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**Treatment of Douglas-fir Heartwood with Disodium Octaborate Tetrahydrate
(Tim-Bor®) to Prevent Attack by the Formosan Subterranean Termite**

by

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Treatment of Douglas-fir Heartwood with Disodium Octaborate Tetrahydrate (Tim-Bor®) to Prevent Attack by the Formosan Subterranean Termite^{1,2}

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ABSTRACT

Toxicity of disodium octaborate tetrahydrate (Tim-Bor®) to *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), and termite feeding on treated Douglas-fir heartwood were evaluated in laboratory and field tests. Feeding on filter papers impregnated with Tim-Bor® solutions reduced but did not eliminate termite gut protozoan populations. In a forced-feeding laboratory assay, Douglas-fir heartwood treated to Tim-Bor® retentions $\geq 0.35\%$ BAE drastically reduced termite feeding and resulted in 100% termite mortality within three weeks. Gradual and significant mortality (49%) after four weeks of feeding at 0.16% BAE suggests that this or lesser concentrations may be useful in baits for remedial termite control. After 162 days of field exposure to an active *C. formosanus* colony in an accelerated field test, moderate feeding was noted at 0.65% BAE (13.6% weight loss) and 0.73% BAE (16.9% wt. loss), and only slight damage (2.5% wt. loss) at the highest retention field tested of 1.02% BAE. These results indicate that Tim-Bor® provides protection from Formosan termite attack, but that some cosmetic damage occurs even at high retentions. This cosmetic damage is unlikely to create a structural hazard, but additional field evaluations are needed to determine the treatment requirements for timbers visible to the consumer.

KEYWORDS: *Coptotermes formosanus*, *Pseudotsuga menziesii*, termite control, boron, borate, wood preservative, Rhinotermitidae, Isoptera

1 INTRODUCTION

The use of preservative-treated lumber in building construction is an important component of integrated pest management of termites (Isoptera) in Hawaii (Tamashiro *et al.* 1987, 1990). The termite species involved are the highly destructive Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Rhinotermitidae), and the West Indian drywood termite, *Cryptotermes brevis* Walker (Kalotermitidae). Untreated or inadequately treated lumber can be quickly destroyed by these termites.

Although Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) heartwood, the primary construction lumber used in Hawaii (Wilcox 1984), has admirable qualities, it is highly susceptible to Formosan termite attack (Su & Tamashiro 1986) and resistant to preservative penetration. At present, the only treatment providing adequate penetration and protection against *C. formosanus* is ammoniacal-copper-zinc-arsenate (ACZA, or Chemonite® [J.H. Baxter & Co.]) (Tamashiro *et al.* 1988). Unfortunately, the required incisions and discoloration of the treated wood prevents the use of ACZA for exposed building timbers. Moreover, arsenical wood treatments in general have raised environmental and public health concerns.

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Borate wood treatments such as disodium octaborate tetrahydrate (TIM-BOR®) have no aesthetic drawbacks, low mammalian toxicity, and can provide adequate penetration of Douglas-fir heartwood (Lebow & Morrell 1988; Barnes *et al.* 1990; Williams 1990). Although disodium octaborate tetrahydrate is toxic to termites (Williams & Amburgey 1987; Williams *et al.* 1990a, 1990b; Grace *et al.* 1990), there is little data available on its performance under field conditions in areas of high termite hazard.

The current study was performed to determine the effectiveness of disodium octaborate tetrahydrate in protecting Douglas-fir heartwood from feeding by Formosan subterranean termites. This required (1) determining the toxicity of disodium octaborate tetrahydrate to *C. formosanus* and its intestinal protozoa, and evaluating its effectiveness as a treatment in Douglas-fir heartwood in (2) laboratory and (3) field studies.

2 MATERIALS AND METHODS

2.1 Toxicity test

Since borates are stomach poisons, treated filter paper (Whatman No. 2) was fed to *C. formosanus* workers to assess toxicity. A stock solution was made by dissolving 120 g disodium octaborate tetrahydrate (TIM-BOR®, United States Borax & Chemical Corporation, Anaheim, California, USA) in 1 liter distilled water. Test concentrations (120 g/l, 12.0 g/l, and 1.2 g/l) were made by diluting aliquots of this stock solution with distilled water.

