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TOXICITY OF BORON COMPOUNDS IN THE FORMOSAN SUBTERRANEAN TERMITE,
COPTOTERMES FORMOSANUS SHIRAKI (ISOPTERA: RHINOTERMITIDAE)

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By
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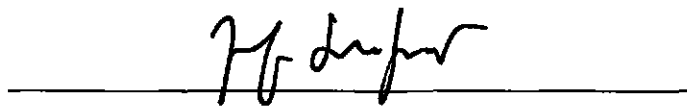
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Chapter I. Review of Boron Toxicity with an Emphasis on Termites

Properties of Boron Compounds

Boron, a ubiquitous element, constitutes a critical component of biological function – but at higher concentrations can be toxic. Found naturally in rocks, soil, and water, boron is present in the soil at an average concentration of 10 to 20 ppm and always as a boron-oxygen complex. These compounds, which are mined in arid areas that previously experienced volcanic or hydrothermal processes, have been used by humans for a variety of reasons: around 4000 years ago Babylonians imported tincal (mineral borax, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) for the manipulation of gold; boron has also been used in mummification rituals in ancient Egypt, as part of medicines, and for metalworking (Woods 1994). In modern history borates have been useful as fire retardants in glass and ceramics; as a component of detergents; a timber treatment to preserve lumber from wood decay; and in insecticides.

Boric acid is a stable and relatively biologically benign compound at levels found in nature (Hall 2005). Boranes are able to complex with a variety of compounds, especially those with adjacent hydroxyl groups, and are mild Lewis acids; most small boronic acids are amphiphilic, with both lipophilic and hydrophilic moieties.

Boron is present as a trace element in many foods and has been shown to be necessary for metabolism (Rainey *et al.* 1999; Lloyd *et al.* 1990). The most important documented role of boron is in stimulatory and inhibitory enzyme function. Without boron, plants and

animals demonstrate decreased metabolic efficiency and sometimes exhibit severe symptoms of cellular level “starvation” (Rainey *et al.* 1999; Woods 1994; Lloyd *et al.* 1990). Lloyd *et al.* (1990) have suggested borates in biological systems produce competitive inhibition due to a substrate-inhibitor complex, rather than the more common enzyme-inhibitor complex. In addition to interfering with enzyme activity, research has suggested that boron deficiency in plants interferes with the integrity of root cells, reducing the ability of cells to absorb ions, and also terminates root growth (Woods 1994).

Boron compounds as wood preservatives

When used appropriately borates are cost-effective insecticides with low toxicity to vertebrates and to the environment. Toxicity is low to mammals, and vertebrates in general, because of rapid excretion of excess boron by the kidneys (Lloyd *et al.* 1990). In rodents the lethal dose of boron is comparable to that of table salt, approximately 4,000 mg/kg for sodium tetraborate decahydrate. Boron treatments are effective at protecting wood from termite attack and colonization by harmful fungi and also act as a fire retardant at high concentrations (*Additives for Polymers* 1995). One of the main problems with the use of borate-treated lumber for construction is leaching; when placed directly in contact with the ground, where moist or wet conditions are possible, the retention of boron in treated wood is reduced (*Additives for Polymers* 1995).

Although borates themselves do not act as feeding repellents, in field tests termites have investigated, fed at, then avoided, those locations where borate-treated (rather than untreated) wood pieces were placed (Grace and Campora 2005). In laboratory choice

tests termites fed less heavily on borate-treated pieces than untreated samples (Kartal and 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 than their untreated counterparts (Tsumoda *et al.* 2000; Grace *et al.* 1995; Tokoro and Su 1993a; Jones 1991).

Because of the physical properties of boron a variety of formulations for superficial application and lumber impregnation have developed. The solvent used may further affect toxicity: Tokoru and Su (1993b) have demonstrated that ethylene glycol, a solvent used with disodium octaborate tetrahydrate (DOT), may act as a synergist and significantly decreased the amount of boric acid needed for the delivery of a lethal dose. The mechanism of this synergism is still unknown and is contrary to what is expected, namely that the ethylene glycol-boric acid complex would mean the boron would be already bound and thus rendered less able to bind *in vivo* and become biologically active.

Many efforts have been made to curtail leaching, the major setback to widespread use of borates as a successful wood treatment for the prevention of both insect and fungal damage. Kartal *et al.* (2004) successfully used chemical modifications of DOT with copolymerizations of allyl glycidyl ether and methyl methacrylate to limit boron leaching in laboratory experiments; even after severe leaching cycles, the wood specimens were effective against *C. formosanus* attack. Kartal and Ayrilmis (2005) demonstrated decreased attack by *C. formosanus*, as well as both brown-rot fungus (*Fomitopsis palustris*) and white-rot fungus (*Trametes versicolor* (L. ex. Fr.) Quel., on blockboard veneer made of untreated fir (*Abies bornmulleriana* M.) strips between borate-treated or

untreated veneers of Ekaba (*Tetraberlinia bifoliolata* Harms.). In laboratory experiments with Tim-Bor (DOT) and Bora-Care (40% DOT in ethylene glycol) treated lumber Tokoro and Su (1993a) showed an accumulation of boron in the wood next to termite galleries, perhaps as a result of termite activity (e.g., gallery construction). Blockwood may provide an alternative to more rigid composites like plywood that can still be treated with boric acid or DOT.

Biological roles and toxicity in living organisms

The pervasive utilization of boron in living organisms makes the specific mechanism of action difficult to delineate. Nzietchueng *et al.* (2002), in order to determine whether boron facilitates wound healing in humans directly or indirectly, demonstrated that extracellular matrix regeneration and protein phosphorylation is increased *in vivo* in borate-treated human fibroblasts. In addition, they described how boron treatment enhances trypsin-like, collagenase, and cathepsin D activities, most likely through a cellular mediator. One of these cellular mediators was determined to be tumor necrosis factor (TNF- α), a cytokine that is also present in insects as part of the immune system (Franchini *et al.* 1996).

Although the mechanism of borates has not been fully elucidated its toxic nature at high concentrations has been well established. Previous research has shown that termite mortality is caused at both high and low concentrations more rapidly than defaunation and starvation would account for alone (Kartal and Ayrilmis 2005; Ahmed *et al.* 2004; Khoo and Sherman 1979). The biochemistry of borates is complicated because of their structure: the tetrahydroxyborate ion $[B(OH)_4]^-$ can complex with any molecule with two

adjacent hydroxyl groups (Kim *et al.* 2004). *In vitro*, borates are toxic to all cells, and the toxicity appears to be a result of complexation through rapid esterification of borates with molecules of biological significance, although those molecules have not been clearly defined. The cells seemed to be “starved,” which points to a biostatic, rather than biocidal, toxicity (Lloyd *et al.* 1990). Borates have been reported to interact with molecules ranging from riboflavin to vitamin B6, coenzyme A, vitamin B-12, and nicotinamide adenine dinucleotide (NAD⁺) (Woods 1994; Lloyd *et al.* 1990; Williams *et al.* 1990). Kim *et al.* (2004) have shown that boron-nucleotide complexes are affected by the pH of the solution, and that at pH 7.4 only borate-NAD⁺ complexes were detected. To further muddy the waters, borates can also act as a purely ionic inhibitor; this may affect membrane stability and the polyols within them (Lloyd *et al.* 1990).

