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Laboratory and Field Studies on Subterranean Termites Native to the Eastern United States

Nathan Shook Little

LABORATORY AND FIELD STUDIES ON SUBTERRANEAN TERMITES NATIVE

TO THE EASTERN UNITED STATES

By

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LABORATORY AND FIELD STUDIES ON SUBTERRANEAN TERMITES NATIVE

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This study investigates modifications to AWPA Standard E18-06 to develop an above-ground field test for subterranean termites native to the eastern United States. AWPA Standard E18-06 was modified in two stages to progressively increase optimum conditions for subterranean termite feeding. Modification II, which incorporated the use of house bricks and feeder strips with a solid ventilated cover, had an increase in termite attack with less variation between tests. This study also investigates the effects of adding antioxidants, both natural and synthetic, and analogues which lack antioxidant properties, to test wood blocks on feeding by *Reticulitermes flavipes* Kollar using AWPA Standard E1-09. Antioxidants had feeding deterrence and mortality properties to *R. flavipes*. Conversely, the non-antioxidant analogues, with the exception of the heterocyclic flavanone, had little effect on *R. flavipes*. It is concluded that *R. flavipes* instinctively avoids wood which contains high levels of antioxidants, such as heartwood with phenolic extractives.

Key words: subterranean termites, antioxidants, benign, extractives

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CHAPTER I

INTRODUCTION

Subterranean termites are highly destructive pests, causing major economic damage to wooden structures in the United States (Gold, 2009). Throughout the past decade, toxicity and environmental concerns have surfaced for some primary wood preservative systems. This has led to the removal or restriction of some key systems for subterranean termite control that the wood products industry and residential termite control companies had come to rely on. With the demand for wood products on the rise, new environmentally benign preservative systems as well as new accelerated termite testing methods are needed.

This is a two part study. First, it examines an above-ground termite test which could act as a preservative pre-screen for potential wood preservative systems against subterranean termites. Second, it examines the potential of wood samples treated with benign antioxidants as termite feeding deterrents.

Above-Ground Test for Subterranean Termites

Current above-ground termite tests are limited in scope by the lack of a field wood preservative pre-screen against subterranean termites. This causes new wood preservative systems to endure a costly battery of often inappropriate tests.

This research, in part, examines modifications to American Wood Protection Association (AWPA) Standard E18-06 to develop an above-ground termite testing platform for wood products intended for Use Category (UC) 1 and UC2 applications against subterranean termites (*Reticulitermes spp.*) native to the eastern United States. While UCI and UC2 applications are the intended focus of this test, the future scope of this test could encompass UC3A and UC3B designations. Each designation is discussed in detail in the following chapter, but all are similar in that they are above-ground applications/conditions for wood products. In developing recommendations for future researchers, this test builds on past research and current standards used for termite testing.

Antioxidants as Termite Feeding Deterrents

The latter part of this research is an investigation, using the American Wood Protection Association (AWPA) Standard E1-09 no-choice laboratory test, to determine feeding deterrence and mortality of select environmentally-benign antioxidant compounds and non-antioxidant analogues to *Reticulitermes spp*. Factors including free radical scavenging (antioxidant) properties along with environmental solidarity of the compounds tested make antioxidants excellent candidates as potential wood preservatives.

Recently, Schultz *et al.* (2008) hypothesized that termites may have learned to avoid extractive-laden heartwood containing varying amounts of extractives that have antioxidant properties. Ragon *et al.* (2008) subsequently published research on the effect of the synthetic and benign antioxidant butylated hydroxytoluene (BHT) on termites, which led to this research into three additional classes of antioxidants. Three classes of antioxidants and a non-antioxidant analogue for each class were used to study the effect of the antioxidants on termites.

CHAPTER II

DEVELOPMENT OF A GROUND PROXIMITY TEST FOR SUBTERRANEAN TERMITES NATIVE TO THE EASTERN UNITED STATES

Introduction/Literature Review

The recent push to rapidly develop new wood preservatives has accelerated the need to test preservatives against both decay fungi and termites in a field setting. Current above-ground termite test methods have practical shortcomings with regard to the scope of each test. One shortcoming of current above-ground termite tests is the inability to act as a pre-screen for new preservative systems. This research is intended to locate the best above-ground termite testing procedures available and modify them to make recommendations for a new above-ground preservative pre-screen test for wood intended for UC1 and UC2 applications (AWPA, 2009) against subterranean termite attack. However, UC3A and UC3B applications should be considered for inclusion in the scope of this test as all are above-ground applications and, therefore, somewhat similar.

UC1 conditions include above-ground interior construction which is protected from moisture while a UC2 designation refers to above-ground interior construction which is subject to occasional dampness (AWPA, 2009). A UC3A designation refers to coated wood products in above-ground exterior construction exposed to all weather cycles such as siding and trim. UC3B conditions have more severe decay exposure;

uncoated wood products in above-ground exterior exposure which are exposed to all weather cycles subject to prolonged wetting such as decking and railing (AWPA, 2009). In terms of wood deterioration caused by subterranean termites, a UC1 exposure has greater or equal deterioration potential as UC3B.

Since this study is one of the first to cover research of this scope, few published works are available to review on this topic. This review is based on current standards, procedures used at certain universities, and past and present proposals for the standardization of test procedures similar to the objective of this study.

Current Test Methodologies

The main authority on information and methods dealing with all types of wood protection tests in North America, including termite tests, is the American Wood Protection Association (AWPA) Book of Standards (AWPA, 2009). This guide offers a variety of testing procedures dealing with termite interactions with wood. However, no current AWPA standard offers the ability to act as a preservative pre-screen for new systems against termites in above-ground UC1 or UC2 applications.

AWPA Standard E18-06 (Standard Field Test for Evaluation of Wood Preservatives Intended for Use Category 3B Applications Exposed, Out of Ground Contact, Uncoated Ground Proximity Decay Method) is a standard field test for evaluation of wood preservatives intended for UC3B applications, which are situations where treated wood is exposed, out of ground contact, and uncoated. This ground proximity decay method (AWPA Standard E18-06) is mainly used to evaluate decay from fungi and associated microorganisms (AWPA, 2009). Although insect attack on samples in the test may be reported, termite resistance is not the intended focus of this test.

AWPA Standard E21-06 (Standard Test Method for the Evaluation of Preservative Treatments for Lumber and Timbers Against Subterranean Termites in Above-Ground Applications) evaluates the performance of preservatives in UC1 and UC2 applications as well as the treatment method (AWPA, 2009). However, it is intended as a commodity test and not a preservative screening test. This method simulates a worse-case condition for framing lumber designed to be protected from water. Unlike AWPA Standard E18-06, this standard primarily evaluates samples based on termite attack rather than decay. Ideally, this test is used to determine termite repellency/toxity characteristics of preservative systems with additional efficacy data obtained from soil bed or field stake tests.

Proposals

In a past AWPA proposal, Amburgey (1988) suggested a new approach to testing subterranean termites in an above-ground situation. This proposed method utilized untreated wooden stakes (2” x 4” or 2” x 2”) containing both vertical and horizontal holes, and a horizontal hole in which to place a sample dowel, to allow termites access through the bait stake (Amburgey, 1988). Amburgey also proposed using PVC pipe with a 90˚ T in which to place the test dowel. The stakes or PVC pipes were driven into the ground to entice randomly foraging termites to feed on the test dowel.

During the 2009 AWPA Standards cycle, Zahora (2009) submitted proposal #09F - P08 - P6 - Exx to the AWPA membership for consideration. This proposal represents a possible new standard for evaluation of wood sample susceptibility to termite attack and damage in a UC1 – UC2 exposure. Zahora (2009) proposed a test designed to allow for testing of a large number of test samples in an environment highly conducive to termite attack. Although not a current standard, this method, or variations thereof, has reportedly been used by various researchers in the past for collection of termite data.

Briefly, both treated and untreated controls are exposed, in addition to test samples, in an area with known termite activity. Samples are arranged on a cinder block platform in close proximity to, but out of direct contact with, the ground and protected from the weather by a box enclosure (Zahora, 2009). Significant amounts of untreated sapwood are used to entice termites into the test array. The test is inspected periodically until severe attack is reached on the untreated control samples. Once this occurs, all samples are rated using the rating scale documented in AWPA Standard E7-09 (Standard Method of Evaluating Wood Preservatives by Field Tests with Stakes). Results are reported and the test can be re-set with new untreated feeder material of a susceptible species.