Each filter paper was completely saturated by dipping in a solution of the desired concentration, and air-dried. It was then placed in a glass petri dish and 2 ml distilled water added to moisten the paper. Thirty *C. formosanus* workers, collected immediately before use from an active field colony (Tamashiro *et al.* 1973), were introduced into each dish. The dishes were held in an unlighted incubator at 29° C and checked daily for mortality and symptoms of toxicity. Eight replicates were prepared of each solution concentration: five were monitored for termite mortality, and the remaining three were used to determine the effect of disodium octaborate tetrahydrate on the symbiotic gut protozoa (*Pseudotriconympha grassi* Koidzumi, *Holomastigotoides hartmanni* Koidzumi, and *Spirotrichonympha leidyi* Koidzumi).

Counts of the protozoa were made in the following manner: At 1, 2, 4, 7, 9, 11 and 16 days, three termites from each solution concentration were dissected and the number of protozoa in the hind gut counted using a modification of the technique developed by Lai *et al.* (1983). The modification was that entire hind gut was triturated as a single unit and the protozoa counted. Counts were terminated when all of the workers exposed to each disodium octaborate tetrahydrate concentration died.

2.2 Laboratory test of treated wood

Laboratory tests on the efficacy of disodium octaborate tetrahydrate as a wood treatment for Douglas-fir heartwood were conducted using 1.9 X 1.9 X 1.9 cm cubes (ca. 2.5 g) pressure impregnated with TIM-BOR® by the US Borax & Chemical Corp. (Anaheim, CA). The cubes were treated to 6 retentions (weight/weight percentage, by chemical analysis) of disodium octaborate tetrahydrate: 0.08, 0.13, 0.29, 0.45, 0.67, and 0.98% (weight/weight). There were 5 replicates for retentions of 0.13% and higher, and 4 replicates for 0.08% and the untreated controls.

The force-feeding assay with *C. formosanus* was a modification of the ASTM D 3345-74 (Reapproved 1980) method (similar to AWPA M12-72). The modifications were (1) oven-dry weight loss instead of visual estimation was used to measure damage, and (2) more termites were used in the test and termite mortality was evaluated.

The treated cubes were oven-dried (63° C for 7 days), weighed, placed individually in plastic containers (9.5 cm diameter X 3.5 cm high), and covered with 150 g coral sand (washed and oven-dried). This was a sufficient amount of sand to cover all but the upper surface of the test block. Thirty milliliters of distilled water were added to each container before adding the termites. Termites were collected as described above, 400 workers placed in each container, and the containers held in an unlighted incubator at 29° C. The containers were examined weekly to estimate termite mortality. Four weeks after the initiation of the test, the cubes were removed, cleaned, oven-dried, and re-weighed, and total termite mortality was determined. Data were subjected to analysis of variance (ANOVA) and significantly different means separated by Duncan's multiple range test (SAS 1985).

2.3 Field test of treated wood

Nonleaching, aboveground field tests were conducted in a vigorous colony of Formosan subterranean termites located on the Manoa campus of the University of Hawaii. Douglas-fir heartwood boards 2.5 X 10.2 X 20.4 cm (ca. 165 g) were pressure impregnated with TIM-BOR® by US Borax & Chemical Corp. to four retentions (by chemical analysis) of disodium octaborate tetrahydrate: 0.18, 0.54, 0.61, and 0.85% (weight/weight).

Rectangular traps or test boxes (10.2 X 10.2 X 20.4 cm) were constructed using two 2.5 X 10.2 X 20.4 cm boards treated to the same retention and two untreated 2.5 X 5.1 X 20.4 cm boards as sides, as described by Tamashiro *et al.* (1988). Each test box was placed within a covered 5 gallon metal can (with the can bottom removed) on the soil surface. Termites had been actively foraging on untreated wooden boxes placed within these cans for several years. To minimize leaching of the preservative, a small hollow concrete block (5.1 cm high) was first placed on the soil surface within the can. A short wooden stake was driven through the hollow center of the block into the soil (and the termite foraging galleries), and a 6 mil polyethylene sheet (with a hole for the stake) laid over the top of the block. The test box was then placed on the plastic sheet, with the wooden stake allowing the termites direct access from the soil to the box.