Recent research by Habes *et al.* (2006) suggests that boric acid induced glutathione S-transferase activity and inhibited acetylcholinesterase activity in the German cockroach, *Blattella germanica* (L.). Zurek *et al.* (2002), also working with *B. germanica*, have found the use of an entomopathogenic fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Deuteromycota: Hyphomycetes), used in concert with topical applications of boric acid dust (12% w/w) or aqueous formulations of boric acid (0.1% w/v) as drinking water increased cockroach mortality over the rate of either alone, showing a synergistic interaction between the two management strategies.

Organismal respiration, which can be used as an indicator of overall fitness, has been shown to be affected in termites exposed to boron or boron-treated lumber. Nunes and

Dickenson (1995) showed an increase in respiration of *Reticulitermes lucifugus* (Rhinotermitidae) at certain boric acid concentrations (0.04, 0.64 % BAE in paper). Toyoshima *et al.* (1997) reported an overall decrease in respiration in termites immediately after feeding on borate-treated timber across concentrations of boric acid (5, 10, and 20 kg/m³), although at 5 kg/m³ respiration at 15 and 30 minutes showed a slight increase from the initial rate. It is unlikely that a decrease in organismal respiration is the primary mechanism of action of boron compounds because those compounds cause death rapidly, while exposure to lethal levels of boron does not.

Toxicity parameters for boron have been well established in the literature and there is a clear dose-dependent mortality relationship. Because of the difficulty in measuring ingestion of boron by individual termites the lethal concentration for 50% of a test group (LC₅₀), rather than the lethal dose of 50% of a test population (LD₅₀), is most commonly reported. Toyoshima *et al.* (1997) reported the LD₉₉ at 16 days as 930 µg/g in *C. formosanus* worker termites, and the lethal accumulative dose as 136 µg/g. Grace (1990) showed mortality >90% in *Reticulitermes flavipes* within 15 days with substrate concentrations of ≥30,000 ppm (or, ≥30,000 µg/g) barium metaborate monohydrate, and mortality of >90% at ≥ 1000 ppm (or, ≥ 1000 µg/g) within 30 days. Grace *et al.* (1992) showed mortality of *C. formosanus* >90% after 16 days of exposure to DOT-impregnated filter paper at a solution concentration of 1.5 g/L (1500 µg/g), and of 100% by day 12 with a 12.0 g/L (12,000 µg/g) DOT solution and 7 days with a 120.0 g/L (120,000 µg/g) solution. Su *et al.* (1994) determined the LD₅₀ of boric acid as 721.29 µg/g in *C. formosanus* workers and 264.02 µg/g in *R. flavipes* (Kollar). Mortality through the

exchange of different boron dust formulations has also been demonstrated, with boric acid powder-treated termites causing mortality more rapidly than DOT dusted ones in laboratory experiments, and proportions as low as 10% of the experimental population treated; the dust may be transferred via the close interactions between these social insects, *e.g.*, mutual feeding or grooming (Grace 1992). In laboratory tunneling assays, DOT (1.20 BAE) showed greater mortality and less tunneling activity than zinc borate (0.86 BAE) with *R. flavipes*, but the reverse pattern was true of *C. formosanus* (Grace 1991).

Boron, like other trace elements needed for proper organismal growth and development, has many biological roles and complex interactions. In this thesis I measured native boron concentrations in three species of termites, *C. formosanus*, *C. acinaciformis* (Froggatt), and *Mastotermes darwiniensis* Froggatt, and gauged their ability to tolerate sublethal exposure to timber treated with different boron compounds. Based on those data, the ability of *C. formosanus* to recover from sublethal exposure to boron-treated timber and whether *C. formosanus* can horizontally transfer quantifiable amounts of boron was assessed.

Chapter II. Native boron levels and the effect of boron treatment on *Coptotermes formosanus* Shiraki, *Coptotermes acinaciformis* (Frogatt), and *Mastotermes darwiniensis* Frogatt

Abstract

Although boron is a ubiquitous element found in rocks, soil, and water, little has been determined about its physiological role in plants and animals. Comparing the effect of sublethal boron exposure on three termite species yields a broader view of the toxicity of boron compounds. *Coptotermes formosanus* (Rhinotermitidae) were collected from colonies maintained in at the University of Hawai'i at Manoa campus in Honolulu, Hawai'i (United States); *C. acinaciformis* (Rhinotermitidae) from Melbourne, Victoria (Australia); and *Mastotermes darwiniensis* (Mastotermitidae) from Darwin, North Territory (Australia). Termites were exposed to anhydrous boric acid (B₂O₃)-treated composite boards or an untreated control composite board in a no-choice test for five days, either in Honolulu (*C. formosanus*) or Australia (*C. acinaciformis* and *M. darwiniensis*); survival rates, wet weight, and boron content were determined. Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES) was used to determine boron content in untreated and experimental termites. There was a significant (P < 0.01) increase in the amount of boron present in boron-treated termites, in wood consumption, and decrease in individual weight loss after five days for *M. darwiniensis*, and a significant decrease in survival between the boron-treated *Coptotermes* and the untreated (no mortality was observed in *M. darwiniensis*). The boron content of both control and boron-treated experimental termites, determined through ICP-AES, was compared to untreated termites; all three species showed a significant (P < 0.01) increase in boron content in the treated individuals, but there were no significant differences observed between the control and untreated termites.

Introduction

In Hawai'i, termites in the Rhinotermitidae and Kalotermitidae families cause significant structural harm but the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is the most important urban pest in the state. *C. formosanus* may be found on the islands of Hawai'i, Kaua'i, Lana'i, Maui, 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 of termite in three families (Grace *et al.* 2002). Australia, by comparison, has several native species of termite. *Mastotermes darwiniensis* Froggatt and *Coptotermes acinaciformis* (Froggatt) have a wide distribution where they are granted status as both urban pests and important members of natural ecosystems (Gay and Calaby 1970).

The objective of this project was to determine whether three termite species in two families respond differently to short-term exposure to boron compounds. A five-day no-choice test, with weight loss, survival rates, wood consumption, and boron content (assayed using ICP-AES) measured at the end of the experiment, was conducted to quantify the variability of the response to ingestion of boron.

Materials and 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 the same field colony at the University of Hawai'i at Manoa campus in Honolulu, HI (Husseneder and Grace 2001a, 2001b). 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 AWP 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.