Objective

The objective is to determine viable test methodologies suitable for testing preservative systems intended for use in UC1 and UC2 applications and to study modifications to improve their usefulness while lowering deterioration or attack variation between test units. This overall objective can be further described as a two step modification of AWPA Standard E18-06 as follows: 1) Modification I - replacing the E18 shade cloth with a ventilated solid cover to prevent excess rain wetting and 2)

Modification II - replacing cinder blocks (101 x 202 x 404 mm) with house bricks (19.7 x 8.3 x 5.7 mm) to allow for more entry sites and adding southern yellow pine (SYP) sapwood feeder strips between the bricks to enhance termite activity.

Experimental

During the spring of 2007, Schultz and Nicholas installed modified AWPA Standard E18-06 tests in hopes of creating a new above-ground testing platform for assessing the efficacies of wood treatments against subterranean termites. This idea was driven by an industry push to obtain a more rapid process for screening new or modified wood preservative systems. With only one above-ground standard for testing subterranean termites, AWPA Standard E21-06, which is not intended for use as a preservative pre-screen, the need for an above-ground preservative screening test was evident.

Location Selection

The site selected for evaluating this experimental termite field testing procedure was the Mississippi State University (MSU) field test site near Saucier, Mississippi. As shown in Figure 2.1, the field test site near Saucier is located in AWPA Decay Hazard Zone 5, the most severe hazard zone on a scale of 1 to 5 as rated by AWPA (2009). This site was chosen because it has termites, which, due to the sandy soil, readily attack newly-installed wood samples which are not or only poorly protected against termite attack.

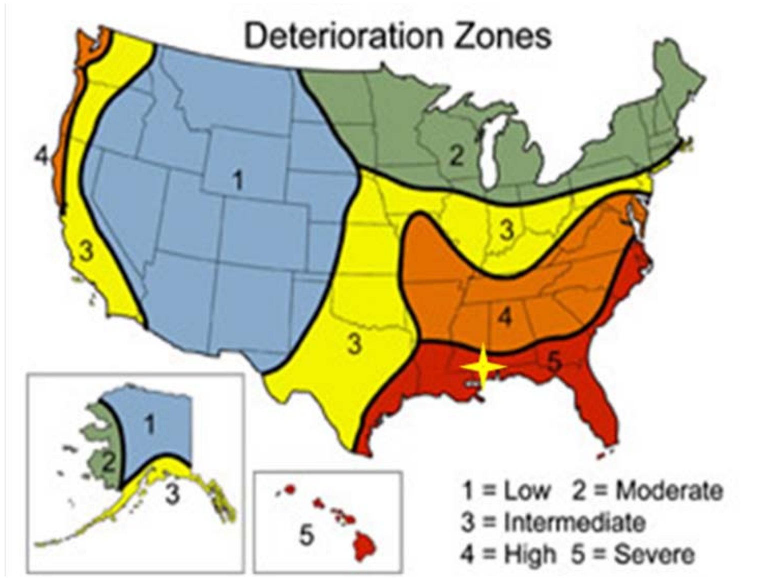

Figure 2.1

Decay Hazard Map of the United States.

The Saucier site has an abundance of native subterranean termites *(Reticulitermes spp.)* in and near the test site. The proposed above-ground termite test is mostly aimed at the genus *Reticulitermes* and not *Coptotermes*; both occur in Mississippi in portions of decay hazard zone 5. Saucier has, at this time, no known infestations of *Coptotermes* nearby.

Modification I: Solid Cover

The AWPA Standard E18-06 was chosen as a starting point for modifications because of its simplicity. Although primarily targeted at decay associated with fungi and microorganisms, this test array had the most practical design for modification. The flat cinder block layout of the Standard E18-06 offers a large testing platform for an array of possible sample layouts. Figure 2.2 shows an active Standard E18-06 test with shade cloth cover.

Figure 2.2

An active AWPA Standard E18-06 test with 80% shade cloth cover.

The first test using modification I took place during August of 2007. Materials for this modification were four cinder blocks 101 x 202 x 404 mm which contained no vertical holes, five untreated SYP sapwood blocks 20 x 50 x 125 mm, five untreated sweetgum blocks 20 x 50 x 125 mm, some small SYP feeder strips, and a square frame 1 m x 1 m constructed with 1” x 6” lumber treated to above-ground retention levels with ACQ and a solid Hardy board (wood-cement composite) top.

As shown in Figure 2.3, four cinder blocks were laid flat in a rectangular pattern. SYP feeder strips were inserted into the small cracks between the blocks and pressed into the soil to help enhance termite activity. The feeder strips were pressed into the soil until the top of the strips were slightly below the surface of the cinder blocks. The remaining cracks were filled with soil to approximately half the depth of the cinder blocks. Five untreated blocks of SYP sapwood and four sweetgum sapwood blocks were laid across the cracks in the blocks to establish termite feeding activity.

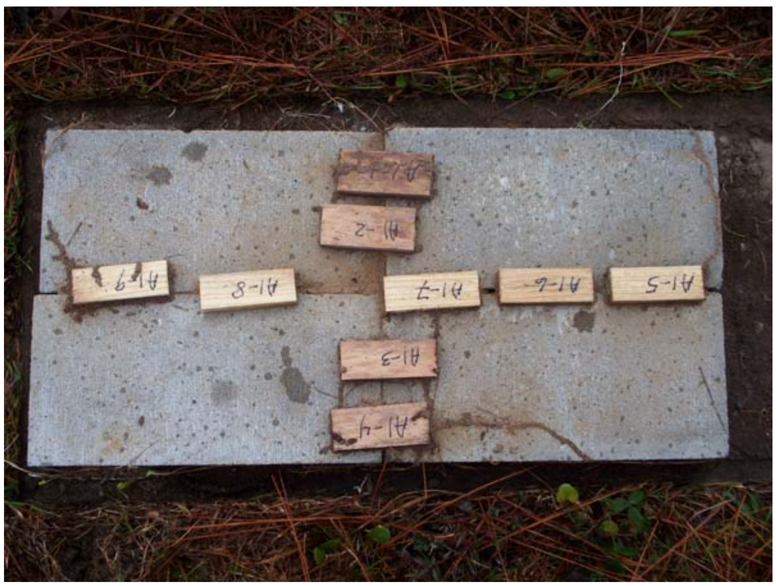

Figure 2.3

Modification I - Four cinder block layout with untreated samples.

Modification I was similar to AWPA Standard E18-06 in that they both used cinder blocks as a foundation for a testing platform. However, the design differed from the AWPA standard in several ways. First, the size of the test platform was smaller. There were only four blocks in the modified test, which can be increased for larger sample numbers, compared to the eighteen cinder blocks typically employed at Saucier in the AWPA Standard E18-06 test. Second, the samples were laid across the cracks between the cinder blocks (Figure 2.3), as opposed to inside the perimeter of the blocks as in AWPA Standard E18-06, to promote termite attack. Finally, the test cover was smaller to accommodate the smaller test and constructed with a solid Hardy board top, as shown in Figure 2.4, to protect the test from damage and excess rain wetting.

Figure 2.4

Test unit with solid Hardy board top to protect test blocks from damage and excess rain wetting.

Modification II: Bricks and Feeder Strips

During December 2008, a second modification to the AWPA Standard E18-06 was performed. This modification took into consideration the need for more termite access routes, as well as a better sample placement on the test platform.

The materials for the second test setup of this modification included 16 house bricks (19.7 x 8.3 x 5.7 mm) (l x w x h) containing ten holes through the center of each brick, 15 SYP untreated test blocks, and the same treated wood frame and Hardy board cover used in the original modification. The same overall shape and size of the test platform was maintained to reuse the same Hardy board test cover from the earlier study. At the same time, fresh SYP blocks were placed on the brick platform to monitor termite feeding progression. Two plots were established, each of which contained six tests, three of modification I and three of modification II. Figure 2.5 shows the brick and feeder strip arrangement of modification II.

Figure 2.5

Modification II – Cinder blocks replaced with house bricks.

By using the house bricks in place of the cinder blocks, potential termite access to the test blocks was increased. Figure 2.6 shows the smaller house bricks with more brick-to-brick gaps and holes to allow for more access sites for termites as compared to the cinder blocks, which only allowed entry through the two cracks and around the edges of the test. The house bricks were also thinner than the cinder blocks and, therefore, shortened the distance needed by termites to reach the wood samples. Finally, as shown in Figure 2.6, untreated SYP samples were laid across cracks in the bricks containing SYP feeder strips.

Figure 2.6

Modification II with test cover removed**.**

Test Evaluation

Samples were tagged, numbered, and mapped to keep track of their location within the test. Untreated SYP and sweetgum samples were evaluated using an “attack” or “no attack” rating, with attack indicating any trace of termite feeding. This is a simple method to evaluate and track termite attack and feeding patterns throughout the test.

Variation between test units was measured using Levene's test for homogeneity of population variances in SAS 9.2 (2010) software for statistical analysis with alpha=0.05.

