Different Boron Compounds Elicit Similar Responses in *Coptotermes formosanus* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Although boric acid and other boron compounds have been used since the 1800s as insecticides, their mode of action is not well understood. Borate salts, in particular sodium and zinc formulations, are effective wood preservatives and are used extensively in Hawai'i to protect building materials from termite attack. In order to determine whether different borate salts elicit different responses in *Coptotermes formosanus* Shiraki, workers (undifferentiated individuals) were collected from field colonies maintained in Honolulu, Hawai'i, and exposed to composite board samples of different borate salt formulations in the laboratory. The treatments included zinc borate (ZB) (0.88% and 0.18%), disodium octaborate tetrahydrate (DOT) (ZB and DOT in a 60/40 and 80/20 ratio), anhydrous boric acid (B_2O_3) (60/40 and 80/20 ZB/B_2O_3), and an untreated composite board control. Activity and mortality data were recorded over a 4-week period; results suggest the concentration of boron in the wood sample, rather than the associated salt, has a greater impact on termite feeding, and that anhydrous boric acid reduces termite feeding more rapidly than the other formulations tested. Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) was used to determine boron ingestion after five days of exposure to high-concentration (ZB 0.88%, 60/40 ZB/DOT or 60/40 ZB/B_2O_3) boron treated timber. DOT consumption resulted in slightly higher boron concentrations than B_2O_3 (324.2 and 306.3 mean µg/g boron, respectively), and ZB 0.88% (170.0 µg/g) was intermediate between those two treatments and the control (30.2 µg/g). The DOT and B_2O_3 treatments were an order of magnitude greater than the composite board control.

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INTRODUCTION

In Hawai'i, termites in the families Rhinotermitidae and Kalotermitidae cause significant structural harm but the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is the most important urban pest in the state. *Coptotermes formosanus* may be found on the islands of Hawai'i, Kaua'i, Lana'i, Maui, Midway, Moloka'i, and O'ahu (HTAC 1999). In addition to approximately $100 million in annual structural damage, termites may also cause economic damage by harming crops and trees (Tamashiro *et al.* 1996). All the termites found in Hawai'i are invasive, and there are currently eight species in three families present statewide (Grace *et al.* 2002).

Although persistent soil termiticides like chlordane were effective and lasted an acceptable amount of time when applied around houses and other structures to prevent termite infestation, most were banned for use in the United States in 1988 (EPA 2000) because of environmental and safety concerns. The expiration of successful but broad-spectrum insecticides have helped drive the search for environmentally friendly and target-specific alternatives, and boron compounds can be considered an effective part of a termite management strategy.

Borates themselves do not act as a feeding repellent: in field tests, termites have investigated, and then avoided, those locations where borate-treated (rather than untreated) wood pieces were placed (Grace & Campora 2005). In long-term (three to eight week) laboratory choice tests termites fed less heavily on borate-treated pieces than untreated samples (Kartal & Ayrilmis 2005; Ahmed *et al.* 2004; Kartal *et al.* 2004). In field tests similar results have been reported, with borate-treated lumber fed on less by termites than their untreated counterparts (Tsunoda *et al.* 2000; Grace *et al.* 1995; Tokoro & Su 1993a; Jones 1991).

The goal of the first trial was to determine which boron formulation would yield the highest boron consumption and to find an appropriate length of no-choice exposure to boron-treated timber for *C. formosanus* to ensure lethal levels of boron consumption would be reached, but survival would remain high enough to obtain termites for ICP-AES analysis. The objective of the second experiment was to test the hypothesis that the ion associated with the borate salt, more than elemental boron content, would affect the amount of boron ingested and retained.

METHODS

Termite collection

All *C. formosanus* workers were collected in Douglas fir (*Pseudotsuga menziesii* Mirb. (Franco)) traps using a technique described by Tamashiro *et al.* (1973), from a field colony in Honolulu, HI. Within 24 hours of collection from the field worker termites were aspirated in groups of 200 and placed into plastic jars (8.5 cm wide x 10 cm deep). The jars contained 150 g silica sand (40 – 100 mesh; Fisher Scientific, Fairlawn, NJ), 30 mL distilled water, and a square piece of wood approximately 2.5 cm x 2.5 cm x 1 cm on an aluminium foil square centered on the sand as outlined in the AWPA Standard E1-97 (2005). During the experiment the jars were placed in an unlighted 28°C incubator at 68% relative humidity in a covered plastic box. The bottom of the box was lined with damp paper towels.

Boron ingestion

In order to determine the optimum parameters (e.g., duration of exposure, type of boron-treated wood to use) for future short-term feeding experiments using borate-treated wood, two tests were conducted using *C. formosanus*. All wood samples were aspen composite particleboard prepared with methylene diphenyl diisocyanate (MDI) and provided by Rio Tinto Minerals (US Borax) unless otherwise noted.