The hollow interior of each test box was filled with paper toweling, and the top of each box was capped with an untreated 2.5 X 10.2 X 10.2 cm Douglas-fir heartwood board. Control test boxes were constructed in a similar manner with untreated wood. Each preservative retention was replicated with 4 test boxes, for a total of 8 boards per treatment.

Traps were examined at weekly intervals to determine when the termites initially attacked each test box. *Coptotermes formosanus* is capricious in its pattern of attack, and termites were noted in some traps within a few days, while others were untouched for several months. In order to standardize the exposure period, each test box was removed 162 days (23 weeks) after the initial termite attack on the untreated wood in that particular test box was observed. Thus, each treated board was exposed for 162 days to actively foraging termites. After removal from the trap, each test box was dismantled, cleaned, oven-dried, and the boards weighed to determine mass loss from termite feeding. Data were subjected to analysis of variance (ANOVA) and significantly different means separated by Duncan's multiple range test (SAS 1985).

3 RESULTS AND DISCUSSION

3.1 Toxicity test

Forced feeding on disodium octaborate tetrahydrate proved toxic to *C. formosanus* workers at all concentrations tested (Table 1). The rate of mortality was concentration dependent.

Prior to death, termites appeared lethargic, and had shortened and flattened dorso-ventrally with a rounded abdomen. This appearance is characteristic of termites that are dehydrated, starved, or feeding on nutritionally inadequate materials. At the highest concentration (120.0 g/l), populations of the gut protozoa *Pseudotrichonympha grassi* Koidzumi and *Holomastigotoides hartmanni* Koidzumi were greatly reduced by the fourth day. However, these protozoa were not all killed or eliminated until the termite died, and some live *P. grassi* and *H. hartmanni* were still found on the seventh day in termites that appeared to be dead. Populations of *Spirotrichonympha leydei* Koidzumi were not affected until the seventh day. By the 9th day, both termites and protozoa were dead.

From this study, we could not determine whether the borate is directly toxic to the protozoa, or whether the protozoa are affected secondarily as a result of borate toxicity to the termite. Kard (1990) also noted reductions in protozoan complement in *Reticulitermes flavipes* (Kollar) workers exposed to soil treated with boric acid. However, frequent fluctuations in intestinal symbiont populations and the difficulty of defining primary effects on

TABLE 1. Mean cumulative percentage mortality of Formosan subterranean termite workers fed filter paper impregnated with TIM-BOR®.^a

DAY	SOLUTION CONCENTRATION (g/l)			
	0.0	1.2	12.0	120.0
1	1.3	2.7	1.3	0.0
2	1.3	3.3	2.0	10.0
3	5.3	4.7	3.3	47.3
4	8.0	9.3	6.7	70.0
5	10.0	17.3	13.3	90.0
6	13.3	29.3	24.7	96.7
7	14.0	38.0	36.7	100.0
8	18.7	42.7	44.7	
9	20.7	46.7	55.0	
10	24.0	54.7	86.7	
11	25.3	64.7	95.3	
12	28.0	72.7	100.0	
13				
14				
15	36.6	88.7		
16	39.3	92.0		
17	44.0	95.3		
18	46.0	96.7		

^aMean of 5 groups of 30 termite workers.

TABLE 2. Mean numbers of protozoa in individual Formosan subterranean workers over the first 11 days, and over the 16-18th days of feeding on filter paper impregnated with TIM-BOR® solutions.^a

PROTOZOA	SOLUTION CONCENTRATION (g/l)				
	DAYS 1-11			DAYS 16-18	
	0.0	1.2	12.0	0.0	1.2
<i>Pseudotrichonympha grassi</i>	442a ±65	493a ±79	200b ±85	207a ±91	0b ±0
<i>Holomastigotoides hartmanni</i>	851a ±129	687a ±107	331b ±80	133a ±46	80a ±55
<i>Spirotrichonympha leydei</i>	1024a ±177	1087a ±148	858a ±184	353a ±163	233a ±122

^aMean (±SEM) per termite. Three termites per treatment were dissected each day. Means within a row in each day category followed by different letters are significantly different (ANOVA blocked by day with Ryan-Einot-Gabriel-Welch Multiple F Test, $\alpha \leq 0.05$).

TABLE 3. Estimated rate of termite mortality (%), final mean percent mortality, and mean amounts of TIM-BOR® treated Douglas-fir heartwood blocks (each ca. 2.5 g) eaten by Formosan subterranean termites in a four week laboratory test (modified ASTM D 3345).