Results and Discussion

Modification I, consisting of three separate plots each containing six tests, was installed in August of 2007. Modification II utilized three active test sites within two of the above-mentioned plots for alterations. In December of 2008, three tests within each of two plots were altered to meet modification II specifications. This yielded a sixteen month time differential between modifications.

A mistake was made in determining the total number of samples needed for the test setup. Enough untreated SYP sapwood samples were cut to satisfy placements on the new modification II tests, but not to resupply the older modification I tests. Samples on modification I were installed when the original test setup was completed in August of 2007, while modification II was started with new SYP sapwood samples. This mistake led to a sixteen month time differential in sample exposures between the two test platform modifications. Therefore, samples for modification II were exposed for about ten months, while samples in modification I were exposed for about twenty six months. Both modifications were intended to create an environment which was progressively more susceptible to termite attack while limiting the effect of decay fungi on the samples. This allowed the scope of the test to be primarily focused on termite degradation with minimal interference from decay fungi.

Upon rating the samples, it was apparent that modification II yielded a higher percentage of attack than modification I, even with the relatively shorter exposure time than modification I. As shown in Table 1, the overall percentage of attack for the samples exposed using modification II, even with the shorter exposure time, was 95 %, which was slightly higher than the modification I average attack of 80 %.

Table 2.1

Average percent of untreated test blocks with termite attack for each test unit.

	% Termite Attack	
Test Set/Rep	Modification I	Modification II
Set A	[redacted]	
Rep 1	100	93
Rep 2	100	100
Rep 3	33	100
Average	78	98
Set B	[redacted]	
Rep 1	100	100
Rep 2	67	86
Rep 3	78	93
Average	82	93
Overall average	80	95

[1]Termite attack was determined by a "hit no-hit" rating assigned to each sample in a test.
[2]At 16 months exposure, modification I had the following percent termite attack: Set A had 67% attack and Set B had 57% attack.

Although the test was only inspected periodically, it was apparent that modification II improved the probability of termite attack on samples and lowered test to test variation. First, more cracks in the bricks allowed for more entry sites for termites compared to the larger cinder blocks. Second, the addition of feeder strips apparently aided termites in locating the test.

Set A

Table 1 illustrates that for Set A, modification II was more effective at initiating termite attack than modification I. Although the average percent attack was greater in modification II, two reps in modification I yielded 100% attack of untreated test samples. This could be due to the fact that termites are random foragers and simply found the third location later than the first two locations, therefore resulting in partial attack.

Set B

As shown in Table 1, the average percent attack was higher for modification II than for modification I. Although it had only a slightly higher percent of attack and was exposed for a shorter duration, modification II proved to be more effective in producing termite attack.

Variation

Variation between test units of modification II were significantly lower than test units of modification I with regard to percent attack in a test unit. The addition of house bricks and SYP feeder strips in modification II increased the overall attack on test units while lowering test to test variation.

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CHAPTER III

EFFECT OF ANTIOXIDANTS ON TERMITE FEEDING

DETERRENCE AND MORTALITY

Introduction/Literature Review

Many bioactive termite control compounds have been removed from the market in the past decade due to environmental or toxicity concerns. New stringent environmental regulations pose an opportunity for developing relatively benign termite control agents. One approach is to examine heartwood of tree species that are naturally resistant to termites.

The exact mechanism(s) by which extractives in durable heartwood affect termites is unclear, but most researchers assume that the extractives have some termite toxicity and/or repellency properties. The activity of most natural extractives against termites is generally low relative to commercial insecticides, however. Alternatively, many previous studies have shown that the same class of natural extractives often has both termite resistance and free radical scavenging (antioxidant) properties (Ragon *et al.,* 2008, and references therein). 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) and tannins (Fava *et al.*, 2006, Oszmianski *et al.*, 2006). Based on the above, Schultz *et al.* (2008) recently proposed that the extractives' antioxidant properties may repel termites. This property, along with any insect toxicity, might explain why the heartwood of some species is resistant to termites.

Schultz *et al.* (2008) first tested their hypothesis in laboratory and field studies employing the antioxidant BHT. BHT was chosen since it is approved as a human foodstuff additive and, consequently, should be benign. BHT-treated wood did have feeding deterrence which supported the hypothesis; however, it also unexpectedly resulted in elevated mortality of termites (Schultz *et al.,* 2008, Ragon *et al.,* 2008).

This study further tests Schultz's *et al.* (2008) hypothesis by determining termite feeding deterrence and mortality of wood treated with three different classes of benign natural or synthetic antioxidants, and non-antioxidant analogues. *Reticulitermes flavipes* was employed for the laboratory no-choice AWPA E1-09 tests. Three classes of compounds were examined: 1) the synthetic butylated hydroxyanisole (BHA), a commercial food antioxidant, and its non-phenolic analogue; 2) three naturally-occurring plant flavonoids and a non-antioxidant flavonoid analogue; and 3) tannic acid and the semi-synthetic food antioxidant propyl gallate which is made from tannic acid, and a non-antioxidant analogue of propyl gallate. All the antioxidants examined are present in plant-derived foods or employed as food additives and, consequently, are benign to humans.

Experimental

Pinus sp. wafers, 25 x 25 x 6 mm (1 x 1 x ¼ in.) (t x r x l), were cut from one sapwood lumber piece to avoid any effect by the extractives (Dahlen, 2009). The chemicals were obtained from Sigma-Aldrich. The solvents employed were toluene for

BHA, butyl benzene, flavanone and propyl benzoate; isopropanol for propyl gallate; acetone for quercein and tannic acid; and ethanol for catechin and morin.

Wafers were treated to five different retention levels with a vacuum/atmospheric pressure method except for the flavonoid quercetin, which was only soluble up to 3% (all treating solutions are wt/wt%) in acetone so that just three treatment levels were done. Other solvents did not dissolve quercetin to a level above 3%. Controls were treated with the solvent only. Retentions for each compound were calculated based on initial and final weight of each wafer and the treating solution concentration. After treatment, the wafers were placed in a hood for 7 days to evaporate the solvent, then put in a conditioning chamber for 14 days to obtain the initial weight.

AWPA Standard E1-09 no-choice test (AWPA, 2009) was followed, with batches of one to three compounds tested as termites became available. The initial tests had some mold and relatively low mass losses for the untreated controls; when the amount of deionized water added was reduced from the E1 specified amount of 30 mls to 25 mls in later tests mold was not observed and the control mass losses were much higher. BHA was treated at 0.5, 1, 2, 3, and 5% and quercetin to only 1, 2, and 3% due to the limited solubility described above. All other antioxidants and non-antioxidant analogues were treated at 1, 2, 3, 4, and 6%. The relatively low treatment level of 0.5% employed only for BHA was based on prior test results with the less-effective antioxidant BHT, and the other retentions were employed to obtain the range of extractives generally found in most termite-resistant heartwood of 2 to 5 wt%.

Immediately following the 28 day test, the wafers were again placed in the conditioning chamber for 14 days to determine mass loss of the wafers. The wafers were then visually rated using the AWPA E1 scale of 10 to 0, beginning with 10 (no attack), 9.5 (trace attack), 9 (slight attack with no more than 3% of cross sectional area affected), etc. In general, ratings of 7 or below are considered to be significant when determining which treating materials should be tested further. Samples were subsequently photographed and retained for future reference.

Analysis

This experiment was run in a series of completely randomized designs. Each chemical was analyzed separately using a one-way classification fixed effects model with SAS 9.2 (2010) software for statistical analyses. The treatments within each compound were analyzed at an alpha=0.05 significance level.

Results and Discussion

Average treatment levels, retentions, mass losses, AWPA E1-06 ratings, and termite mortalities are given in Table 3.1, with individual values given in Appendix A. Least significant difference results for each compound can be found in Appendix B.

