explanation of why we observed increased termite efficacy.

However, we also noted that the stakes treated with BHT alone had less termite damage than the control/untreated stakes, as shown in Table 1 below.

Table 1. Average termite rating for southern pine sapwood ground-contact field stakes that were treated with only 4% BHT. Decay ratings are not given.

	Termite Ratings[1]	
<u>Exposure Time, Months</u>		
	Dorman Lake	**Saucier**
30 months, untreated	1.6	0
30 months, 4% BHT treated	9.5	6.4
33 months, untreated	0.4	0
33 months, 4% BHT treated	9.4	5.5
36 months, untreated	0	0
36 months, 4% BHT treated	9.3	3.6

[1]A rating of "10" denotes sound, a rating of "9" trace to 3% damage, etc., to a "0" rating which denotes failure.

One possible explanation for the above results is that subterranean termites are attracted to some species of decay fungi or products associated with decaying wood (*e.g*., Amburgey et al. 1977, Cornelius et al. 2002, Nan-Yao 2005, and references therein), and BHT alone imparts some protection against wood-decaying fungi. Therefore, it is possible that the greater termite efficacy of the BHT-treated stakes compared to the control stakes was due to these stakes having less decay fungi than the control stakes. Fortunately, we were still curious and skeptical, so we searched the literature and conducted some preliminary laboratory termite studies designed to test the above assumption. This led us to realize that another factor may be involved in the enhanced termite efficacy of both BHT- and BHT/biocide-treated stakes.

HYPOTHESIS

It is well documented that many of the extractives in termite-resistant heartwood have excellent antioxidant properties, in addition to having some toxicity. Further, antioxidants may interfere with lignocellulosic digestion by the termites and their symbiotic microbes. For example, antioxidants could meddle with the 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 and Brune 2002, Ohkuma 2003).

Based on the above we propose that:

- **Extractives with antioxidant properties interfere with lignocellulose digestion by termites and their associated microbes, so termites have evolved the ability to detect wood which contains a certain level of antioxidants. Consequently, termites will avoid wood that contains some minimal level or more of antioxidant compounds, whether the antioxidants are natural extractives or man-made compounds, and toxic or benign.**

Thus, we propose that it is not solely the **amount and toxicity** of extractives in a heartwood sample that gives a heartwood termite resistance, but the extractives' **combined toxicity and antioxidant properties**. Extractives may also have other properties besides toxicity and antioxidant that could also affect termites.

LITERATURE SUPPORTING OUR HYPOTHESIS

A number of studies have examined the effect of extractives on termites (*e.g.*, Arndt 1968, Harun and Labosky 1985, Taylor et al. 2006, to list only a few.) Also, many studies have examined the antioxidant properties of plant extractives (*e.g.*, Ho et al. and chapters therein, Gao et al. 2006). These studies have separately shown that the same class of extractives often has both termiticidal and antioxidant properties. This includes flavonoids (Morimoto et al. 2006, Doi et al. 2002, Chen et al. 2004, 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 and Hausen 1971, Carter et al. 1978, Sizova and Popova 2006), stilbenes in general (Morimoto et al. 2006, Torres et al. 2003) and the stilbene chlorophorin (Arndt 1968, Minn et al. 1996). Not all heartwood extractives have antioxidant properties, of course. One example is the tropoline extractives in western red cedar; however, other phenolics in western redcedar heartwood are antioxidants (*e.g*. Gardner et al. 1959).

That extractives often have antioxidant properties and affect termite behavior may be due to either: 1) coincidence, or 2) evolutionary design. As wood preservationists our research into natural durability has made us aware of nature's many effective and elegant solutions; thus, we favored the latter possibility.

