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Section 1 **Biology**

Response of the Formosan Subterranean Termite (*Coptotermes formosanus*) to Cellulose Insulation Treated with Boric Acid in Choice and No-Choice Tests.

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ABSTRACT

The tunneling ability of the Formosan subterranean termite *Coptotermes formosanus* Shiraki through a cellulose insulation material containing11.1% boric acid was tested in choice and no-choice bioassays. We examined tunneling behavior and mortality of termites exposed to treated and untreated insulation material in miniature simulated wall voids. In a choice test termites tunneled through untreated insulation in all but one of the replicates used. Termites were unable to fully penetrate any of the replicates containing treated insulation and experienced a significantly higher mortality (78.4 ± 18.4%) than termites exposed to untreated insulation (11.6 ± 5.6%, F = 60.4, df = 1, P < 0.0001). In a no-choice test termites fully penetrated all replicates containing untreated insulation and experienced 37.1 ± 37.2% mortality. Termites exposed to treated insulation in this test experienced a significantly higher mortality of 100.0% (F = 14.3, df = 1, P < 0.005), and did not fully penetrate the treated insulation.

Keywords: Boric acid, cellulose insulation, choice test, *Coptotermes formosanus*, Formosan termites.

1. INTRODUCTION

Construction materials used in tropical and subtropical areas are exposed to severe risk of subterranean termite attack. The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, in particular is a severe pest in many parts of the world. Its cryptobiotic lifestyle and aggressiveness make it difficult to manage and control (Yates et al., 1999). In Hawaii, this termite is the most economically damaging insect pest, costing over $100 million for control and damage repairs each year. In other regions, termites cause several billion dollars in damage and repair annually. Inhibiting termite penetration of the structure, and the ability of building materials to resist termite attack are critical factors in architectural design and construction decisions.

Many homes employ insulative materials to prevent heat loss. One of these materials, rigid board insulation (RBI) made from polystyrene, is used to control heat loss around foundations. Rigid board insulation is placed against foundation walls around the exterior perimeter of a structure and is often covered over with siding, stucco, or wood (Smith and Zungoli, 1995). It has been discovered that termites frequently tunnel into this insulation material and although it is not consumed they will travel through it or along the joint with the wall surface to a wood source in the structure. Since construction codes require RBI to extend downward to, and below grade, it is difficult to monitor for the presence of termites in a structure with this material (Guyette, 1991). According to Smith and Zungoli (1995) building codes have created conditions conducive to termite infestation in structures built with RBI and have increased the liability of pest control operations.

Another common insulating material, cellulose insulation, is milled from recycled newsprint into a fibrous form that is cost effective, has a high thermal value, and excellent sound deadening properties (Fogel and Lloyd, 2002). In most countries cellulose insulation materials are pretreated with borate compounds at high retentions as flame-retardants and fungal inhibitors since the increased water holding capacity, surface area and cellulose content are conducive to fungal growth (Viitenen, 1991).

As general insecticides borates are often applied as dusts in isolated dry areas, particularly in wall voids during construction. This form of "built in pest control" has long been used to combat cockroach and other pest problems (Mallis, 1997). As long as treated areas remain dry the effectiveness of the initial treatment can last many years. Dip-diffusion or pressure treatment of lumber with disodium octaborate tetrahydrate (DOT) or application of water or glycol based solutions of DOT or boric acid to wood or cellulose material can provide an effective deterrent against insect and fungal attack (Grace, 1997). Due to the non-repellent nature of borates, termites will attempt to feed when borate treated wood is first discovered. This feeding is superficial and effects on the initial foragers are slightly delayed. Mori (1987) found that pulverized newspaper treated with boric acid and applied in a layer under new construction eliminated visible activity of *Coptotermes formosanus* after six months to a year. Viitenen (1991) found that borate compounds effectively prevented fungal growth when added to insulation fiber. However, no studies have examined the use of treated pulverized paper used as insulation material to deter subterranean termites. In the present study, we tested the ability of an insulation material composed of pulverized, recycled newsprint (cellulose material) treated with boric acid to control tunneling Formosan subterranean termites in choice and no choice tests. TAP (Thermal-Acoustical-Pest Control) insulation, combines the use of cellulose insulation with a borate insecticide and consists of 85% pulverized newsprint treated with a flame retardant and 11.1% (w/w) pure boric acid. The insulation is manufactured by Cellulose Technologies Group, Inc. (CTG) and registered by the US Environmental Protection Agency (EPA Reg. 72787-1).

The material is blown into attics and crawlspaces filling existing voids and can give an increased R-value of up to 45% (Van Klaveren, 2003).