Table 3.1

Average retentions, mass loss, termite mortality, and block ratings for AWPA Standard E1-09 no-choice termite tests employing *Reticulitermes flavipes.*

Compound	Trt. soln. (%)	Avg. rtn. kg/m³)	Avg. mass loss (%)	Avg. mortality (%)	Avg. rating
BHA	0	0	32	<5	3
	0.5	1.77	10	100	8
	1	4.21	8	100	8
	2	7.99	4	100	9
	3	13.01	4	100	9.5
	5	22.02	2	100	9.5
Butyl benzene	0	0	35	<5	2
	1	3.84	39	10	2
	2	7.81	40	<5	1
	3	12.53	33	12	2
	4	15.73	32	12	3
	6	27.07	15	77	7
Quercetin	0	0	61	<5	0
	1	3.57	60	<5	0
	2	7.30	61	<5	0
	3	13.36	57	<5	0
Morin	0	0	58	<5	0
	1	4.09	53	<5	0
	2	8.09	49	<5	4
	3	11.21	43	<5	4
	4	14.64	36	<5	6
	6	21.34	22	8	7
Catechin	0	0	52	<5	0
	1	3.72	51	<5	0
	2	8.00	43	6	4
	3	12.12	35	12	6
	4	16.81	24	50	7
	6	26.43	10	100	8.6

Table 3.1 Continued

Flavanone	0	0	17	<5	8
	1	3.47	4	100	9.9
	2	7.10	4	100	9.9
	3	11.21	4	100	10
	4	14.48	4	100	10
	6	22.52	4	100	10
Tannic acid	0	0	60	<5	0
	1	3.96	55	<5	0
	2	8.05	46	<5	2
	3	12.35	21	75	6
	4	16.92	10	99	9
	6	26.08	8	100	9.2
Propyl gallate	0	0	15	8 [1]	7
	1	3.82	11	100	8
	2	7.45	10	100	8
	3	11.19	8	100	9
	4	14.96	7	100	9
	6	23.12	6	100	10
Propyl benzoate	0	0	22	<5 [1]	6
	1	4.26	27	25	6
	2	8.48	28	<5	5
	3	12.46	21	27	6
	4	16.60	19	26	7
	6	25.38	11	88	8

The averages are based on five replicates, except where noted due to mold contamination in one or two replicate bottles.
[1]Averages were obtained from fewer than five replicates due to mold contamination in some tests.
[2]The ratings are based on AWPA Standard E1-09, where a 10 is no attack, 9 a trace to 3% damage, etc., down to failed (0).

Antioxidants are substances which are added in relatively small levels to materials that react with atmospheric oxygen, such as petroleum products, plastics, and fatty foods. Antioxidants prevent plastics from slowly losing their mechanical properties, and foods from developing off-flavor rancidity. Approximately 95% of materials which antioxidants are added to are highly hydrophobic, so commercial antioxidants are also highly hydrophobic to prevent antioxidants from migrating to the materials' surface, or "blooming". Three different classes of antioxidants, described below, were employed in this study. All of the antioxidants are approved for or naturally present in human foodstuffs and, therefore, benign. For comparison, a similar analogue which does not have antioxidant properties was also tested with each antioxidant class.

BHA Set

The first antioxidant examined was BHA, a totally-synthetic hindered phenolic antioxidant which is very similar to butylated hydroxytoluene (BHT). BHT was the antioxidant first employed at MSU for termite studies (Ragon *et al.,* 2008). BHA is more effective as an antioxidant than BHT, but is also more expensive. BHT and BHA are added to many different materials, including plastics and foodstuffs, with both approved by the US Food and Drug Administration (FDA) for human foods.

Mortality

As shown in Figure 3.1, wood treated with only 0.5% of the antioxidant BHA had 100% termite mortality and less deterioration than the untreated controls in AWPA E1-09 tests. Conversely, the non-antioxidant analogue butyl benzene had only 77% mortality at the highest treatment level of 6%. It is obvious that BHA in this present work has higher termite activity than previous results with the similar antioxidant BHT (Ragon *et al.,* 2008).

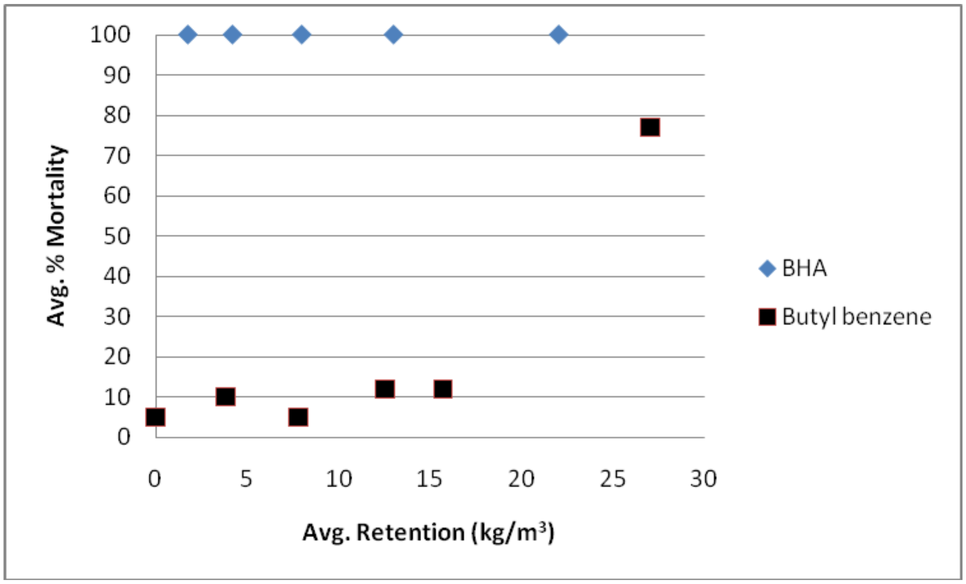

Figure 3.1

Average percent mortality of *R. flavipes* versus average chemical retention for the antioxidant BHA and its non-antioxidant analogue butyl benzene.

Mass Loss

As shown in Figure 3.2, BHA has less mass loss than the non-antioxidant analogue butyl benzene. Since less mass loss occurred with BHA than with the non-antioxidant butyl benzene at all treatment levels, BHA appears to be more effective at preventing termite feeding than the similar non-antioxidant analogue. Linear regressions are shown for this and the following two mass loss graphs.

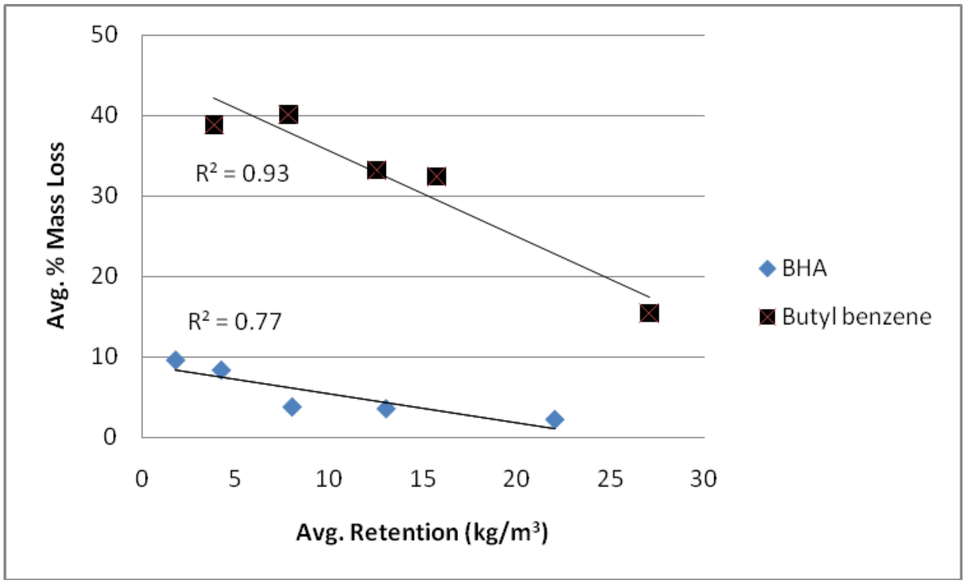

Figure 3.2

Average percent mass loss due to *R. flavipes* versus average chemical retention for the antioxidant BHA and its non-antioxidant analogue Butyl benzene.

Sample Ratings

Figure 3.3 shows that BHA, at the lower 0.5 and 1% treatment solutions, had an average sample rating of 8. As retention was increased from 1 to 3%, the average sample rating increased to 9.5, which indicates a trace of attack. Conversely, for the non-antioxidant butyl benzene, the average rating decreased with an increase in solution from 1 to 2%, but an increase from 2 to 6% treatment solution produced a somewhat linear increase in ratings to a maximum “7” rating at the highest retention examined.