In earlier studies on extractives and fungal activity we reported that most extractives which protect wood against decay fungi have only minimal fungicidal activity – with a few exceptions - but are excellent antioxidants and metal chelators and so help prevent the fungal free radical degradation of wood. Continuing this thought with termites, we wondered if these non-biocidal properties may be more important than many prior researchers realized who simply measured the extractives' toxicity to termites (Carter et al. 1978, Harun and Labsky 1985). Specifically, many researchers reported that extractives are repellents and/or feeding deterrents and toxic only if termites are forced to have direct or indirect contact with extracts (Rudman and Gay

1961, Reyes-Chilpa et al. 1995, Blaske and Hertel 2001, Chen et al. 2004, Ganapaty et al. 2004, Morimoto et al. 2005). We feel that this repellent effect may be one of the key aspects of the natural durability of the heartwood of some species against termites.

LABORATORY STUDIES SHOWING *R. flavipes* AVOIDS WOOD TREATED WITH AN ARTIFICAL AND NON-BIOCIDAL ANTIOXIDANT

To test our hypothesis we decided to conduct a choice test where two wood wafers are present in one bottle, with one wafer treated with a man-made nontoxic antioxidant and the other wafer untreated or treated with the solvent employed to dissolve the antioxidant. We chose the commercial antioxidant butylated hydroxytoluene (BHT) because it was only developed about 50 years ago and, therefore, termites have had little exposure to it compared to the multiple millennia that termites have encountered heartwood extractives. Also, BHT has no biological activity that we are aware of. Indeed, BHT is classified as a GRAS (Generally Recognized as Safe) compound by the US Food and Drug Administration (FDA) and so about 20% of the BHT produced annually is added to human foodstuffs. Since BHT is benign to humans, as per the US FDA, we felt that it should likely also be nontoxic to the microbes in the termite gut (*e.g.*, Trevors et al. 1981). Finally, the low vapor pressure and high hydrophobicity of BHT would likely prevent any sublimation or water-borne migration that could adversely affect the test.

Defect-free SYP sapwood was first cut into 2.54 cm x 2.54 cm sticks and then 0.64 cm thick (longitudinal direction) wafers were cut. Four wafers were treated with 1% and six wafers with 3% BHT dissolved in isopropanol using a full-cell vacuum/atmospheric pressure treatment. In addition, four wafers were treated with isopropanol alone and six wafers were untreated. After treatment the wafers were air-dried.

The wafers were employed in a laboratory termite choice test (AWPA E1-06). One set consisted of four bottles each containing two wafers, one treated with 1% BHT and the other treated with only isopropanol. The second set consisted six bottles each with one wafer treated with 3% BHT and the other wafer untreated. Three bottles were also set up with two untreated wood wafers each to determine if there was any difference in the degradation of untreated wafers with and without a second BHT-treated wafer present and to observe if termite mortality in these bottles was similar to that in the other bottles. Finally, four bottles were also set up with only wafers that had been first treated with isopropanol then air-dried, to determine if the solvent had any possible effect.

A response was first noted when native USA subterranean termites, *R. flavipes*, were introduced into the test bottles where the termites avoided the treated wafers but not the untreated controls. On day five the bottles were inspected and it was observed that no termites were under or on the antioxidant-treated samples. However, termites had tunneled under the untreated samples which they had partially covered with sand and on which they had begun to feed. Termites were alive with the exception of three bottles that had 2-3 dead termites near the BHT-treated block. It is normal for some termites to die from handling stress, but it was interesting that the dead were segregated near the BHT-treated wafer.

After 28 days the wafers were inspected and given a rating of "10" if no attack, "9" for a slight, "8" for moderate, etc., down to a "0" for failure, and the termite mortality determined. Wafers from the three control jars that contained two untreated wafers had extensive damage ranging from 4 to 7. In contrast, the average termite damage rating for the 1% BHT-treated wafers was 10.0 while the untreated wafers had an average rating of 8.0. In the six jars with a 3% BHT- and untreated wafer side by side, the average rating of the BHT-treated wafer was 10.0 and 9.5 for the untreated controls.

Consequently, based on the results of this preliminary choice test using *R. flavipes* it appears that the nontoxic hindered phenolic antioxidant BHT may be a feeding deterrent. Furthermore, some literature suggests that plant extracts also repel subterranean termites from penetrating treated soil (Blaske and Hertel 2001, Kitchens 2006), suggesting that antioxidants might also be repellents and, if so, might serve as a non-biocidal soil barrier treatment.