We designed a laboratory choice test to mimic the field situation in which foraging termites enter a building and encounter a wall void filled with insulation. Food (wood) was available to the insects in their original nest container, so that they were provided with the opportunity to tunnel into the insulation, but were not forced to do so out of starvation. Additional food (wood) was made available to them on the opposite side of the “tunneling arena” containing the insulation. We also performed a no-choice test that rigorously tests the ability of the material to resist termites since no food (wood) is available to them in their nest container, and they must either tunnel through the insulation in search of food or feed upon the insulation directly. Both tests are based upon a standard experimental design in our laboratory that was originally developed to evaluate termite tunneling into sand treated with borates and other insecticides (Grace 1990, 1991; Grace et al. 1992; Grace & Yates 1992; Tamashiro et al. 1996).

2. MATERIALS AND METHODS

2.1 Choice Test

The choice and no-choice tunneling assays followed the design described by Grace (1990, 1991), and also used by Grace et al. (1992), Grace & Yates (1992) and Tamashiro et al. (1996) to evaluate termite penetration of insecticide-treated sand. In this assay, standard glass microscope slides (2.5 x 7.5 cm) are spaced 3-4 mm apart and secured in a horizontal upright position along one edge by silicone sealant to a base consisting of a third flat microscope slide. The ends of the tunneling arena are sealed with plastic spacers and silicone caulking. A 1.5 cm long Tygon tube is inserted at the base of each end of the arena leading into the base of one of two 55 ml polystyrene vials, each containing 20 g silica sand and 4 ml water (Figure 1).

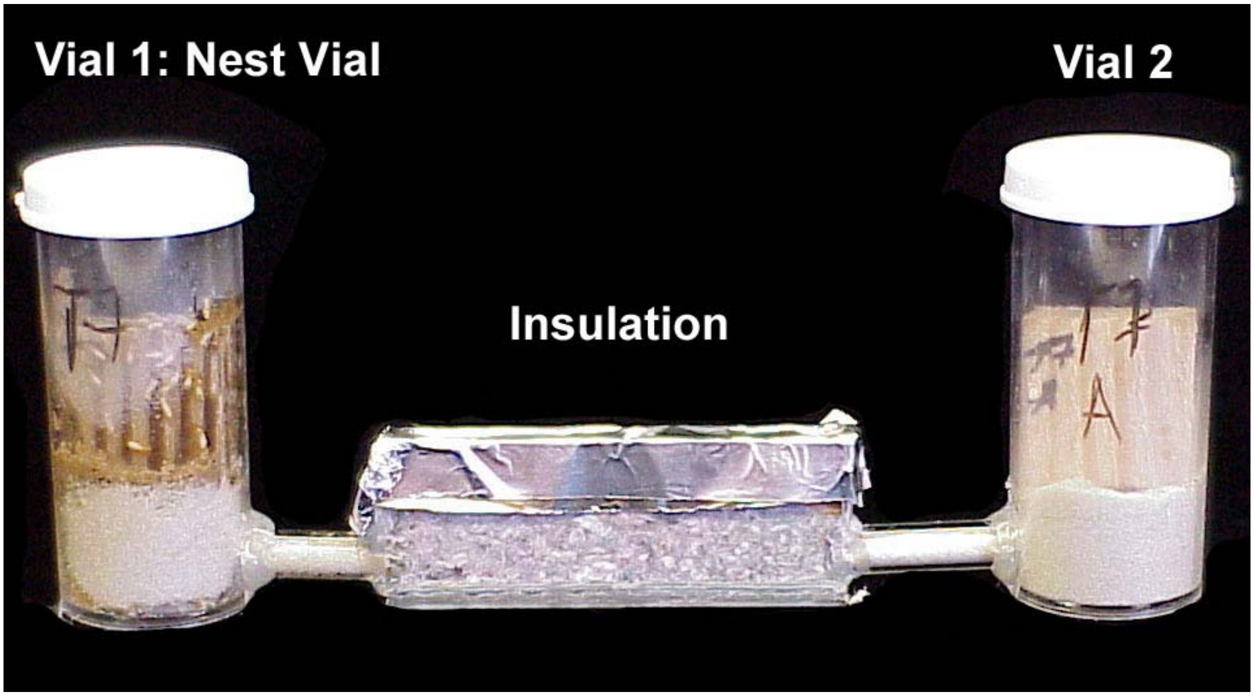

Figure 1. Test apparatus for measuring termite tunneling in insulation for the choice test. A no-choice test was performed with an identical apparatus minus wood in vial 1 (nest vial).