Coptotermes acinaciformis and *M. darwiniensis* were collected from colonies maintained by the Australian Commonwealth Scientific and Research Organization (CSIRO) and used within two weeks of collection. *C. acinaciformis* was collected from a mound on a stringy bark tree, halfway between the Elizabeth River and Channel Island (approximately 30 km from Darwin, Northern Territory, Australia). These termites were maintained in jars approximately 9 cm wide x 10 cm deep on moist carton material and kept in a dark incubator at 28°C and 72% relative humidity. *M. darwiniensis* was collected from a dead mango tree in Howard-Springs, approximately 30 km from Darwin (Northern Territory, Australia). These termites were maintained in plastic trays approximately 20 x 20 x 6 cm, filled with grade 4 vermiculite, *Eucalyptus raglans* (Victorian ash) sawdust, and 138 mL distilled water, and kept in a dark incubator at 32°C and 76% relative humidity.

Boron ingestion

All wood samples were aspen composite particleboard prepared with methylene diphenyl diisocyanate (MDI) and were provided by Rio Tinto Minerals (US Borax).

In Hawai'i, three replicates of each of the four wood samples were used in a 5 day no-choice test, with 200 *C. formosanus* termites (collected as previously explained) per replicate; the boron-treated samples were ZB 0.88%, ZB/DOT 60/40, and ZB/B₂O₃

60/40, plus an untreated MDI control. Before and after the experiment the wood blocks were oven dried at 90°C for 24 hr, placed into a desiccator for one hour, and weighed to determine the amount of feeding on each wood sample. At the completion of both experiments experimental (control or boron-treated) termite samples were dried at 50°C for three hours and retained for ICP-AES analysis 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). Additional samples of untreated (field-caught) termites were dried and prepared for ICP-AES analysis in the same way.

In Australia, five replicates for each treatment (zinc borate/anhydrous boric acid (B_2O_3) in a 60/40 ratio or a control composite board) were assembled each for *C. acinaciformis* and *M. darwiniensis*; only B_2O_3 was used because in *C. formosanus* it was the formulation shown to yield the highest wood consumption and boron ingestion. The 9 x 10 cm glass jars used in the experiments were filled with 150 g washed river sand, 30 mL deionized water, and one wood block (either untreated or boron-treated composite board) was placed on top a square of aluminium foil, which was centered on the sand. Because of the large difference in size and weight between *M. darwiniensis* and *Coptotermes*, 200 *C. acinaciformis* workers or 20 *M. darwiniensis* workers were placed into each replicate. Before and after the experiment the wood blocks were desiccated at 40°C and weighed. After the experiment survival and wet weight were recorded; both experimental (control or boron-treated) and untreated (wild-caught) termite samples were dried at 50°C for three hours, separated into groups (200 for *Coptotermes*, 20 for *Mastotermes*), and

retained 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 termite samples were dissolved in 2.5 mL of concentrated nitric acid (HNO₃) in digestion tubes and heated in an autobloc at 98°C for 15 minutes. The tubes were allowed to cool and 7.5 mL of sterile water was added to make a solution of 25% nitric acid; the solution was filtered and 5 mL of sample was injected into the Thermo Fisher IRIS Intrepid II (Thermo Scientific, Waltham, MA) ICP-AES machine. The machine was calibrated using 0.0, 0.2, 0.5, and 1.0 ppm boron calibration standards in a 25% nitric acid matrix. These analyses were performed at Rio Tinto Minerals (Mark Mankowski, personal communication).

Consumption of boron

The mean amount of boron theoretically ingested by the termites in each treatment (200 *Coptotermes* or 20 *Mastotermes*) 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 exposed to borate-treated timber was obtained by a calculation based on the amount of boron assayed by ICP-AES. 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 *Coptotermes* workers or 10 *Mastotermes* 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 2004).

Results

Feeding tests

A significant decrease in survival between the boron-treated and control *Coptotermes* species was observed (there was no mortality in *M. darwiniensis* after five days), as well as a significant decrease in wood consumption and individual mass for treated *M. darwiniensis* (Table 2.1).

Table 2.1. Mean weight, wood consumption, and survival rate of worker termites after five days of continuous exposure to wood samples. For *C. formosanus*, n = 3 replicates of 200 workers; for *C. acinaciformis*, n = 5 replicates of 200 workers; for *M. darwiniensis*, n = 5 replicates of 20 workers. Treatments with the same letter after the numeric value did not have significant differences where $P < 0.01$; the standard deviation is given in parentheses.

Species	Treatment	Mean weight (mg/termite)	Mean wood consumption (mg/termite)	Mean Survival (%)
<i>C. formosanus</i>	Control	0.379a (0.02)	0.311a (0.48)	95.6a
<i>C. formosanus</i>	B ₂ O ₃	0.167a (0.03)	0.134a (0.06)	90.8b
<i>C. acinaciformis</i> ,	Control	0.216a (0.26)	0.201a (0.03)	98.7a
<i>C. acinaciformis</i>	B ₂ O ₃	0.368a (0.11)	0.124a (0.02)	89.1b
<i>M. darwiniensis</i>	Control	3.2a,b (4.2)	3.94c (0.65)	100a
<i>M. darwiniensis</i>	B ₂ O ₃	6.4b (3.3)	1.59b (0.337)	100a

Species comparison of boron exposure

In *C. formosanus*, the boron concentration was an order of magnitude greater than the control, and the general toxicity trend based on weight loss and survival places DOT approximately intermediate to ZB 0.88% and B₂O₃ (Table 2.1). ZB and DOT had the lowest variability in mean weight loss per termite of the treated wood blocks, but the boron concentration variability was lowest with DOT. Although variability within species and caste was small, the weight difference between *Coptotermes* and *Mastotermes* was over an order of magnitude (Table 2.2).

Table 2.2. Mean weight of untreated termites in the three species used in this experiment. *C. formosanus* was collected in Honolulu, HI, and both *C. acinaciformis* and *M. darwiniensis* near Darwin, Northern Territory, Australia. Groups of 50 termites were weighed for *Coptotermes*; for *Mastotermes* 10 workers or soldiers were weighed. The standard deviation is in parentheses; for workers, n = 5; for *C. formosanus* soldiers, n = 3, for *C. acinaciformis* and *M. darwiniensis*, n = 1 due to low soldier ratios in collected termites.

Species, caste	Mean weight (mg/termite)
<i>C. formosanus</i> , worker	3.90 (0.13)
<i>C. formosanus</i> , soldier	3.97 (0.12)
<i>C. acinaciformis</i> , worker	3.61 (0.098)
<i>C. acinaciformis</i> , soldier	3.73
<i>M. darwiniensis</i> , worker	46.7 (2.0)
<i>M. darwiniensis</i> , soldier	56.7

There was a significant (P < 0.01) increase in boron content in the termites exposed to borate-treated wood from the control MDI, at the level of approximately one order of magnitude (Table 2.3).

Table 2. 3. Mean boron content of untreated termites and termites of each species exposed for five days to control composite and boron-treated boards. Means with the same letter after the numeric value are not significantly different ($P < 0.01$); standard deviations are in parentheses.