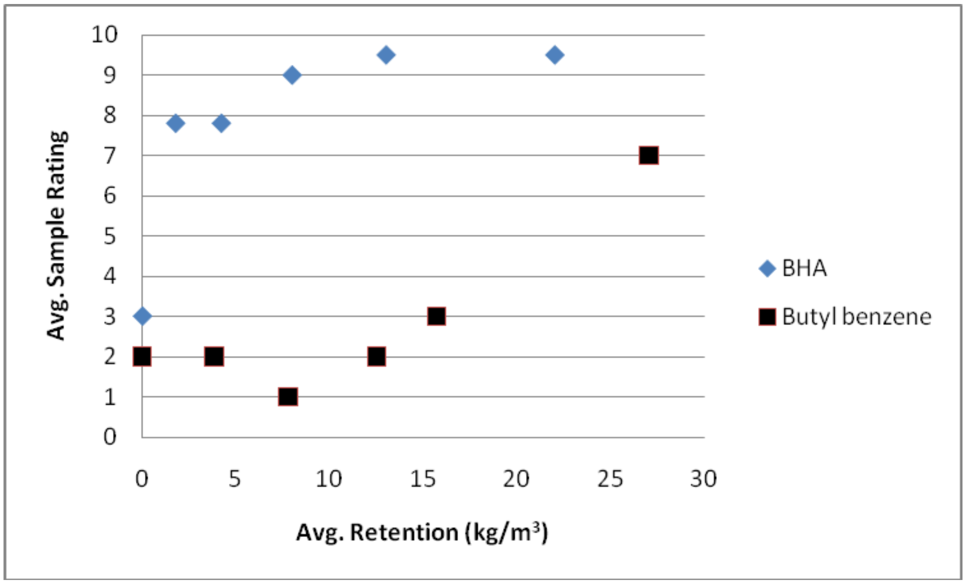

Figure 3.3

Average sample rating versus average chemical retention
for the antioxidant BHA and its non-antioxidant
analogue butyl benzene.

Prior Termite Literature on BHA

In an earlier study, Grace (1990) found that BHA placed in termite paths completely suppressed termite response while BHT had only some limited effect on termite orientation. Further, BHA, due to its antioxidant properties, has been reported to affect termites (Abdul Khalil *et al.,* 2009). BHA is an artificial antioxidant, unlike the antioxidants studied below, so termites cannot have a learned "antipathy" against it. The termite feeding deterrence and mortality properties observed with BHA are thus likely due to its antioxidant property, but other factors cannot be excluded.

Tannic/Gallic Acid Set

Tannins are natural plant-derived materials that can be separated into two distinct classes, condensed and hydrolyzable tannins, with the latter sometimes called tannic acid. Hydrolyzable tannins are composed of two or more gallic acids linked by ester bonds to glucose. Tannins are naturally present in plant foodstuffs, such as wines, berries, vegetables, and chocolates. Propyl gallate is a semi-synthetic antioxidant in which the benzylic acid group has been esterfied with a three-carbon side chain to make gallic acid more hydrophobic and, thus, more suitable as a food antioxidant. It is approved by the US FDA for food use.

Mortality

Tannic acid had good termite mortality at a treatment of 3% and higher concentrations. The commercial antioxidant propyl gallate, which is synthesized from tannic acid, also showed good activity with 100% mortality observed at all retentions examined. Conversely, Figure 3.4 indicates that the non-antioxidant analogue propyl benzoate only had high termite mortality at the maximum treatment level of 6%.

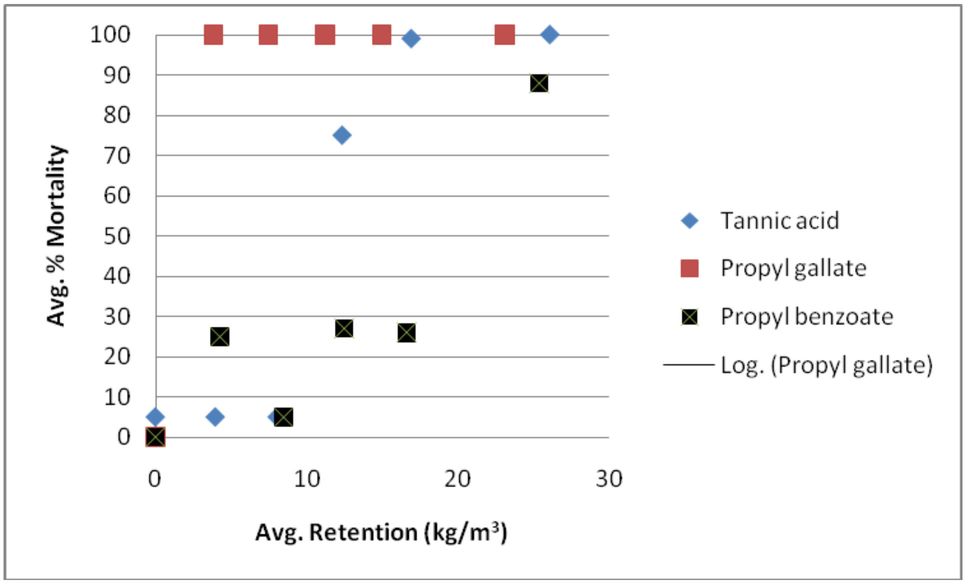

Figure 3.4

Average percent mortality of *R. flavipes* versus average chemical retention for the antioxidants tannic acid, propyl gallate, and a non-antioxidant of analogue of propyl gallate, propyl benzoate.

Mass Loss

As shown in Figure 3.5, tannic acid exhibited good feeding deterrence at treatment levels of 4% and higher concentrations. Propyl gallate also showed increased feeding deterrence when compared to its non-antioxidant analogue propyl benzoate, but showed a more gradual dose-response slope compared to tannic acid. No single treatment level of propyl gallate optimumly prevented termite degradation; however, all levels of propyl gallate were statistically better than the non-antioxidant control. However, the non-antioxidant propyl benzoate did produce significant reductions in termite degredation at treatment levels of 4 and 6%.

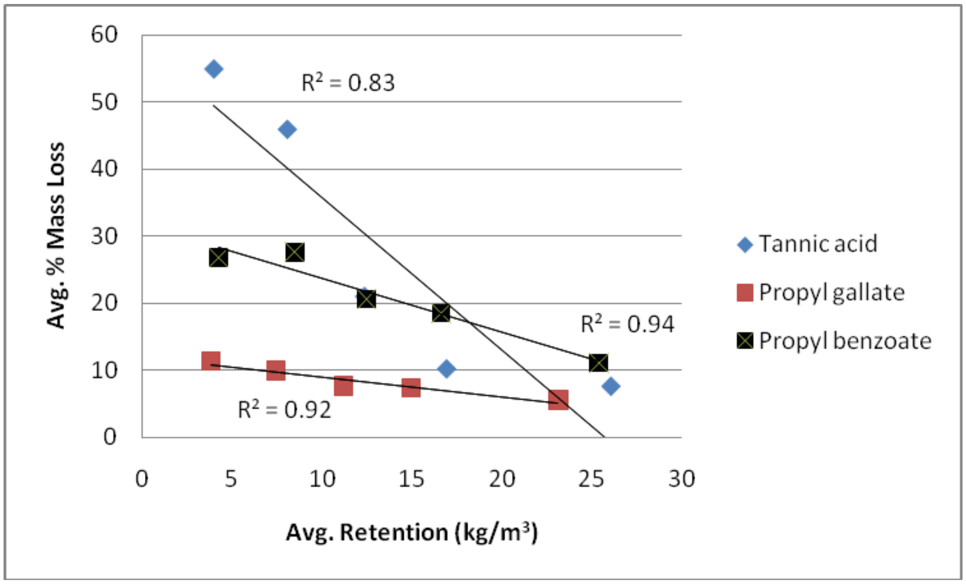

Figure 3.5

Average percent mass loss due to *R. flavipes* versus average chemical retention for tannic acid, propyl gallate, and a non-antioxidant analogue of propyl gallate, propyl benzoate.

Sample Ratings

Figure 3.6 shows that tannic acid had a rating of zero, or complete failure, at the lowest level tested of 1%. As solution concentration increased from 1 through 6%, there was an increase in sample ratings up to an average rating of 9 for the 6% solution. Propyl gallate had a slight increase in sample ratings between 2 and 3% solutions and 4 and 6% solutions, with the highest rating at 6% being a 9.5 rating. Conversely, the non-antioxidant analogue of tannic acid and propyl gallate, propyl benzoate, had a decrease in sample rating between solutions of 1 and 2%, then an increased rating of 5 to 8 with retention increases from 2 to 6%.