Unexpectedly, 100% termite mortality was observed in all jars that contained a BHT-treated wafer, while termite mortality was only about 2% in the jars with two control wafers and no BHT-treated wafer. We suggest this may be due to some initial direct (eating) and/or indirect (walking) contact with the BHT-treated wafer by some of the termites. These termites then passed on the small amount of BHT to all other termites through trophylaxis by various social activities such as grooming and anal feeding so that the BHT was introduced into the termites' guts. Cyclic redox reactions are known to be involved in the complex transformation of the woody polysaccharides into acetate, which is the caloric energy source for termites, and the methane by-product (Abe et al. 2000, Kappler and Brune 2002, Ohkuma 2003). It is possible that the antioxidant participated and altered the normal pathway of the redox mechanisms, thus literally forcing the reaction into a non-beneficial alternate route which ultimately caused the termites to starve. Future experiments that measure the calorie value gained by termites that consume normal sapwood versus BHT-treated sapwood may provide some information on this point. Alternatively, it is possible that the BHT could have migrated from the treated wafer to the sand via water-borne diffusion or vapor sublimination. However, this is unlikely as BHT is highly hydrophobic and has a very low vapor pressure, as shown by the low depletion observed with BHT-treated stakes after more then four years of ground-contact exposure at two locations in Mississippi (Schultz et al. 2006). Other factors could also explain the unanticipated mortality.

Several earlier studies appear to provide additional evidence for our hypothesis. First, Grace (1990) showed that adding BHT to *Gloeophyllum trabeum* fungal extracts slightly decreased subterranean termite trail-following activity, and using the more active and expensive analogue BHA totally inhibited the activity. Further, Grace et al. (1992) reported that wood decay siderophores impregnated into paper deterred Formosan feeding. While these siderophores chelate metals involved in the wood decay pathway, they are also redox reagents and, as such, may have antioxidant properties. We studied the influence of resin acids, known metal complexing compounds, on *R. flavipes* and Formosan termite feeding with the choice test and observed no effect. Thus, we suggest that possible antioxidant properties of the siderophores, rather than their metal complexing properties, affected the termites.

Another study appears to contradict our results, however. Specifically, Henderson et al. (2002) claimed that BHT, among other organic compounds, increased the rate of consumption of toxicants by termites. That is, BHT was reported to be a feeding stimulant.

Some readers may question if termites could have "learned" to identify, and thus avoid, wood that contains extractives with antioxidant properties. We note that antioxidant measurements of plant extractives are commonly done in many laboratories studies, and would not be surprised if nature endowed one or more organisms with the ability to conduct these "experiments" first if it depends on their survival.

Additional laboratory no-choice tests with *R. flavipes* and *C. formosanus* subterranean termites, and an exterior test with *C. formosanus* at Viance's Hilo termite test site, have recently confirmed the above initial results.

QUALIFIERS AND OTHER FACTORS/COMMENTS THAT MAY AFFECT/INFLUENCE FURTHER STUDIES OF THIS DUAL ANTIOXIDANT/ TERMITE REPELLENT HYPOTHESIS

Termite studies, as with all biological studies, can be difficult with many different factors potentially influencing the data and observations. Among items which need to be considered in further studies of our hypothesis are:

- Our hypothesis suggests that both the bioactivity and antioxidant properties of extractives affect termite behavior. While most extractives have relatively poor termiticide activity compared to commercial insecticides, they generally do have some toxicity. Further, some extractives have good termiticide activity. These include the terpenoids that generally have no antioxidant properties but some have very high insecticidal activities (*e.g.* Ishida et al. 1992 and 1993). In addition, some extractives that have antioxidant properties may also have relatively good biocidal activity. In our fungal activity studies, we usually found that the fungicidal activities of most extractives were extremely low relative to commercial biocides (*e.g.*, Schultz et al. 1995). However, one extractive, chlorophorin, was a superb antioxidant and had relatively high fungicidal activity (Minn et al. 1996, Schultz et al. 1995), and Arndt (1968) reported that chlorophorin had high termite bioactivity. Consequently, any study that employs natural extractives designed to test our hypothesis may find it difficult to separate out the toxicity versus antioxidant effects, in addition to any other properties of extractives – as yet unknown - that could further affect termite behavior.
- Extractive research can be extremely difficult. For example, the word "extractive" is misleading as not all extractives are extractable by exhaustive sequential extraction of ground heartwood with various solvents due to apparent covalent bonds between lignocellulose and extractives (*e.g.*, Schultz et al. 1995). Thus, extracting and isolating extractives followed by re-