Species, caste	Treatment	Mean boron content ($\mu\text{g/g}$)
<i>C. formosanus</i> , worker	Untreated	7.1a (0.6)
<i>C. formosanus</i> , soldier	Untreated	7.3a (2.65)
<i>C. acinaciformis</i> , worker	Untreated	2.6a (1.1)
<i>C. acinaciformis</i> , soldier	Untreated	4.0a (1.1)
<i>M. darwiniensis</i> , worker	Untreated	0.6a (0.1)
<i>M. darwiniensis</i> , soldier	Untreated	1.1a (0.3)
<i>C. formosanus</i> , worker	Control	30.2a (6.2)
<i>C. formosanus</i> , worker	B ₂ O ₃	306.3b (90.3)
<i>C. acinaciformis</i> , worker	Control	20.5a (4.4)
<i>C. acinaciformis</i> , worker	B ₂ O ₃	122.1b (17.8)
<i>M. darwiniensis</i> , worker	Control	32.7a (14.2)
<i>M. darwiniensis</i> , worker	B ₂ O ₃	181.1c (43.6)

The boron content of both control and boron-treated experimental termites was compared to untreated termites; all three species showed a significant ($P < 0.01$) increase in boron content in the treated individuals, but there were no significant differences observed between the control and untreated termites. Additionally, although there was a large size difference, there were no significant differences in the boron content across the species.

Table 2.4. Observed boron ingestion and theoretical boron available from the wood consumed by each termite exposed to the borate-treated wood. The observed boron ingestion was calculated from the mean actual boron content assayed using ICP-AES (Table 2.3), compared to the theoretical boron ingestion by each of the termites exposed to borate-treated lumber; see the *Methods* section of this chapter 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.

Species, caste	Observed boron ingestion ($\mu\text{g/termite}$)	Theoretical boron available ($\mu\text{g/termite}$) from wood consumed	Difference (theoretical - observed)
<i>C. formosanus</i> , worker	1.23	0.673	-0.557
<i>C. acinaciformis</i> , worker	0.488	0.338	-0.150
<i>M. darwiniensis</i> , worker	0.362	4.34	3.978

In each of the species, the boron-treated lumber was fed on less than the control composite board, although the only significant decrease was in the boron-treated *M. darwiniensis* compared to the control.

Discussion

This set of experiments determined several effects of borates on termites that appear to hold true across families, including a decrease in weight and an increase in boron content immediately after feeding of about an order of magnitude from the controls. *Coptotermes formosanus* and *C. acinaciformis* displayed a similar pattern of response to boron, but the lack of mortality or significant individual weight loss in *M. darwinensis* raises some interesting questions about the toxicity of boron to different termite species. The termites used in ICP-AES analysis were not washed or degutted, but the boron was impregnated in the composite board so large scale contamination was unlikely. Although boron compounds are not repellent to termites, it has been shown that they will cease feeding on borate-treated woods after a several days (Grace and Campora 2005), minimizing the amount of residual wood that may have been left in the gut.

Along with morphological and behavioral differences between species there are relevant physiological distinctions between these three species of termites as well. Termites have a range of reported gut pH values (Brune *et al.* 1995; Brune and Kuhl 1996), which may further complicate the understanding of the mechanism of an oral toxicant. This is further complicated by the presence of different symbiotic protozoa and bacterial

communities, although the flora populations are often highly reproducible within field and laboratory colonies (Yoshimura *et al.* 1993). Despite the many different substrates termites exploit, including live and dead wood, the bacteria found in their gut serve quite homogenous roles in digesting cellulose (Eutick *et al.* 1978). It has been established that death of the gut protozoa is not the primary mechanism of action for boronic compounds, based on time to death; in addition, starvation produces a different protozoa decline than exposure to boron compounds (Grace *et al.* 1992; Yoshimura *et al.* 1994a). Seasonal fluctuations in the field of protozoa also have been noted (Yoshimura *et al.* 1994b), perhaps in response to changes in feeding during the year, identifying another variable that may play a role in how the boron compounds were processed by the termites.

The much greater mass of *M. darwinensis* may have been able to compensate for high boron concentrations without causing significant mortality or high levels of boron ingestion per termite, in contrast to the *Coptotermes* species. *C. formosanus* workers and soldiers had the highest native boron levels of the three species, which could be in part due to boron content of the volcanic soil in which they live (Hue *et al.* 1988). In a no-choice laboratory test with boron-treated wood, Ahmed (2000) found that Australian termites from areas with high boron content in the soil took slightly longer to die than those from soil with low boron content, likely the result of a dose-dependent response. This dose-dependent response may be further complicated by species, and gross mass or physiological differences.

Nation (2002) provides an excellent review of the existing literature on the ability of insects to self-select nutritional elements from both natural foods and prepared diets; and the possibility that at least some of the termites used in the experiments are purposely feeding on sections of the composite board, thereby modulating boron intake, must not be overlooked. *M. darwiniensis* is known to be a robust feeder, able to feed on live plants (including eucalyptus, sugarcane, and mango) – not to mention leather, ivory, lead-sheathed cables, and even can corrode glass and some metal surfaces (Gay and Calaby 1970). The two *Coptotermes* species may also attack live wood, although not with the same voracity as *M. darwiniensis*.

Differences in gut pH, as well as bacterial symbionts that may differ interspecifically, could mediate the effect of boron on these three species; more derived termites, in the family Termitidae, have gut pH that is as alkaline as any found in biological systems (Brune and Kuhl 1996). Changes in gut pH could alter the toxicity of boron *in vivo*, as pH changes would affect the charge and conformation of a boronic acid (Hall 2005). Kim *et al.* (2004) showed that at pH 7.4 only the borate-NAD⁺ complex was observed *in vitro*; even if other species are present at that pH *in vivo*, it is likely the borate-NAD⁺ complex will be the most biologically relevant. Further work with a variety of species, including perhaps those in the family Termitidae, could help to determine the relevant parameters for understanding boronic toxicity in insects.

**Chapter III. The response and recovery of the Formosan subterranean termite
(*Coptotermes formosanus* Shiraki) to sublethal doses of boron compounds**

Abstract

Controlling the Formosan subterranean termite, the most structurally damaging pest in the state of Hawai'i, is an important priority for homeowners and commercial builders alike. Boron-treated lumber is often part of the management strategy. In this study, termites from field colonies maintained in Honolulu, HI were collected and exposed to one of four composite boards: zinc borate (ZB) 0.88%, ZB/disodium octaborate tetrahydrate (DOT), anhydrous boric acid (B_2O_3), or an untreated composite board control. After five days, the wood pieces were removed and all replaced with untreated control boards. The impact of boron ingestion on termite fitness was examined by comparing the wet mass of termites at ten days, wood consumption of boron-treated composite boards from the start of the experiment through day five, wood consumption of untreated composite boards from day five to ten, survival at ten days, and boron content of the termites at ten days. The analyses at both five and ten days revealed significantly less boron in the control than in the treated termites ($P < 0.05$), and the general trend based on all the parameters analyzed was that the zinc borate treatment was less detrimental to the termites than the DOT, both of which were less harmful than the boric acid. The boron content of the termites at day ten (five days after removal from the treated boards) were compared with day five data from a previous experiment conducted under the same experimental conditions, and there was a decrease in mean boron content on day ten of 12.8 μg boron/g termites for the control, 114.5 μg boron/g termites for ZB 0.88%, and 227 μg boron/g termites for both DOT 60/40 and B_2O_3 . These results suggest that termites are able to eliminate ingested boron over time.