The center tunneling area between the two vials was filled with ca. 1 g of either dry TAP insulation or untreated insulation, and the top of the arena sealed with steel wool and covered with aluminum foil. The TAP insulation was treated to a target concentration of 11.1% (w/w) BAE and was provided by Cellulose Technologies Group Inc. A 1 x 1 x 0.25-inch Douglas-fir wood wafer was oven-dried (90° C for 24 hours), weighed, and placed in each vial. Formosan subterranean termites, *Coptotermes formosanus* Shiraki, were collected from an active field colony on the Manoa campus of the University of Hawaii (Oahu, Hawaii) immediately before the laboratory test using a standard trapping technique (Tamashiro et al. 1973). 200 termites (180 workers and 20 soldiers, to mimic natural caste proportions) were placed in one vial, as shown in Figure 1.

Five replicates were constructed with TAP insulation in the center arena, and 5 with untreated insulation. All replicates were held in an unlighted incubator at 27° C for 4 weeks (28 days). After this period we measured the distance the termites had tunneled through the arena, termite mortality, and the amount of feeding on the wood wafers in the vials. Wood wafers were again oven-dried and weighed to determine the mass loss from termite feeding. This setup mimicked a field situation in which termites entering a building encounter a wall void filled with insulation. This is a "choice test" in that the termites may either attempt to tunnel into the insulation in search of additional food, or may avoid the insulation and feed on the nearby wood that is already available to them.

2.2 No-Choice Test

This test was set up exactly as above except that vial 1, the nest vial, contained only sand and no wood. This no-choice test setup represents a rigorous exposure, in which the termites have no choice but to explore the insulation in order to find food. A 1 x 1 x 0.25-inch Douglas-fir wood wafer was placed in vial 2. Termites were placed in vial 1 and thus forced to explore or tunnel through the cellulose insulation material.

Five replicates were constructed with TAP insulation in the center arena, and 5 with untreated insulation. All replicates were held in an unlighted incubator at 27° C for 4 weeks (28 days). At the end of that period, we measured the distance the termites had tunneled through the arena, termite mortality, and the amount of feeding on the wood wafer in vial 2. Wood wafers were oven-dried (90° C for 24 hours) and weighed to determine the mass loss from termite feeding.

2.3 Data Analysis

Wood mass loss, tunneling length and mortality data were analyzed using ANOVA in SAS (SAS Institute, 2001). Means were compared using Ryan-Einot-Gabriel-Welsh multiple range tests.

3.0 RESULTS AND DISCUSION

3.1 Choice Test

The extent of termite tunneling through both the treated TAP insulation and the untreated insulation, resulting termite mortality, and termite feeding on the wood wafers in the "nest" vial (vial 1), and vial 2 are presented in Table 1. Termites fully penetrated all of the 7.5 cm long tunneling arenas containing the untreated cellulose insulation. In contrast, termites were unable to fully penetrate any of the tunneling arenas containing treated TAP insulation and tunneled an average of 2.8 ± 2.2cm. Termites penetrated the untreated insulation significantly further than termites in arenas containing the treated insulation ($F = 22.0$, $df = 1$, $P < 0.0016$). In four of the five control (untreated) replicates, feeding was almost entirely confined to the wood wafer placed in the original termite nesting container (vial 1). However, in one of these untreated replicates, the termites transferred almost all of their feeding activity to the wood wafer on the opposite side of the untreated insulation (vial 2). Thus, the untreated cellulose insulation alone did not represent a barrier to termite penetration, nor prevent feeding on adjacent wood by the termites following such penetration.

Contact with the treated insulation resulted in a significantly higher termite mortality (mean of 78.4 ± 18.4%), in comparison to the low mortality (mean of 11.6 ± 5.6%) observed in the untreated control replicates ($F = 60.4$, $df = 1$, $P < 0.0001$). In three of the five treated replicates we observed a correlation between increased mortality and tunnel length. This suggests that mortality increased as more of the TAP insulation was consumed.

There was evidence ($F = 5.08$, $df = 1$, $P < 0.05$) that termites exposed to the untreated insulation ate significantly more of the wood in vial 1 than termites from treated arenas. This greater feeding is indicative of the greater survival of these termites over the test period. Termites exposed to the treated insulation did not tunnel through the material and were left to feed on the block in vial 1. However, since the numbers of these termites were reduced by exposure to treated insulation there were fewer termites to feed on the wood source. Termites in the untreated insulation tunneled through and fed on the wood in vial 2. Termites exposed to the treated insulation did not tunnel through and thus did not feed on any wood in vial 2. Interestingly there was no significant difference ($F = 1.6$, $df = 1$, $P < 0.24$) between wood consumption in vial 2 for the treated and untreated exposures. This appears to be due to the high variation observed in the untreated replicates given the fact that ample wood was available in vial 1 for a test of this duration. Some of the termites that tunneled through the arenas fed on the wood while in other replicates no feeding occurred during the test period. Since termites penetrated none of the treated TAP insulation arenas, no feeding occurred on the wood wafers on the opposite side of the tunneling arenas.