impregnating them into non-durable sapwood at the “original” retention results in the wood having less termite resistance than the original wood (Onuorah 2001), and exhaustively-extracted heartwood can have more fungal and termite resistance than sapwood. Also, extractives can be easily degraded in the heartwood or during extraction by many factors including light, oxidation, heat, drying the wood before extraction, isolation procedure, exposure to the natural acidic conditions in wood or bases during isolation, etc., and any degradation would lower the extractive’s termite bioactivity (Chen et al. 2004, Rudman and Gay 1961, Yazaki 1983, Doi et al. 1998, Bavendamm and Arndt 1964). Furthermore, the extractive concentration in heartwood of a particular species can vary widely. For example, growing conditions often affect the heartwood extractive level which likely explains why heartwood from old-growth trees frequently has more natural resistance against termites and/or decay fungi than heartwood from plantation-grown trees (Hillis 1987a, Ohtani et al. 1996). Further, the within-tree variation of heartwood extractives may be another problem. For example, extractives can undergo slow auto- or enzymatic oxidation and acidic polymerization due to the naturally acidic conditions in wood (Hillis 1987b, Raiskila et al. 2006). Therefore, the older inner heartwood can have less overall antioxidant capacity than the younger outer heartwood. This may explain the lesser termite resistance of older inner heartwood compared to the younger outer heartwood of various trees in the data of Rudman (1963) and Taylor et al. (2006). Further confounding this is that in fast grown trees, with possible reduced heartwood extractives compared to slow growth trees, the heartwood would be younger (i.e., the extractives would have experienced less oxidative and/or acidic degradation compared to slower growth trees with older heartwood).

- Feeding deterrence and repellency are not absolute terms but, like most biological properties, a matter of comparative degree.

POSSIBLE APPLICATIONS

This work originated because we were perplexed by the resistance of BHT-treated stakes to native subterranean termites in Mississippi. While our intent was to understand our confusing observations it appears that this concept may have some practical applications. That is, BHT, BHA, and other hindered phenolic antioxidants are benign, economical, and stable in wood in multi-year outdoor exposure (Schultz et al. 2005 and 2006). The use of these benign and economical antioxidants alone, or combined with other bioactive compounds, may serve as a treatment to reduce termite damage to wood products. [Another advantage of employing antioxidants may be reduced biodegradation of organic biocides when BHT is present (Schultz et al. 2006)]. Going further out on a wooden limb [pun again intended], artificial and benign antioxidants might possibly impede termite movement through soil in much the same way as with extractives (Blaske and Hertel 2001, Kitchens 2006). If this speculation is correct, antioxidants might serve as a soil perimeter treatment to protect buildings, again either alone or with reduced levels of organic biocides co-added. This concept would likely have a smaller environmental impact than the bioactive compounds currently employed to surround homes. Finally, BHT has been listed as an additive in termite bait patents for use as a stabilizer, and was also recently proposed as a feeding stimulate for termite toxicant bait traps (Henderson 2002). While we are not sure if any bait systems currently use BHT, our work and an earlier study (Grace 1990) suggest that further experiments are warranted before it is added to commercial bait systems.