Introduction

Boron, a ubiquitous element, constitutes a critical component of biological function and is present as a trace element in many foods and has been shown to be necessary for metabolism (Rainey *et al.* 1999; Lloyd *et al.* 1990). However, at higher concentrations boron can be lethal. The most important role of boron is in stimulatory and inhibitory enzyme function; without boron, plants and animals demonstrate decreased metabolic efficiency and sometimes exhibit severe symptoms of cellular-level "starvation" (Rainey *et al.* 1999; Woods 1994; Lloyd *et al.* 1990). Examining whether termites exposed to

sublethal amounts of borates for short periods can recover may help determine whether either excretion or detoxification occurs, which may help guide future research on physiological responses to boron.

Subterranean termites have a global economic impact due to the damage caused to homes and other structures and the amount of money spent to prevent, manage, and repair manifestations of termite infestation. In 1997, these costs were estimated at over one billion dollars annually in the United States alone (Osbrink *et al.* 2001), and at approximately \$100 million in Hawai'i (Tamashiro *et al.* 1996). In Hawai'i, termites in both the Rhinotermitidae and Kalotermitidae families cause significant structural harm, but the invasive Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is the most important urban pest in the state.

Although the mechanism of action has not been fully elucidated, termite mortality after exposure to boron occurs more rapidly than would occur with defaunation and subsequent starvation alone (Kartal and Ayrilmis 2005; Ahmed *et al.* 2004; Khoo and Sherman 1979). The goal of this research was to examine the ability of termites to recover from sublethal doses of three borate formulations by comparing the weight, survival rate, and boron content of treated termites in comparison to termites fed upon untreated control wood.

Materials and Methods

Termite collection

Coptotermes formosanus workers were collected from a field colony in Honolulu, HI in Douglas fir (*Pseudotsuga menziesii* Mirb. [Franco]) traps, using a technique described by Tamashiro *et al.* (1973). Within 24 hours of collection from the field, worker termites were aspirated in groups of 100 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 placed on an aluminium foil square centered on the sand as outlined in AWWA Standard E1-97 (2005). 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 to maintain humidity.

Ingestion of boron

The treated wood samples contained either zinc borate (ZB), disodium octaborate tetrahydrate (DOT), or boric acid (B_2O_3). All wood samples were aspen composite particleboard prepared with 6% methylene diphenyl diisocyanate (MDI), provided by Rio Tinto Minerals (formerly US Borax), Valencia, CA: ZB 0.88% (0.75% boric acid equivalent (BAE)), ZB/DOT 60/40 (0.88% BAE), ZB/ B_2O_3 60/40 (0.88% BAE), and an untreated composite board control.

Recovery test

Four replicates of each of the four wood treatments were prepared. Before and after the experiment, the wood blocks were oven dried at 90°C for 24 hr, placed into a desiccator for one hour, and weighed. Jars were assembled as previously explained, according to

AWPA E1-97 Standard (2005). One hundred worker termites were added to each jar and the jars were stored in a 28°C incubator in a plastic box with damp paper towels lining the bottom of the box.

After five days of exposure to 100 termites, the borate-treated wood from each of the jars was carefully removed and replaced with untreated composite samples, with minimal disturbance of the termites and their galleries. The wood pieces that had been removed were oven dried at 90°C for 24 hours and weighed.

After five days of exposure to the untreated composite boards (day ten of the experiment), the number of live workers in each replicate and the wet weight of ten randomly selected termites recorded. The wood pieces were desiccated at 90°C for 24 hours and weighed. The live termites were placed into an oven at 50°C for three hours to dry. After drying, they were separated into groups of 50, and retained in 1.5 mL polypropylene microcentrifuge tubes. The samples were sent to Rio Tinto Minerals (formerly 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 termite samples were dissolved in 2.5 mL of concentrated nitric acid (HNO₃) in digestion tubes and heated in an autobloc at 98°C for 15 minutes. The tubes were allowed to cool and 7.5 mL of sterile water was added to make a solution of 25% nitric acid; the solution was filtered and 5 mL of sample was injected into the Thermo Fisher IRIS Intrepid II (Thermo Scientific, Waltham, MA)

ICP-AES machine. The machine was calibrated using 0.0, 0.2, 0.5, and 1.0 ppm boron calibration standards in a 25% nitric acid matrix. These analyses were performed at Rio Tinto Minerals (Mark Mankowski, personal communication).

Determination of boron content per termite

The boron content of each termite exposed to borate-treated timber was obtained by a calculation based on the amount of boron assayed by ICP-AES. The results from the ICP-AES assay were given as ppm, converted to $\mu\text{g/g}$ using the average weight of the termites, and divided by the number of termites in the assay (100 per jar) to calculate the amount of boron in each termite.

Statistical analyses

The mean wood consumption per termite, termite weight, 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 2004).

Results

After exposure to any of the three boron compounds tested the termites displayed a decrease in their mean wet mass and survival rate (Table 3.1).

Table 3.1. Effect of boron exposure on mean weight and survival of *C. formosanus* after 5 days of exposure to boron-treated lumber and 5 days subsequent exposure to untreated composite boards. Each treatment represents four independent replicates of 100 workers each. Different letters after the numeric value indicate a significant difference ($P < 0.05$) from the ANOVA test; standard deviations follow in parentheses.

Treatment	Mean individual weight (mg), day 10	Mean 10 day survival (no.) (out of 100)
USB Control	3.55a (0.4)	91a (5)
ZB 0.88%	3.09b (0.1)	89a (3)
ZB/DOT 60/40	3.17b (0.2)	79a,b (11)
ZB/B ₂ O ₃ 60/40	3.01b (0.2)	70b (9)
Untreated	3.40 (0.05)	

There were no significant differences in wood consumption among the borate-treated formulations, although all three were significantly less than the untreated composite control (Table 3.2). During the last five days of the experiment ZB 0.88% and B₂O₃ showed significantly less wood consumption and DOT was intermediate between the composite control and the other two boron treatments.

Table 3.2. Mean wood consumption by *C. formosanus* during the first five days (exposure to borate-treated timber) or the second five days (exposure to untreated composite boards). Different letters after the numeric value indicate a significant difference ($P < 0.05$) from the ANOVA test; standard deviations follow in parentheses.