% Retention ^a (wt./wt)		Mean Percent Mortality ^b				Wood Weight Loss ^c	
%DOT	%BAE	Week 1	Week 2	Week 3	Week 4	Mean Wt. Loss (g)	Percent Wt. Loss
0.00	0.00	0	0	0	18	1.231 a	53.4
0.08	0.10	0	0	0	23	1.339 a	47.6
0.13	0.16	0	0	0	49	0.784 b	33.4
0.29	0.35	0	39	100		0.211 c	8.4
0.45	0.54	0	73	100		0.141 c	5.4
0.67	0.80	0 ^d	94	100		0.091 c	3.6
0.98	1.18	0 ^d	99	100		0.074 c	2.9

^aDOT = disodium octaborate tetrahydrate. BAE = boric acid equivalents. DOT content was determined by chemical analysis.

^bMean of 4-5 (see text) replicates per treatment of 400 *C. formosanus* workers.

^cMean weight losses followed by different letters are significantly different at the P = 0.05 level.

^dTermites affected. Activity slowed but no mortality.

Table 4. Mean amounts of TIM-BOR® treated Douglas-fir heartwood boards (mean weight = 165 ± 16 g) eaten by Formosan subterranean termites during 162 days of exposure to an active termite colony in a field test.

% Retention ^a (wt./wt.)		Weight Retention ^a				Wood Weight Loss ^b	
%DOT	%BAE	(lbs/ft ³)		(kg/m ³)		Mean Wt. Loss (g)	Percent Wt. Loss
		DOT	BAE	DOT	BAE		
0.00	0.00	0.00	0.00	0.00	0.00	115.9 a	70.0
0.18	0.22	0.05	0.06	0.80	0.96	105.5 a	60.2
0.54	0.65	0.15	0.18	2.40	2.88	24.1 b	13.6
0.61	0.73	0.17	0.20	2.72	3.27	26.9 b	16.9
0.85	1.02	0.24	0.29	3.84	4.61	3.7 c	2.5

^aDOT = disodium octaborate tetrahydrate. BAE = boric acid equivalents. DOT content was determined by chemical analysis.

^bMean weight losses followed by different letters are significantly different at the P=0.05 level. N = 8 boards per treatment.

obligative symbionts have complicated other attempts to determine the mode of action of borate toxicity ((Williams *et al.* 1990). In this study, apparent susceptibility of the protozoa was directly proportional to their size and location in the hindgut. *Pseudotriconympha grassi*, the largest species, is predominant in the anterior part of the hindgut; *H. hartmanni*, the medium sized species, is found in the middle; and *S. leydei*, the smallest and least susceptible species is predominantly found in the posterior part of the hindgut (Lai *et al.* 1983). Analysis of protozoan counts for the two lowest concentration (12.0 and 1.2 g/l) over 11 days, and over the 16-18th days of exposure (i.e., sublethal effects) demonstrated reductions in *P. grassi* and *H. hartmanni* numbers, but no change in *S. leydei* relative to the control (Table 2). Termite mortality is not the direct result of starvation due to the reduction in protozoa numbers, since defaunated Formosan subterranean termites can survive as long as 30 days (Khoo & Sherman 1979).

The results of this study indicate that disodium octaborate tetrahydrate is a slow acting Type 3 material, according to the classification of Su *et al.* (1982). Even at 120 g/l, it took 9 days of force-feeding to kill all the termites. As has been suggested elsewhere (Grace *et al.* 1990; Jones 1990), TIM-BOR® may find a role in termite baiting systems, or other remedial control methods where a slow acting compound is required.

3.2 Laboratory test of treated wood

Results of the modified ASTM D3345 force-feeding test with TIM-BOR® treated Douglas-fir cubes are presented in Table 3. Prior to the fourth week of the test, cumulative mortality was estimated visually without dismantling the containers. Although at the higher retentions termites were visibly affected after the first week, no mortality was noted until the second week. Some feeding was apparent at all retentions (Table 3), and the amount of feeding was correlated with the rate of termite mortality. This slow action and non-repellence suggest again that disodium octaborate tetrahydrate may be useful as a bait toxicant for remedial termite control.