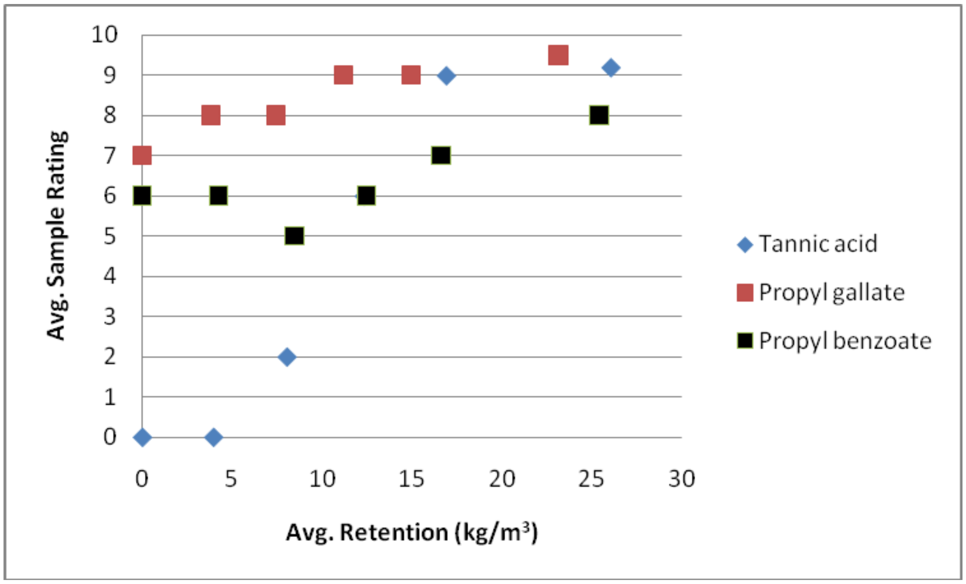

Figure 3.6

Average sample rating versus average chemical retention for tannic acid, propyl gallate, and a non-antioxidant analogue of propyl gallate, propyl benzoate.

Prior Termite Literature on Tannic/Gallic Acid

Tang *et al.* (2007) reported that tannic acid, and the flavonoid quercetin, inhibited an S-transferase enzyme in termites. Tannic acids are well known to have excellent antioxidant properties (Vinson *et al.,* 1995, Rice-Evans *et al.,* 1996). Tannins are present in many plant-derived human foodstuffs and propyl gallate is an approved food antioxidant, making these two antioxidants benign to humans.

Flavonoid Set

Flavonoids are omnipresent, naturally-occurring plant antioxidants which have activity against fungi and insects. They are commonly found in high levels in blackberries, blueberries, cranberries, and other colored fruits, and in lower levels in essentially all plant-derived foodstuffs. Flavonoids have a wide range of potential health benefits.

Mortality

The flavonoid quercetin is only soluble in acetone up to 3%, and at this level only a slight effect on average ratings and termite mortality was observed in Figure 3.7. Morin and catechin, however, were sufficiently soluble to obtain treating solutions up to 6%, and had good toxicity at treatment levels of 4 and 6%. The non-antioxidant analogue, flavanone, however, also showed good mortality. It was previously demonstrated that this heterocyclic compound is active against wood decay fungi (Binbuga *et al.,* 2008), and it is possible that it also has some termite activity.

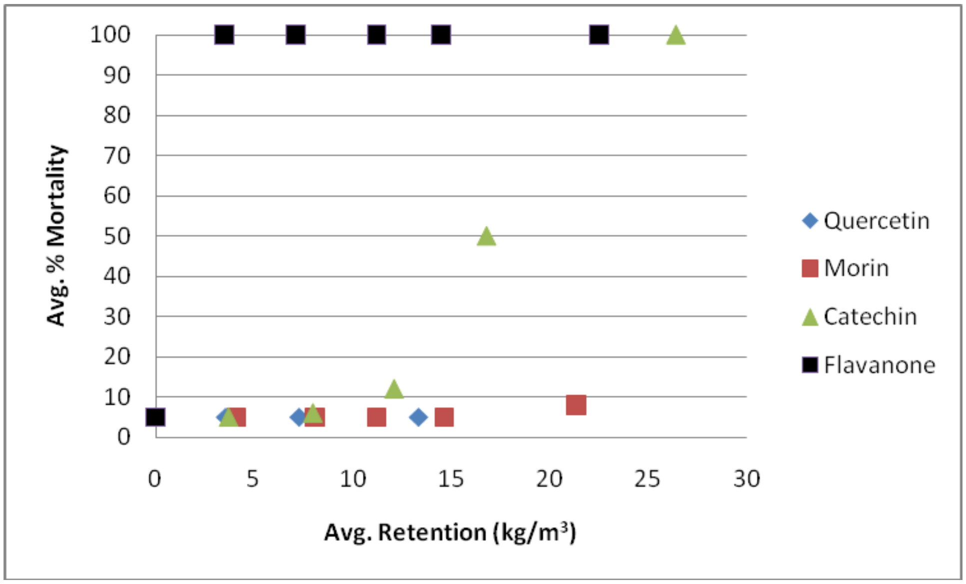

Figure 3.7

Average percent mortality of *R. flavipes* versus average chemical retention for the antioxidants quercetin, morin, catechin, and a non-antioxidant analogue of quercetin, flavanone.

Mass Loss

As shown in Figure 3.8, the flavonoid quercetin only showed a slight decrease in mass loss for each treatment level up to 3%. Chen *et al.* (2004) reported that the flavonoid quercetin, in a termite choice test with paper discs treated by immersion in a 1% quercetin solution, resulted in termite feeding deterrence. This study did not observe any significant effect on termite feeding deterrence for quercetin up to the highest treatment level of 3%. While no treatment level of morin was significantly different, treating with morin did significantly lower mean mass loss due to termite feeding. For each increase in treatment level higher than 1% for catechin, on average, there was a significant and relatively linear decrease in mass loss due to termite degradation. A 2%

or greater solution resulted in a significant reduction in mass loss; there was no significant difference between the 1% solution and the control.

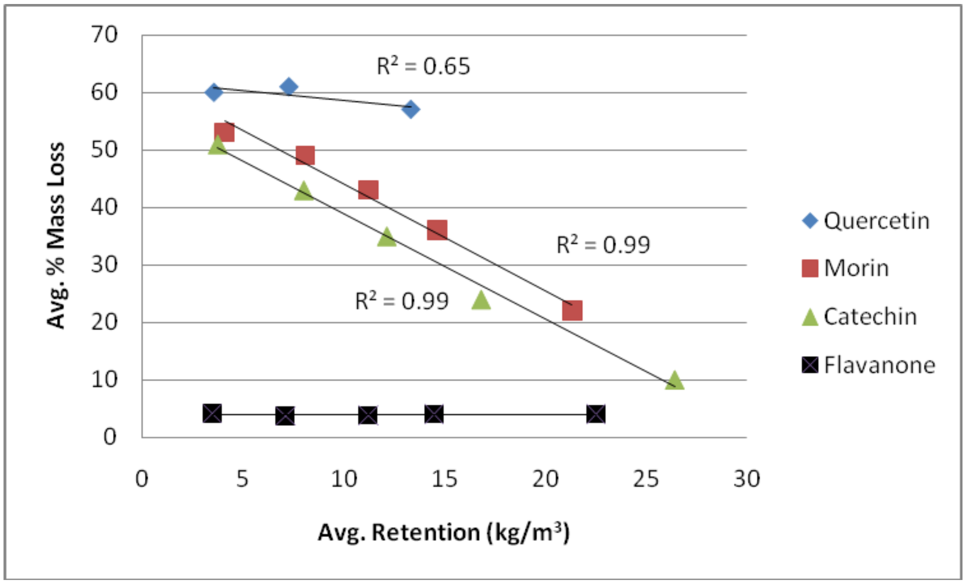

Figure 3.8

Average percent mass loss due to *R. flavipes* versus average chemical retention for quercetin, morin, catechin, and a non-antioxidant analogue of quercetin, flavanone.

The non-antioxidant analogue, flavanone, showed good termite feeding deterrence at the lowest treatment level of 1%. This was the only non-antioxidant which had termite activity.

Sample Ratings

Quercetin, which is only soluble in up to a 3% solution, had ratings of 0, or complete failure, for each level tested, as shown in Figure 3.9. Morin at a 1% treatment solution also yielded a rating of 0. With additional increases in solutions from 1 to 6%, however, morin yielded a somewhat linear increase in ratings up to a rating of 7 for the 6% treatment solution. Catechin had a rating of 0 for the 1% solution, but an increased rating of 4 at 2% solution up to a rating of 8.5 at the 6% treatment solution. Conversely, flavanone, for all treatment levels, had a rating of 10, or no attack.