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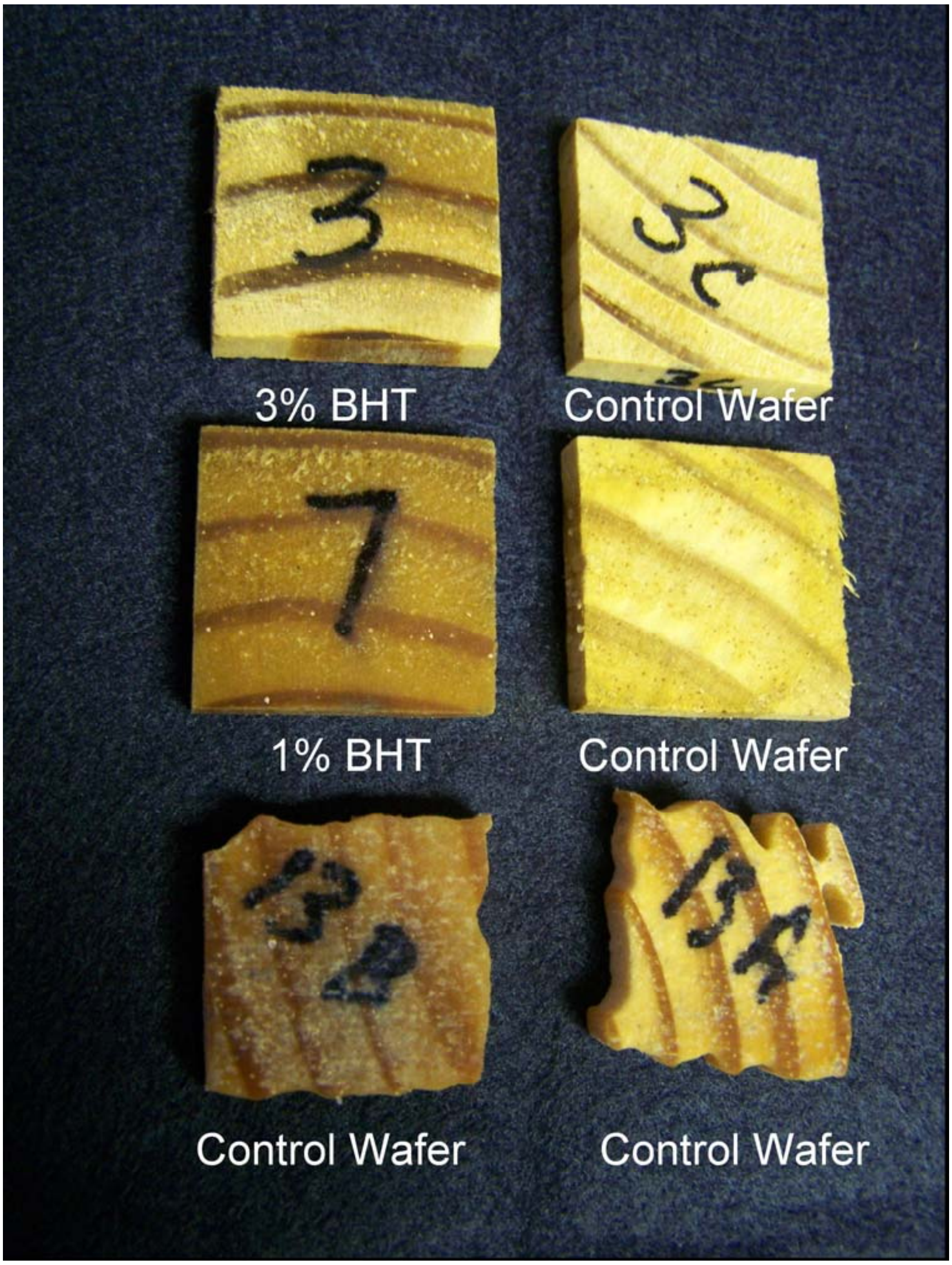

Figure 1. Wafers from three bottles of the termite choice test, with the bottom two wafers from a jar with two untreated wafers and the other wafer sets from jars with an untreated wafer and BHT-treated wafer. The untreated wafer in the jar with the 1% BHT-treated wafer was turned upside down to show moderate grazing (an "8" rating) on the underside.