Termite feeding on the treated cubes was unaffected by 0.10% BAE, but was significantly reduced at 0.16% BAE (Table 3). However, despite 49% termite mortality at 0.16% BAE, a mean 33.4% weight loss was observed. At retentions $\geq 0.35\%$ BAE, all the termites died within 3 weeks, and wood weight losses did not exceed 10%.

3.3 Field test of treated wood

Results of exposing treated wood to an active field colony of Formosan subterranean termites are summarized in Table 4. All of the boards, both treated and untreated, were attacked to some extent, with analysis indicating three damage levels (Table 4). The controls and those containing 0.22% BAE were essentially destroyed (range of 32.8 - 94.8% weight loss). Weight losses at 0.65% BAE and 0.73% BAE ranged from 4.3 - 34.9%. At the highest retention of 1.02% BAE, an average 2.5% weight loss was recorded, with individual board weight losses ranging from 0.2 - 6.8%. Although the damage at 1.02% BAE was cosmetic and did not effect the structural integrity of the boards, this damage was easily noticeable.

This was a rigorous field test, in that the test samples were placed directly into active Formosan subterranean termite feeding sites and then monitored to insure that foraging termite workers contacted the samples. Each "test box" contained both treated and untreated wood pieces. During the 162 days of exposure, the untreated boards in each test box were almost completely consumed, with no statistical differences among the traps.

There is good correspondence between our results obtained with the highest TIM-BOR® retentions tested in the laboratory (1.18% BAE) and the field (1.02% BAE). However, significant damage occurred with 0.65% BAE and 0.73% BAE in the field test, even though comparable retentions in the laboratory test killed all feeding termites and prevented much damage. Mark-release-recapture estimation of termite populations in the field colony

before and after this study did not indicate any decline in termite numbers, and no reduction in feeding on wood in the traps was noted that would indicate a decline in colony vigor. It is apparent that, in the field, termites feed alternately at many sites on both treated and untreated wood. The random foraging pattern of *C. formosanus* (Su *et al.* 1984) would reduce the odds of a termite continuing to return to the same feeding site. Thus, a long feeding period and a large number of poisoned food sources would be required to affect colony vigor, and preservative retentions either toxic or repellent on the basis of a single exposure might be necessary to prevent any damage to the treated wood.

3.4 Conclusions

In our laboratory test, forced feeding by *C. formosanus* on TIM-BOR® treated Douglas-fir containing 0.16% BAE resulted in significant termite mortality (49%) within 4 weeks, while concentrations $\geq 0.35\%$ BAE killed all termites within 3 weeks, and resulted in less than 10% weight loss in the treated blocks. These results are in agreement with the conclusions from similar laboratory tests of Williams *et al.* (1990) that *C. formosanus* failed to survive for 7 weeks on banak (*Virola* sp.) wood with $\geq 0.125\%$ BAE, and of Williams & Amburgey (1987) that retentions in banak $> 0.17\%$ BAE were toxic to *Reticulitermes flavipes* (Kollar). However, none of these laboratory tests, including our own, accurately predict the retentions necessary for protection from termite feeding under field conditions.

In our field test, 1.02% BAE was required to limit *C. formosanus* feeding on the treated Douglas-fir to the status of cosmetic damage. This is in agreement with the observations of Preston *et al.* (1985, 1986) from an aboveground "graveyard" field test in Hawaii that 1.24% BAE was required to protect southern yellow pine from Formosan subterranean termite feeding, although lesser concentrations provided protection for a period of several years. Our observations of *C. formosanus* foraging behavior indicate that this termite does not necessarily locate or feed on all available food in a given area, and that the results of standard preservative field tests with this insect should be interpreted with caution. For example, we are currently testing the Basaltic Termite Barrier® (Ameron HC&D), a physical barrier to foraging termites, in this fashion at a heavily termite-infested location, yet only half of our control stakes have been attacked at this site in almost 5 years (Tamashiro *et al.* 1991). Thus, although our results indicate that TIM-BOR® can protect Douglas-fir heartwood from Formosan subterranean termite attack, we cannot predict a retention where absolutely no feeding would occur. Field evaluations of retentions $> 1.02\%$ BAE are needed to determine the treatment requirements for visible timbers in areas of high Formosan subterranean termite hazard. Cosmetic damage to hidden structural timbers, on the other hand, should not pose a problem so long as repeated termite attacks on those timbers do not occur.

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