The objective of the first experiment was to determine wood mass loss after exposure to 200 worker termites (collected as previously explained) for four weeks, and used five independent replicates of each type of wood. The wood types were aspen composite particleboards, treated with different boron formulations at different concentrations, one untreated particleboard control, and an additional Douglas Fir control. The formulations were: ZB (zinc borate) 0.18%, ZB 0.88%, zinc borate and disodium octaborate tetrahydrate in a 60/40 ratio (ZB/DOT 60/40), ZB/DOT 80/20, ZB and anhydrous boric acid (B_2O_3) 60/40, and ZB/B_2O_3 80/20.

The second feeding test used those particleboard formulations from the first test that contained the highest boron content and were consumed the least by the termites. The objective was to determine which lumber treatment would yield the highest boron ingestion by the termites. Three replicates of each of the four wood samples were used in a five-day no-choice test, with 200 termites (collected as previously explained) in each independent replicate. The wood types were: an untreated MDI control, ZB 0.88%, ZB/DOT 60/40, and ZB/B_2O_3 60/40. Before and after the experiment the wood blocks were oven dried at 90°C for 24 hr, placed into a desiccator for 1 hr, and weighed to determine the amount of feeding on each wood sample. The mean amount of wood consumption was calculated by dividing the mean wood mass loss by the total number of termites used in the experiment.

The number of live workers in each replicate was recorded, and the unwashed live termites were placed into an oven at 50°C for three hours to dry, separated into groups of 50, and retained in 1.5 mL polypropylene microcentrifuge tubes. The samples were sent to Rio Tinto Minerals (US Borax) for boron content analysis using Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES).

Determination of boron content using ICP-AES

In this low-level elemental analysis technique, the dried samples were dissolved in a 25% nitric acid (HNO_3) solution, mixed with argon gas, filtered, and the only the finest parts of the sample were allowed to enter the torch as a mist. The fine aerosol sample/argon gas mist was injected into plasma torch; the electrons in the sample were excited, and when returned to ground state they emitted energy at element-specific wavelengths. The light emitted from the plasma was focused through a lens, the computer synchronized the light refracted by the plasma torch and the quantitative result obtained from the machine was compared to a reference standard. Twenty-five percent nitric acid was used as a blank. A dilution factor of 1 yields a detection limit (DL) of 0.04 μg/g boron; since the samples were diluted by the assay solution the DL was multiplied by the dilution factor (DF, solution weight / sample weight); the DF of 300 multiplied by the DL of 0.04 produced a detection limit of 12 μg/g for these samples using ICP-AES.

Consumption of boron

The mean amount of boron ingested by the termites in each treatment was stoichiometrically calculated from the quantity of wood consumed, and that value was compared to the boron content assayed using ICP-AES.

Boron content per termite. The boron content of each termite was obtained by a calculation based on the amount of boron assayed by ICP-AES. Based on previous measurements of termites from the same colony, the average mass of an individual C. formosanus worker termite was estimated as 4.0 mg (unpublished data). The results from the ICP-AES assay were given as ppm, converted to µg/g, and divided by the number of termites in the assay (50 workers) to calculate the amount of boron in each individual termite.

Statistical analyses

The mean wood consumption per termite, survival rate, and boron content were analysed for significant effects. The Ryan-Einot-Gabriel-Welsch Multiple Q-test ANOVA ($\alpha = 0.05$), which controls for Type 1 experiment error rate, was used to compare means (SAS Institute 2003).

RESULTS

Together these feeding trials demonstrated that the termites fed on borate treated woods in high enough concentrations to yield a noticeable elevation of boron content. Table 1 shows the results of the first feeding test, and significant wood consumption by individual termites was shown between the Douglas fir, the control MDI board, and the six boron-treated composite boards.

By the end of the third week mortality was high in all of the borate-treated experiments, so differences in mass and boron content could not be assessed. Those treatments with the highest boron concentration and highest wood consumption per individual were used in the second feeding test: the untreated control MDI, ZB 0.88%, ZB/DOT 60/40, and ZB/B_2O_3 60/40.

In the second feeding trial the actual boron content was determined using ICP-AES. The boron concentration in treated termites was over an order of magnitude greater than in the untreated MDI control, and the general trend of the data based on mass loss and survival places DOT approximately intermediate to ZB 0.88% and ZB/B_2O_3 (Table 2).

Of the borate-treated samples, zinc borate (0.88%) and DOT had the lowest deviation from the mean for wood consumption per individual termite; the variability of the actual boron content was lowest with DOT.