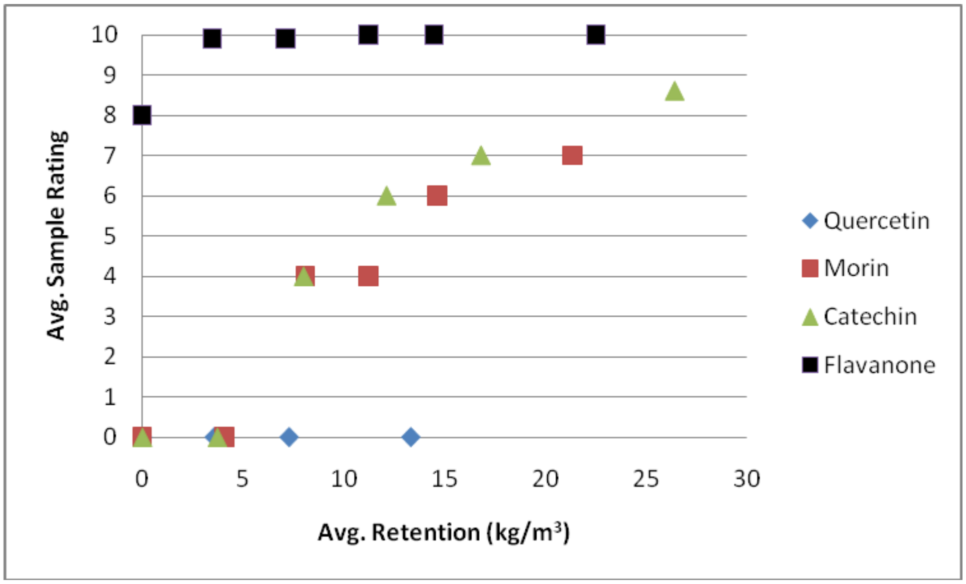

Figure 3.9

Average sample rating versus average chemical retention for quercetin, morin, catechin, and a non-antioxidant analogue of quercetin, flavanone.

Prior Termite Literature on Flavonoids

In the studies of Boue and Raina (2003), quercetin caused the second highest termite mortality of five flavonoids examined, and was the most potent antifeedant with much higher activity than catechin (Ohmura *et al.,* 2000). Chen *et al.* (2004) and Morimoto *et al.* (2006) also reported that flavanoids have termite activity. Many other studies have also shown that flavonoids, including those studied herein, are excellent antioxidants (e.g. Vinson *et al.,* 1995, Rice-Evans *et al.,* 1996, Pietta, 2000, Cao *et al.,* 1997, Chua *et al.,* 2008). As mentioned earlier, flavonoids are present in many plant-based human foodstuffs and have generally reported to have many health benefits.

CHAPTER IV

CONCLUSIONS

Above-Ground Test for Subterranean Termites

Results from this study indicate that modification II of the AWPA Standard E18-06 ground proximity decay test, consisting of house bricks and SYP feeder strips, is a viable field testing platform for native subterranean termites for UC1 and UC2 applications. Although the tests were only inspected a few times, and the two modifications had different exposure times, the smaller house bricks coupled with the addition of feeder strips increased the percentage of termite attack on the untreated test samples for modification II compared to modification I. More testing is needed to verify that this testing platform is a suitable means of testing wood preservatives against native subterranean termites. To increase termite attack in future studies, the use of small house bricks with SYP feeder strips in all gaps and holes, and a ventilated solid test cover, is recommended

Antioxidants as Termite Feeding Deterrents

This, and earlier results, show that termites avoid wood which contains relatively high levels of synthetic or natural antioxidants which are benign to humans. Consequently, one explanation for the termite resistance of the heartwood of some naturally durable tree species is that they contain relatively high levels of phenolic extractives which have antioxidant properties; other properties of the extractives may also contribute to the termite resistance of the heartwood of some tree species.

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APPENDIX A

DATA FROM AWPA STANDARD E1-09 NO-CHOICE TESTS

BHA SET

BHA

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
A-0.5-1	1.92	7.3	100	8
A-0.5-2	1.95	13.5	100	8
A-0.5-3	1.91	8.0	100	8
A-0.5-4	1.56	9.0	100	8
A-0.5-5	1.52	10.1	100	7
A-1-1	4.06	8.3	100	9
A-1-2	4.22	7.2	100	8
A-1-3	4.24	6.9	100	9
A-1-4	4.22	11.9	100	8
A-1-5	4.33	7.3	100	8
A-2-1	7.94	3.8	100	9
A-2-2	8.12	3.7	100	9
A-2-3	7.89	3.6	100	9
A-2-4	7.89	3.2	100	9
A-2-5	8.12	4.4	100	9
A-3-1	13.25	3.0	100	9.5
A-3-2	13.19	3.1	100	9.5
A-3-3	12.72	3.5	100	9.5
A-3-4	12.92	3.6	100	9.5
A-3-5	12.98	4.3	100	9.5
A-5-1	21.30	2.0	100	9.5
A-5-2	21.98	2.3	100	9.5
A-5-3	22.20	2.1	100	9.5
A-5-4	22.65	2.2	100	9.5
A-5-5	21.98	2.3	100	9.5
AC-1	0.00	37.4	5	4
AC-2	0.00	40.1	2	0
AC-3	0.00	58.0	1	0
AC-4	0.00	18.0	8	6
AC-5	0.00	7.5	90	7

Butyl benzene – A non-antioxidant analogue of BHA

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
BB-1-1	3.95	40.7	10	0
BB-1-2	3.72	28.2	5	4
BB-1-3	3.92	46.8	3	0
BB-1-4	3.81	40.1	20	0
BB-1-5	3.81	37.9	10	4
BB-2-1	7.58	34.9	10	4
BB-2-2	8.03	37.9	5	0
BB-2-3	7.76	41.1	2	0
BB-2-4	7.67	44.7	2	0
BB-2-5	8.03	41.9	5	0
BB-3-1	12.78	28.4	15	4
BB-3-2	12.58	21.9	16	4
BB-3-3	12.38	40.3	12	0
BB-3-4	12.38	31.9	10	0
BB-3-5	12.51	43.5	5	0
BB-4-1	15.97	35.3	8	4
BB-4-2	16.15	33.4	10	4
BB-4-3	15.70	44.2	5	0
BB-4-4	15.61	19.9	20	4
BB-4-5	15.25	29.3	18	4
BB-6-1	27.18	4.8	100	8
BB-6-2	27.45	22.8	35	6
BB-6-3	27.18	21.6	75	7
BB-6-4	27.05	5.4	100	8
BB-6-5	26.51	22.6	75	6
BBC-1	0.00	30.6	3	4
BBC-2	0.00	25.3	5	4
BBC-3	0.00	39.5	1	0
BBC-4	0.00	31.5	3	4
BBC-5	0.00	46.3	1	0

FLAVOVOID SET

Quercetin

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
Q-1-1	3.61	62	2	0
Q-1-2	3.39	56	2	0
Q-1-3	3.66	58	2	0
Q-1-4	3.57	61	2	0
Q-1-5	3.61	64	2	0
Q-2-1	7.36	67	2	0
Q-2-2	7.40	64	2	0
Q-2-3	7.40	53	2	0
Q-2-4	7.09	63	2	0
Q-2-5	7.27	59	2	0
Q-3-1	11.30	54	2	0
Q-3-2	11.57	63	2	0
Q-3-3	11.37	61	2	0
Q-3-4	11.17	58	2	0
Q-3-5	11.37	51	2	0
QC-1	0.00	59	2	0
QC-2	0.00	60	2	0
QC-3	0.00	61	2	0
QC-4	0.00	57	2	0
QC-5	0.00	65	2	0

Morin

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
M-1-1	4.10	48	3	0
M-1-2	3.95	53	3	0
M-1-3	4.08	56	3	0
M-1-4	4.17	53	3	0
M-1-5	4.17	55	3	0
M-2-1	7.67	52	3	4
M-2-2	8.25	48	3	4
M-2-3	8.30	47	3	4
M-2-4	8.34	48	3	4
M-2-5	7.89	52	3	4
M-3-1	11.44	45	3	4
M-3-2	10.83	49	3	4
M-3-3	11.17	38	3	4
M-3-4	11.10	42	3	4
M-3-5	11.50	39	3	4
M-4-1	14.80	32	3	6
M-4-2	14.71	36	3	6
M-4-3	14.62	34	3	6
M-4-4	14.71	39	3	6
M-4-5	14.35	39	3	6
M-6-1	21.13	25	8	7
M-6-2	20.86	27	8	7
M-6-3	21.80	20	8	7
M-6-4	21.39	18	8	7
M-6-5	21.53	18	8	7
MC-1	0.00	56	3	0
MC-2	0.00	57	3	0
MC-3	0.00	60	3	0
MC-4	0.00	60	3	0
MC-5	0.00	57	3	0