Treatment	0 to 5 D mean wood consumption (mg) per termite	5 to 10 Day mean wood consumption (mg) per termite
USB Control	0.530a (0.09)	0.808a (0.13)
ZB 0.88%	0.234b (0.05)	0.324b (0.04)
ZB/DOT 60/40	0.195b (0.03)	0.577a,b (0.32)
ZB/B ₂ O ₃ 60/40	0.247b (0.03)	0.305b (0.26)

Exposure to any of the boron-treated samples resulted in an increase in boron content compared to termites exposed to the untreated composite board (Table 3.3). Boric acid (B_2O_3) had the greatest effect on mass and survival, and resulted in higher boron content in comparison to the other treatments. The mean individual mass per termite decreased with all the treatments, but the survival rates indicated that the DOT treatment was intermediate between the control and ZB 0.88% and B_2O_3 . The untreated composite board appeared to increase the boron concentration from the wild-caught concentration (approximately 7 $\mu\text{g/g}$ B per 50 workers (Chapter II)), although the difference was not significant ($P < 0.01$) due to high variance.

Table 3.3. Observed boron content in treated worker termites. The observed boron content was calculated from the mean actual boron content assayed using ICP-AES, an assay that used 50 workers from each independent replicate. See the *Methods* section of this chapter for more details on the calculations.

Treatment	Mean boron content ($\mu\text{g/g}$) on day 10	Mean boron content ($\mu\text{g/termite}$)
Untreated MDI	17.4a (15.4)	0.0348
ZB 0.88%	55.5b (17.1)	0.111
ZB/DOT 60/40	79.0b,c (20.2)	0.158
ZB/ B_2O_3 60/40	97.0c (15.6)	0.194

After a five-day recovery period, at day ten of the experiment, the mean boron content of the termites decreased from those values obtained during a separate five-day trial with the same experimental conditions (Chapter II) across boron formulations (Table 3.4).

Table 3.4. Comparison of boron content after five days of continuous exposure and an additional five days of recovery period on untreated composite board. The observed boron content was calculated from the mean actual boron content assayed using ICP-AES, an assay that used 50 workers from each independent replicate. See the *Methods* section of this chapter for more details on the calculations.

Treatment	Mean boron content ($\mu\text{g/g}$) on day 5	Mean boron content ($\mu\text{g/g}$) on day 10	Mean difference of boron content ($\mu\text{g/g}$) between days 5 and 10
USB Control	30.2 (6.2)	17.4 (15.4)	12.8
ZB 0.88%	170.0 (36.7)	55.5 (17.1)	114.5
ZB/DOT 60/40	306.3 (90.3)	79.0 (20.2)	227.3
ZB/B ₂ O ₃ 60/40	324.2 (12.4)	97.0 (15.6)	227.2

The boron content of the control termites, as well as those exposed to boron-treated timber, decreased from five to ten days. For termites exposed to both DOT and B₂O₃ the mean amount of boron that the termites were able to eliminate between day five and day ten was almost identical, approximately 227 $\mu\text{g/g}$.

Discussion

Borate compounds have been well established as causing mortality in *C. formosanus*, with an estimated lethal dose of 721.29 $\mu\text{g/g}$ (Su *et al.* 1994). This set of experiments demonstrates several effects of borates on termites, including a decrease in mass and an increase in boron content. However, after five days of recovery time the termites showed a decrease in boron content, indicating the possibility of excretion. Since treated termites appeared to eliminate approximately the same amount of boron in five days, with all treatments, it seems the concentration of boron, rather than the associated ion, is responsible for toxicity.

Many organisms have mechanisms for processing excess nutrients in their diets and termites may have a similar excretion mechanism. If the termites are able to eliminate, excrete, or otherwise process excess amounts of boron then the reported time to mortality of around 15 days might suggest that a gradual buildup to a certain threshold, rather than a single acute dose, of a boron compound would prove fatal in termites. Both the DOT and B₂O₃-treated lumber had the same BAE (0.88%), but the B₂O₃ treatment yielded higher mortality, weight loss, and boron content. There was variability in the amount of wood that was consumed by termites exposed to borate-treated wood, and the trend suggested that the boron formulation might affect subsequent feeding and activity levels; Nunes and Dickinson (1995) reported similar results, where certain boron concentrations increased the respiratory quotient.

Previous research (Grace and Campora 2005) has shown that in field tests termites will superficially explore borate-treated boards but will not feed continuously. Although it is well documented in other types of insects that self-selection from available food sources occurs to meet their nutritional needs (Nation 2002), the mechanism for this behavior remains unknown. Future physiological and behavioral studies may be able to determine whether there is a boron content threshold that termites reach before a cessation of feeding. The concentrations of certain essential but trace elements, like boron, could be one of the indicators termites use to judge the quality of feeding sources, and may help future applied technologies become more effective.

Chapter IV. The ability of the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) to engage in horizontal transfer of boron compounds

Abstract

Managing infestations of the Formosan subterranean termite, *Coptotermes formosanus*, is critically important in Hawai'i. Boron-treated timber has become a widely used prevention strategy although the mode of action of boron is not well understood. Borates are not repellent, nor do they cause mortality quickly, making them excellent candidates for horizontal transfer from donor termites that have fed upon the treated wood to other naïve recipient members of the colony. In the present study, termites from field colonies maintained in Honolulu, HI were collected and dyed with Sudan Red 7B over a seven-day period, and then either exposed to a composite board containing anhydrous boric acid (B_2O_3) in a 60/40 ratio with zinc borate or to an untreated control composite board for three days. Termites exposed to each treatment were then placed with an equal number of naïve termites for either five or ten days. Sham-dyed donors and recipients were used to negate any possible impacts of the dye on horizontal transfer. Termite weight, survival rates, and boron content were used to assess the rate of horizontal transmission of boron. After five days, both the dyed donors and recipients (c.a. 92 and 40 μg boron/g dyed termites) and the undyed donors and recipients (c.a. 84 and 30 μg boron/g undyed termites) had higher boron content than untreated, field-caught termites; the same pattern was observed after ten days (c.a. 61 and 46 μg boron/g dyed donors and recipients to 60 and 24 μg boron/g undyed donors and recipients). The data suggest that boron can be horizontally transferred after at least five days.

Introduction

Horizontal transfer refers to the transfer of an ingested (or otherwise contacted) insecticide from the insect originally coming in contact with it to other naïve members of its species, and possibly from those individuals to others, as well. Understanding the horizontal transfer of insecticides is especially important for the management of social insects with non-repellent and slow-acting insecticidal compounds. Boron compounds are known to be nonrepellent (Grace and Campora 2005) and to cause high levels of mortality in termites in approximately two weeks (Kartal and Aryilmis 2005; Ahmed *et al.* 2004). In a laboratory study Su (2005) observed total mortality of the test termite

population with the bait toxicant noviflumuron, but “splitting” of the population into exposed and unexposed groups with concomitant low mortality (35%) following exposure to the liquid soil treatments fipronil or thiamethoxam. He considered this to demonstrate the significance of horizontal transfer in the former case, and its reduction or absence in the latter.