Based on the boron content of the wood consumed in the second feeding trial, the theoretical amount of boron that was ingested by each of the 200

Table 1: Results of the first, four-week feeding test with *C. formosanus*; five independent replicates of 200 worker termites were used. Treatments with the same letter after the numeric value were not significantly different, $P < 0.05$; the standard deviation is given in parentheses. The mean wood consumption per termite was calculated by dividing the wood mass loss at the end of the experiment by the number of termites used in each replicate (200 workers). Rio Tinto Minerals, manufacturers of the composite boards, provided the boric acid equivalent.

Treatment	Mean Wood Consumption [mg/termite] after 4 weeks	Boric Acid Equivalent (BAE) [%]	Theoretical Boron Content [µg/termite]
Douglas Fir	4.919a (1.12)	--	--
Untreated MDI	7.657b (2.32)	--	--
ZB 0.18%	1.550c (0.21)	0.15	0.724
ZB 0.88%	1.049c (0.10)	0.75	2.44
ZB/DOT 60/40	0.860c (0.15)	0.88	2.36
ZB/DOT 80/20	1.092c (0.27)	0.88	2.98
ZB/B_2O_3 60/40	0.870c (0.08)	0.88	2.38
ZB/B_2O_3 80/20	1.128c (0.32)	0.88	3.08

Table 2. Mean wood consumption, survival, and boron content of *C. formosanus* after five days of continuous exposure to borate-treated lumber in the second feeding trial; three independent replicates of 200 worker termites were used. Treatments with the same letter were not significantly different, $P < 0.05$; standard deviation is given in parentheses.

Treatment	Mean wood consumption [mg] per termite after 5 days [a]	Mean actual boron [µg/g] per 50 workers	Mean Survival [no.] out of 200 [a]
Untreated MDI	0.311 (0.48)	30.2a (6.2)	197 (3)
ZB 0.88%	0.151 (0.07)	170.0b (36.7)	184 (3)
ZB/B_2O_3 60/40	0.134 (0.06)	306.3c (90.3)	184 (9)
ZB/DOT 60/40	0.659 (0.91)	324.2c (12.4)	184 (8)

[a]No significant differences between treatments were observed.

termites in the experiment was calculated (Table 3). In the ZB 0.88% and B_2O_3, the observed boron ingestion was greater than the theoretical amount of boron available in the wood consumed; the reverse was true in the case of DOT: the theoretical amount of boron available was greater than the boron content observed.

CONCLUSIONS

Exposure to any of the borate-treated woods in these feeding trials reduced survival rates; in the second, five-day feeding test, elevated boron content was observed in those termites exposed to borate-treated timber. The highest

Table 3. Theoretical boron available from the wood consumed during the second feeding trial by each termite exposed to the borate-treated wood (200 for each experiment), calculated from the mean actual boron content assayed using ICP-AES (Table 2), compared to the theoretical boron ingestion by each of the 200 termites exposed to borate-treated lumber; the average weight of individual workers was estimated to be 4.0 mg (see section 2.3.2. and 2.3.3. of this paper for more details on the calculations). The difference between the two values was obtained by subtracting the observed boron ingested from the theoretical boron content available.

Treatment	Theoretical boron [μg/termite] available from wood consumed	Observed boron ingestion [μg/termite]	Difference (theoretical-observed)
Untreated MDI	--	0.12	--
ZB 0.88%	0.410	0.68	-0.27
ZB/B_2O_3 60/40	0.410	1.23	-0.82
ZB/DOT 60/40	1.80	1.30	0.50

boron content of the treated timber was in the ZB/DOT 60/40 formulation, followed by B_2O_3, and finally ZB; all these values were approximately an order of magnitude greater than the untreated composite board.

Interestingly, the amount of boron theoretically available to each termite from the wood consumed was less than the observed value in ZB and B_2O_3, possibly as a result of bioaccumulation. In the case of DOT, the data shows the actual boron content was lower than what was theoretically available from the wood consumed, suggesting excretion or some other mechanism is in place to decrease the overall boron content. These values were calculated using the initial number of termites used in each experiment, not the final number of survivors, because borates have not been shown to immediately deter termite feeding – although mortality was observed even given the short duration of the experiment and may have affected the calculation.

The termites were not degutted prior to ICP-AES analysis and so the boron content may have been artificially inflated by undigested borate-treated wood remaining in the gut. Additionally, termites were not washed prior to analysis so boron residues on the cuticle may have increased the boron content observed. However, due to mutual grooming and trophallaxis these concerns were ameliorated and may more accurately mimic field conditions. The objective of the experiment was to determine whether boron ingestion is increased when the associated salt, but not the boron content, of the formulation is varied; the data suggest that the associated salt, rather than the boron content alone, may impact boron ingestion and retention.

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