Catechin

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
C-1-1	3.66	52	3	0
C-1-2	3.68	50	3	0
C-1-3	3.81	51	3	0
C-1-4	3.81	52	3	0
C-1-5	3.66	51	3	0
C-2-1	7.80	47	6	4
C-2-2	7.80	43	6	4
C-2-3	8.12	42	6	4
C-2-4	8.34	42	6	4
C-2-5	7.94	42	6	4
C-3-1	11.84	35	12	6
C-3-2	12.51	35	12	6
C-3-3	12.18	36	12	6
C-3-4	12.31	33	12	6
C-3-5	11.77	39	12	4
C-4-1	16.68	21	50	7
C-4-2	16.42	22	50	7
C-4-3	17.40	24	50	7
C-4-4	16.95	26	50	7
C-4-5	16.60	26	50	7
C-6-1	26.10	9	100	9
C-6-2	26.37	12	100	8
C-6-3	27.18	7	100	9
C-6-4	26.10	12	100	8
C-6-5	26.37	8	100	9
CC-1	0.00	52	3	0
CC-2	0.00	51	3	0
CC-3	0.00	53	3	0
CC-4	0.00	49	3	0
CC-5	0.00	53	3	0

Flavanone – A non-antioxidant flavonoid

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
F-1-1	3.54	3.8	100	9.5
F-1-2	3.43	4.3	100	10
F-1-3	3.39	4.4	100	10
F-1-4	3.48	3.6	100	10
F-1-5	3.50	4.3	100	10
F-2-1	7.27	3.8	100	10
F-2-2	7.00	4.1	100	10
F-2-3	6.95	3.4	100	9.5
F-2-4	7.09	3.7	100	10
F-2-5	7.18	3.4	100	10
F-3-1	11.37	3.7	100	10
F-3-2	11.03	3.8	100	10
F-3-3	11.17	3.9	100	10
F-3-4	11.17	3.9	100	10
F-3-5	11.30	3.8	100	10
F-4-1	14.80	4.1	100	10
F-4-2	14.62	3.6	100	10
F-4-3	14.80	4.3	100	10
F-4-4	14.08	3.5	100	10
F-4-5	14.08	3.7	100	10
F-6-1	22.74	3.8	100	10
F-6-2	22.61	3.9	100	10
F-6-3	21.39	4.3	100	10
F-6-4	23.01	3.9	100	10
F-6-5	22.87	4.7	100	10
FC-1	0.00	9.6	90	8
FC-2	0.00	10.0	85	8
FC-3	0.00	11.5	85	8
FC-4	0.00	25.4	2	7
FC-5	0.00	25.7	5	7

TANNIC/GALLIC ACID SET

Tannic acid

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
T-1-1	3.86	54.9	2	0
T-1-2	3.81	54.3	2	0
T-1-3	3.97	59.8	3	0
T-1-4	4.08	49.9	2	0
T-1-5	4.06	55.5	3	0
T-2-1	7.94	49.5	3	0
T-2-2	7.89	43.1	3	4
T-2-3	8.12	41.6	2	4
T-2-4	8.48	43.8	2	4
T-2-5	7.80	51.6	2	0
T-3-1	12.11	39.1	7	4
T-3-2	12.65	19.2	90	6
T-3-3	12.18	15.3	95	7
T-3-4	12.45	12.9	95	8
T-3-5	12.38	18.7	90	7
T-4-1	17.31	8.7	100	8
T-4-2	16.60	9.5	100	9
T-4-3	16.95	9.4	96	9
T-4-4	17.22	10.4	100	9
T-4-5	16.51	12.9	100	8
T-6-1	26.51	7.4	100	9
T-6-2	25.70	7.5	100	9
T-6-3	25.16	7.4	100	9.5
T-6-4	27.05	9.3	98	9
T-6-5	25.97	6.6	100	9.5
TC-1	0.00	54.2	2	0
TC-2	0.00	64.7	1	0
TC-3	0.00	57.6	1	0
TC-4	0.00	65.4	1	0
TC-5	0.00	60.2	1	0

Propyl gallate

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
PG-1-1	3.86	10	100	8
PG-1-2	3.79	7	100	9
PG-1-3	3.90	7	100	9
PG-1-4	3.86	23	5	7
PG-1-5	3.70	9	100	8
PG-2-1	7.36	7	100	9
PG-2-2	7.45	10	100	8
PG-2-3	7.45	7	100	9
PG-2-4	7.49	14	50	8
PG-2-5	7.54	11	100	8
PG-3-1	11.10	9	100	8
PG-3-2	11.71	5	100	9
PG-3-3	10.90	7	100	9
PG-3-4	11.10	10	100	8
PG-3-5	11.17	7	100	9
PG-4-1	14.71	7	100	9
PG-4-2	14.89	7	100	9
PG-4-3	14.98	7	100	9
PG-4-4	15.61	9	100	9
PG-4-5	14.62	8	100	9
PG-6-1	23.28	6	100	9.5
PG-6-2	23.41	5	100	9.5
PG-6-3	22.87	5	100	9.5
PG-6-4	23.01	5	100	9.5
PG-6-5	23.01	6	100	9.5
PGC-1	0.00	13	100	7
PGC-2	0.00	27	5	6
PGC-3	0.00	11	100	7
PGC-4	0.00	16	10	6
PGC-5	0.00	8	100	7

Propyl benzoate – A non-antioxidant analogue of propyl gallate

Sample #	RTN (kg/m³)	% Mass Loss	% Mortality	Rating
PB-1-1	4.33	14.8	100	7
PB-1-2	4.17	25.0	5	7
PB-1-3	4.28	35.6	3	4
PB-1-4	4.17	15.9	12	6
PB-1-5	4.33	42.5	7	4
PB-2-1	8.66	24.4	5	6
PB-2-2	8.61	40.3	3	4
PB-2-3	8.43	27.1	3	6
PB-2-4	8.25	19.4	8	7
PB-2-5	8.43	26.7	3	4
PB-3-1	12.72	21.4	10	6
PB-3-2	12.45	10.3	100	7
PB-3-3	12.58	22.0	12	6
PB-3-4	12.18	30.5	3	4
PB-3-5	12.38	18.7	10	6
PB-4-1	15.43	21.0	3	6
PB-4-2	17.13	19.4	2	6
PB-4-3	17.04	29.2	1	6
PB-4-4	16.42	11.9	50	7
PB-4-5	16.95	11.1	75	8
PB-6-1	25.43	9.6	90	7
PB-6-2	25.70	17.8	75	7
PB-6-3	25.03	8.8	100	8
PB-6-4	25.30	15.5	75	7
PB-6-5	25.43	3.6	100	9
PBC-1	0.00	14.7	100	7
PBC-2	0.00	16.5	100	7
PBC-3	0.00	12.1	70	8
PBC-4	0.00	33.5	2	6
PBC-5	0.00	31.3	2	4

APPENDIX B

LEAST SIGNIFICANT DIFFERENCE RESULTS FOR EACH COMPOUND TESTED

BHA SET

BHA, LSD = 10.70

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	32.206	5	0
B	9.565	5	0.5
B			
B	8.328	5	1
B			
B	3.741	5	2
B			
B	3.518	5	3
B			
B	2.169	5	5

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Butyl benzene, LSD = 10.28

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	40.106	5	2
A			
A	38.763	5	1
A			
A	34.646	5	0
A			
A	33.204	5	3
A			
A	32.386	5	4
B	15.439	5	6

FLAVONOID SET

Quercetin, LSD = 5.72

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	61.180	5	2
A			
A	60.562	5	0
A			
A	60.299	5	1
A			
A	57.422	5	3

-

Morin, LSD = 4.45

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	16.459	5	0
B	4.128	5	6
B			
B	4.091	5	1
B			
B	3.841	5	4
B			
B	3.828	5	3
B			
B	3.670	5	2

Catechin, LSD = 2.57

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	51.550	5	0
A			
A	51.125	5	1
B	43.345	5	2
C	35.389	5	3
D	23.930	5	4
E	9.462	5	6

-

Flavanone, LSD = 4.45

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	16.459	5	0
B	4.128	5	6
B			
B	4.091	5	1
B			
B	3.841	5	4
B			
B	3.828	5	3
B			
B	3.670	5	2

TANNIC/GALLIC ACID SET

Tannic Acid, LSD = 6.86

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	60.407	5	0
A			
A	54.874	5	1
B	45.919	5	2
C	21.028	5	3
D	10.177	5	4
D			
D	7.631	5	6

-

Propyl gallate, LSD = 5.59

Means with the same letter are not significantly different.

t Grouping			Mean	N	Pct_Soln
	A		15.016	5	0
	A				
B	A		11.405	5	1
B	A				
B	A	C	9.873	5	2
B		C			
B		C	7.599	5	3
B		C			
B		C	7.397	5	4
		C			
		C	5.499	5	6

Propyl benzoate, LSD = 11.27

Means with the same letter are not significantly different.

t Grouping	Mean	N	Pct_Soln
A	27.597	5	2
A			
A	26.760	5	1
A			
B A	21.633	5	0
B A			
B A	20.581	5	3
B A			
B A	18.523	5	4
B			
B	11.052	5	6