Shelton and Grace (2003) demonstrated at least a small amount of transfer of the non-repellent termiticides fipronil and imidacloprid between donors and recipients in laboratory tests, even at concentrations as low as one part per million (ppm). Hu *et al.* (2005) reported that the concentration of indoxacarb and the period of recipient exposure to donors had a significant impact on mortality in *C. formosanus*. Buczkowski and Schal (2001a) demonstrated emetophagy (the ingestion of insecticide-induced regurgitate) in *Blattella germanica* (L.), the German cockroach, as an important mechanism of horizontal transfer of fipronil between adults and nymphs. Insecticide formulation and the method of delivery has also been shown to impact horizontal transfer; *B. germanica* transfers ingested fipronil more effectively than topically applied material (Buczkowski and Schal 2001b). Synthetic insecticides are not the only compounds of interest with respect to horizontal transfer; Long *et al.* (2000) demonstrated horizontal transmission of the insect pathogen *Beauveria bassiana* between larval cadavers and prepupae in the soil.

Understanding horizontal transfer of insecticides in social insects is particularly important in order to evaluate their field efficacy and potential for integration into management programs. The purpose of this experiment was to determine whether donor *C.*

formosanus workers exposed to boron-treated wood were capable of transferring boron to naïve recipient termites.

Materials and Methods

Termite collection

Coptotermes formosanus 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 field collection, 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 AWWA Standard E1-97 (2005). During the experiment, the jars were placed in a covered plastic box within an unlighted 28°C incubator at 68% relative humidity. The bottom of the box was lined with damp paper towels to maintain humidity.

Dyeing termites with Sudan Red 7B

Using methods adapted from Lai *et al.* (1983), Whatman No. 1 filter paper circles (55 mm diameter, Whatman International Ltd., Maidstone, England) were dyed with Sudan Red 7B (Sigma Chemical Co., St. Louis, MO) dissolved in acetone (HPLC-grade, Fisher Scientific, Fairlawn, NJ) to achieve a concentration of 1% of the filter paper weight as dye. One milliliter of dye solution was applied to each circle of filter paper, and once the acetone had evaporated two circles of filter paper were placed in each Petri dish with an added 1 mL dH₂O per top piece of filter paper and 2 mL dH₂O per bottom piece of filter

paper. Approximately 2 g *C. formosanus*, collected as outlined above, were added to each Petri dish.

Termites were exposed to the dyed filter paper for seven days in an unlighted 28°C incubator, monitored daily, and the dyed filter paper was replaced as needed. After the seven-day exposure period termites were transferred to the boron ingestion phase of the experiment.

Ingestion of Boron

All wood samples were aspen composite particleboard prepared with methylene diphenyl diisocyanate (MDI) and provided by Rio Tinto Minerals (US Borax). Two replicates of boards containing zinc borate / anhydrous boric acid (B_2O_3) (0.88% boric acid equivalent, or BAE) in a 60/40 ratio were prepared with donor termites (one replicate dyed, one undyed), and two replicates of recipient termites (two dyed, two undyed) were exposed to untreated Douglas fir for three days. Each of the replicates consisted of two grams of *C. formosanus* workers, placed in a plastic jar with the wood sample (2.5 x 2.5 x 1 cm), prepared as described above. Before and after the experiment the wood blocks were oven dried at 40°C for 24 hr, placed into a desiccator for 1 hr, and weighed to measure wood consumption.

Test for horizontal transfer

Five replicates of either dyed donors or dyed recipients were used for each of the five- and ten-day transfer experiments. Replicates consisted of 100 donors and 100 recipients, with either the donors or recipients dyed. The 200 *C. formosanus* workers were placed into large 15 cm Petri dishes with Whatman No. 2 (90 mm diameter) filter paper circles

moistened with 1 mL distilled water. Every fourth day, 0.5 mL of distilled water was added to the filter paper in each replicate to prevent desiccation.

Five (half) of the replicates were destructively sampled after five days, and the other five after ten days. The number of live workers in each replicate was recorded, 50 termites from each replicate weighed, and the live termites were placed into an oven at 50°C for three hours to dry. The dried termites were separated into groups of 50 and analyzed for boron content ICP-AES (Inductively Coupled Plasma - Atomic Emission Spectrometry, Rio Tinto Minerals).

Determination of boron content with ICP-AES

In this low-level elemental analysis technique the dried termite samples were dissolved in 2.5 mL of concentrated nitric acid (HNO₃) in digestion tubes and heated in an autobloc at 98°C for 15 minutes. The tubes were allowed to cool and 7.5 mL of sterile water was added to make a solution of 25% nitric acid; the solution was filtered and 5 mL of sample was injected into the Thermo Fisher IRIS Intrepid II (Thermo Scientific, Waltham, MA) ICP-AES machine. The machine was calibrated using 0.0, 0.2, 0.5, and 1.0 ppm boron calibration standards in a 25% nitric acid matrix. These analyses were performed at Rio Tinto Minerals (Mark Mankowski, personal communication).

Statistical analyses

The mean wet weight per termite, survival rate, and boron content were analyzed for significant effects. SAS (v 9.1) was used for the analyses; the Ryan-Einot-Gabriel-Welsch Multiple Q-test, which controls for Type 1 experiment error rate ($\alpha = 0.01$), was used to compare means (SAS Institute 2004).

Also using SAS 9.1, the orthogonal GLM test was used to compare means for the amount of boron in donor and recipient termites, wet weight, and survival rates of both dyed and undyed termites after five and ten days exposure.

Results

Exposure to boron-treated timber for three days significantly decreased individual weight and survival but not as much as exposure to the dye (Table 4.1). Boron content was significantly increased in both donors and recipients over field-caught termites; recipients also showed significant increases in boron content. After ten days of feeding on untreated composite board and exposure to donor termites, the recipient termites had elevated boron levels over field-caught untreated termites, and the boron content of donor termites (dyed and undyed) had decreased.

Table 4.1. Mean boron content, individual weight, and survival rates for the five and ten day exposure periods after termites had been dyed with Sudan Red 7B and exposed to either B₂O₃-treated or control composite boards for three days. After the initial exposure period termites were combined in a 1:1 donor:recipient ratio to a total of 200 termites for each of the five independent replicates; during the horizontal transfer phase of the experiment, termites were fed on untreated composite MDI boards. Data from untreated, field-caught termites is provided as a comparison. Treatments with the same letter after the numeric value did not have significant differences ($P < 0.01$) using the REGW ANOVA; the standard deviation is given in parentheses.