TABLE 1: Results of choice and no-choice tests. 200 Formosan subterranean termites were placed in Vial #1, with either a wood wafer as food or with no wood wafer. Vial #1 was connected to Vial #2, containing a second wood wafer, by a 7.5-cm tunneling arena containing cellulose insulation. Feeding on wood wafers, distance tunneled through the arena, and termite mortality were recorded after 4 weeks (28 days).

TREATMENT/TEST	VIAL #1 (nest)		TUNNELING ARENA		VIAL #2		TERMITE MORTALITY	
	Mean % Wood Mass Loss	SD	Mean Distance Tunneled (cm)	SD	Mean % Wood Mass Loss	SD	Mean % Mortality	SD
CHOICE TEST								
Untreated Insulation	19.14	5.57	7.50	0.00	4.35	7.72	11.60	5.57
Treated Insulation (TAP)	12.66	3.21	2.80	2.24	0.00	0.00	78.40	18.40
NO-CHOICE TEST								
Untreated Insulation	na	na	7.50	0.00	8.49	6.00	37.10	37.22
Treated Insulation (TAP)	na	na	2.76	0.60	0.25	0.23	100.00	0.00

As is the case with borate wood treatments (Grace 1997), the boric acid content of the TAP insulation did not deter termites from initially exploring the material. However, that exploration resulted in very high termite mortality. It is likely that termite mortality in the vicinity of the exploratory tunnels into the TAP insulation repels other foragers from continuing the tunneling effort. As a result, even though some termites (50% in one case) still remained alive in the TAP replicates at the conclusion of this 4-week test, the insulation barrier was never fully penetrated, and wood on the opposite side was never located nor fed upon.

3.2 No-Choice Test

Table 1 shows the extent of termite tunneling through both treated TAP insulation and untreated insulation, resulting termite mortality, and termite feeding on the wood wafer in the end vial (Vial #2) for the no-choice test. Termites fully penetrated all of the 7.5 cm long tunneling arenas containing the untreated cellulose insulation. As observed in the choice test, the boric acid content of the TAP insulation did not deter termites from initially exploring and tunneling into the treated material. Termites exposed to the treated insulation tunneled an average of 2.76 ± 0.6 cm. This was significantly less than termites in the untreated insulation ($F = 313.8$, df = 1, $P < 0.0001$).

Termites in the untreated insulation exposure consumed significantly more wood in vial 2 than termites in the treated insulation ($F = 9.4$, df = 1, $P < 0.015$). In four of the five control (untreated) replicates, the termites proceeded to feed on the wood wafer. However, in one of these untreated replicates, termites tunneled the length of the arena, but failed to locate the tube leading into the end vial containing wood. Although termite mortality was fairly low in the other 4 control replicates, in this particular replicate, all termites died during the 4-week exposure, apparently as a result of starvation when the wood wafer was not located. These results indicate that the untreated cellulose insulation does not prevent termite tunneling, but may not be an unacceptable food source to termites. No termites fed on wood in vial 2 in the treated replicates. In the absence of termite feeding, three wafers exhibited a very slight mass loss (0.4%), most likely due to slight fluctuations in moisture content.

Termites exposed to the treated insulation had a significantly higher mortality (100.0%) than termites exposed to the untreated insulation (37.1 ± 37.2%, $F = 14.3$, df = 1, $P < 0.005$). Termites exposed to the treated insulation experienced 100.0% mortality, probably as the combined result of toxicity of the TAP insulation and starvation. This is similar to the high mortality observed in the choice test (mean of 78.4 ± 18.4%).

Our results indicate that installation of TAP insulation could aid in the protection of structures from destruction by Formosan subterranean termites by killing those termites that attempt to tunnel through it. This is not a stand-alone termite protection treatment, but could help to inhibit termite activity in wall voids, including tunnel (shelter tube) construction on the surface of wood within such voids that is in direct contact with the TAP insulation. The TAP insulation does not provide a food source for termites, nor a suitable tunneling medium due to the insecticidal activity of the borate treatment. Moreover, although termites will tunnel through the untreated cellulose-based insulation, it does not appear to be an adequate food source for them, and they will die if no other food is available. These are significant benefits in an integrated approach to termite protection.

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