Field-caught

Untreated	Mean boron content ($\mu\text{g/g}$) in 50 W	Mean individual weight (mg)
Workers	7.1a (0.64)	3.40 (0.1)
Soldiers	7.3a (2.65)	3.52 (0.01)

5 Day Exposure

Treatment	Mean boron content ($\mu\text{g/g}$) in 50 W	Mean individual weight (mg)	Mean survival (no.)
Recipient, not dyed	29.5b,c (2.5)	3.42a (0.1)	956a (2)
Recipient, dyed	38.4c,d (6.6)	2.85b,c (0.04)	92a (11)
Donor, not dyed	84.3f (8.1)	3.30a,b (0.1)	91a,b (3)
Donor, dyed	92.1f (4.1)	2.64c (0.01)	74c (4)

10 Day Exposure

Treatment	Mean boron content ($\mu\text{g/g}$) in 50 W	Mean individual weight (mg)	Mean survival (no.)
Recipient, not dyed	24.2b (3.9)	3.30a,b (0.2)	90a,b (1)
Recipient, dyed	45.9d (8.2)	2.58c (0.2)	88a,b (3)
Donor, not dyed	58.9e (8.8)	2.94b,c (0.2)	80b,c (3)
Donor, dyed	60.5e (7.4)	2.55c (0.1)	55d (5)

The boron content of the undyed recipients at both five and ten days was closest to the observed boron content in untreated, field-caught termites (Table 4.2). Dyed recipient termites had individual boron content levels increased by nearly one order of magnitude from the undyed recipients. Dyed recipients at five and ten days had comparable

individual boron content, and similar boron content was also recorded between dyed and undyed donors at five and ten days.

Table 4.2. Observed boron content per individual termite. The observed boron content was calculated from the mean actual boron content assayed using ICP-AES (Table 4.1). See the *Methods* section of this chapter for more details on the calculations.

Treatment	Observed boron content ($\mu\text{g}/\text{termite}$)
Field-caught	
Workers	0.0284
Soldiers	0.0292
5 Day	
Recipient, not dyed	0.0295
Recipient, dyed	0.154
Donor, not dyed	0.338
Donor, dyed	0.368
10 Day	
Recipient, not dyed	0.0968
Recipient, dyed	0.184
Donor, not dyed	0.236
Donor, dyed	0.242

An orthogonal contrast was performed to determine the effect of the boron formulation on termite weight (Table 4.3), survival rates (Table 4.4), and boron content (Table 4.5). Significant decreases in weight were observed between the dyed and undyed donor and recipient termites at five and ten days (Table 4.3). The dyed donor termites at five and ten days also exhibited highly significant decreases in survival rates (Table 4.4). Compared to untreated, field-caught termites, all the experimental termites had significantly increased boron content. Highly significant ($P < 0.0001$) increases in boron content were observed at both five and ten days between the donor and recipient termites (Table 4.5).

Table 4.3. Orthogonal contrast of termite weight after five or ten days of engaging in horizontal transfer of boron after termites had been dyed with Sudan Red 7B and exposed to either B₂O₃-treated or control composite boards for three days. Data from untreated, field-caught termites is provided as a comparison.

NS = no significant difference; * = P < 0.01; ** = P < 0.05 ; *** = P < 0.001 ; † = P < 0.0001.

		5 Day						10 Day			
		Untreated	Recipient		Donor		Recipient		Donor		
			Not Dyed	Dyed	Not Dyed	Dyed	Not Dyed	Dyed	Not Dyed	Dyed	
5 Day	Recipient	Not Dyed	NS								
		Dyed	†	**							
	Donor	Not Dyed	NS	†	†						
		Dyed	†	†	†	†					
10 Day	Recipient	Not Dyed	NS	NS	**	NS	†				
		Dyed	†	**	**	†	NS	†			
	Donor	Not Dyed	***	†	†	*	**	*	**		
		Dyed	†	†	†	†	NS	†	NS	**	

Table 4.4. Orthogonal contrast of survival after five or ten days of engaging in horizontal transfer of boron after termites had been dyed with Sudan Red 7B and exposed to either B₂O₃-treated or control composite boards for three days.

NS = no significant difference; * = P < 0.01; ** = P < 0.05 ; *** = P < 0.001 ; † = P < 0.0001.

			5 Day				10 Day			
			Recipient		Donor		Recipient		Donor	
			Not Dyed	Dyed	Not Dyed	Dyed	Not Dyed	Dyed	Not Dyed	Dyed
5 Day	Recipient	Not Dyed								
		Dyed	NS							
	Donor	Not Dyed	NS	NS						
		Dyed	†	†	†					
10 Day	Recipient	Not Dyed	NS	NS	NS	†				
		Dyed	*	NS	NS	**	NS			
	Donor	Not Dyed	†	**	**	NS	**	*		
		Dyed	†	†	†	†	†	†	†	

Table 4.5. Orthogonal contrast of boron content after five or ten days of engaging in horizontal transfer of boron after termites had been dyed with Sudan Red 7B and exposed to either B₂O₃-treated or control composite boards for three days. Data from untreated, field-caught termites is provided as a comparison.

NS = no significant difference; * = P < 0.01; ** = P < 0.05 ; *** = P < 0.001 ; † = P < 0.0001.

		5 Day						10 Day			
		Untreated	Recipient		Donor		Recipient		Donor		
			Not Dyed	Dyed	Not Dyed	Dyed	Not Dyed	Dyed	Not Dyed	Dyed	
5 Day	Recipient	Not Dyed	**								
		Dyed	†	**							
	Donor	Not Dyed	†	†	†						
		Dyed	**	†	†	**					
10 Day	Recipient	Not Dyed	***	NS	**	†	†				
		Dyed	†	***	NS	†	†	†			
	Donor	Not Dyed	†	†	†	†	†	†	**		
		Dyed	†	†	†	†	†	†	†	NS	

Discussion

Horizontal transfer of insecticides can be especially useful in the control of pestiferous social insects. Nonrepellence and delayed toxicity are among the most important characteristics of such an insecticide, and boron-treated lumber fits that profile. The goal was to determine whether boron can be horizontally transferred between worker *C. formosanus* termites, and the data suggest that transmission is indeed possible, even with limited short-term exposure.

Sudan Red 7B is a dye that is known to affect termite fitness but was believed to be the best candidate for dyeing termites. The biologically active borane can complex with any compound with adjacent hydroxyl groups; Sudan Red 7B, the lipophilic dye used in this experiment, contains such a moiety. Delaplane and La Fage (1989) demonstrated that *C. formosanus* workers dyed with Sudan Red 7B had lower numbers of symbiotic protozoa, feeding rates, and survivorship than non-dyed termites. Raina *et al.* (2004) showed that defaunation resulted in cannibalism by primary reproductives of *C. formosanus*, and it is likely that termites defaunated by other causes would also cannibalize other members of the colony, perhaps increasing the dose of insecticide they ingest. Lai *et al.* (1983) demonstrated that the dye was found in fat bodies, the gut, muscle, brain, and protozoa; boron compounds, because of their wide biological distribution, could easily enter and intercalate themselves with the dye in these locations. A comparison of these data presented here with a similar experiment using another type of dye, like commercial gouache (Brunow *et al.* 2005), could help determine whether Sudan Red dyeing had an impact on boron ingestion and retention.

Boron, although necessary as a micronutrient, causes mortality after exposure even at lower concentrations. A three-day exposure period enabled donor termites to transfer significant or doses of boron to recipient termites; and even ten days post-exposure donor termites were affected, as seen by the reduced weight, reduced survival rates, and elevated boron content. The boron-treated termites also showed a decrease in boron content after the five- and ten-day transfer period, suggesting that the boron was being excreted or otherwise metabolized; this excretion may have affected the horizontal transmission that was observed.

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