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The response and recovery of the Formosan subterranean termite (*Coptotermes formosanus* Shiraki) from sublethal boron exposures

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Controlling the Formosan subterranean termite, a cosmopolitan pest and the most structurally damaging pest in the state of Hawaii, is an important priority for homeowners and commercial builders alike. Boron-treated lumber is often part of an effective integrated pest management (IPM) strategy, owing to its effect of reducing attack by subterranean termites and preventing wood decay due to fungi and bacteria. Termites from field colonies maintained in Honolulu were collected and exposed to one of four composite boards: zinc borate (ZB)/disodium octaborate tetrahydrate (DOT) (boric acid equivalent or BAE 0.75%), anhydrous boric acid (B_2O_3) (BAE 0.75%), or an untreated composite board control; after 5 days the wood pieces were replaced with untreated composite boards. The effect of 5 days of boron exposure was examined by comparing the wet weight of termites, wood consumption, survival, and termite boron content. The analyses at both 5 and 10 days revealed significantly more boron in the termites exposed to treated timber than those not exposed, and decreased weight in those termites exposed to borates. The general trend was for the ZB treatment to have less of an effect on the termites than the DOT treatment, while were both less harmful than boric acid in these experiments. The boron content of the exposed termites declined by 66–74% after 5 days of feeding on untreated wood, indicating that termites can excrete or metabolize ingested boron over time. This ability to recover from sublethal exposure to boron may explain the gradual avoidance of borate-treated wood noted by other authors, and has implications for the inclusion of borate-treated timber in an IPM programs for preventing infestation by subterranean termites.

Keywords: Isoptera; Rhinotermitidae; boric acid; disodium octaborate tetrahydrate (DOT); zinc borate; termite behavior

1. Introduction

Boron, a ubiquitous element present as a trace element in many plants, constitutes a critical component of biological function and has been shown to be necessary for metabolism (Lloyd et al. 1990; Rainey et al. 1999). However, at elevated 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' (Lloyd et al. 1990; Woods 1994; Rainey et al. 1999). Examining whether termites exposed to sublethal amounts of boron compounds for short periods can recover may shed light on whether either excretion or detoxification occurs, and may help to guide future research on physiological responses of insects to boron, and so improve management techniques.

In 1997 the costs of subterranean termite damage and control were estimated at over one billion dollars annually in the United States alone (Osbrink et al. 2001), and at least \$100 million in the state of Hawaii (Tamashiro et al. 1996) where all termites are invasive (HTAC c1996–2001). In Hawaii, termites in the

families Rhinotermitidae and Kalotermitidae families cause significant structural harm, and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is the most economically important insect pest in the state. Along with the use of soil insecticides, physical barriers to subterranean termites, and termite baits, structural lumber pressure-treated with disodium octaborate tetrahydrate is used, statewide, to minimize termite damage (Grace 2002). Although options compatible with integrated pest management (IPM) for termites have been proposed for the prevention and treatment of termite infestation (Su and Scheffrahn 1998; Culliney and Grace 2000), these programs have not been widely implemented. Here we define termite IPM as a strategy to minimize environmental impact of treatments while supplementing traditional chemical control measures with additional measures (e.g. cultural control, biological control) to increase a management programmes chance of reducing pest numbers below an 'action threshold' and also to reduce the possibility of inadvertently selecting for resistance to an insecticide (Su and Scheffrahn 1998).

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Even though the mode of action of boron has not been fully elucidated, termite mortality after exposure to boron occurs more rapidly than would occur with gut microflora defaunation and subsequent starvation alone (as established by Khoo and Sherman 1979) (Ahmed et al. 2004; Kartal and Ayrilmis 2005). The goal of the present study was to examine the ability of termites to recover from sublethal doses of three borate formulations by comparing the weight, survival, and boron content of treated termites in comparison to those of termites fed upon untreated composite boards.

2. Materials and methods

2.1. Termite collection

Coptotermes formosanus workers were collected from a field colony in Honolulu, Hawaii, USA (Husseneder and Grace 2001a, 2001b) in Douglas fir (*Pseudotsuga menziesii* Mirb. [Franco]) traps, using a technique described by Tamashiro et al. (1973). Within 24 h of collection from the field, worker termites were aspirated in groups of 100 and used in the manner described in the AWP A E1-97 (2005) standard method (see below).

2.2. Ingestion of boron

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

2.3. Test for recovery

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 h, placed into a desiccator for 1 h, and weighed. One hundred worker termites were placed into plastic screw-top jars (8.5 × 10 cm, wide × 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 × 2.5 × 1 cm placed on an aluminium foil square centred on the sand. The jars were placed in an unlighted 28°C incubator at 68% R.H. in a covered plastic box. The bottom of the box was lined with damp paper towels to maintain humidity.

The borate-treated wood from each of the jars was carefully removed and replaced with untreated composite samples after 5 days, with minimal disturbance of the termites and their galleries. The

wood pieces that had been removed were again oven-dried at 90°C for 24 h and weighed.

After 5 days of exposure to the untreated composite boards (day 10 of the experiment), the number of live workers in each replicate and the wet weight of 10 randomly selected termites were recorded. The wood pieces were desiccated at 90°C for 24 h and weighed. The live termites were placed into an oven at 50°C for 3 h to dry. After drying they were separated into groups of 50 individuals and retained in 1.5-ml polypropylene microcentrifuge tubes. The samples were sent to Rio Tinto Minerals (formerly US Borax) for boron content assaying using inductively coupled plasma-atomic emission spectrometry (ICP-AES).

2.4. Determination of boron content using ICP-AES

The dried termite samples were dissolved in 2.5 ml of concentrated nitric acid (HNO_3) in digestion tubes and heated in an autobloc at 98°C for 15 min. 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 5.0 ml of sample was injected into a 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 (M. Mankowski, personal communication).

2.5. 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 $\mu g/g$ using the average weight of the termites, and divided by the total number of termites in the assay (100 per jar) to calculate the amount of boron in each termite.

2.6. Statistical analyses

The mean wood consumption per termite, termite weight, survival (number), and boron content (mg/termite) were analysed for significant effects. ANOVA with the Ryan-Einot-Gabriel-Welsch Multiple Q-test (REGWQ) ($P < 0.05$), which controls for Type I experiment error rate, was used to compare means (SAS Institute 2004).

3. Results

The data show that exposure to any boron-treated timber for 5 days had a significant effect on the variables assessed in this experiment, including weight,

survival, wood consumption, and boron content. Even after a 5-day recovery period, where the termites were exposed to untreated wood, only those termites fed ZB/DOT showed boron content, survival (at 10 days), and wood consumption (5–10 days) intermediate between the untreated controls and the other two experimental treatments. Exposure to any of the three boron compounds resulted in a decrease in termite mean wet weight and survival (Table 1).

There were no significant differences in wood consumption among the borate-treated formulations, although all three treatments were fed upon significantly less than the untreated composite board (Table 1). During the last 5 days of the experiment ZB and ZB/B₂O₃ were consumed least, and ZB/DOT was intermediate between the composite control and the other two boron treatments.

Exposure to any of the boron-treated samples resulted in a significant increase in boron content in comparison to termites maintained on untreated composite board (Table 2). Boric acid had a slightly greater impact on survival and, along with ZB/DOT, resulted in the highest boron content when compared across treatments. The mean individual weight per termite decreased with all the treatments but the number surviving indicated again that the ZB/DOT treatment was intermediate between the control and ZB and ZB/B₂O₃. Feeding on the untreated composite board appeared to increase termite boron content over that of the field-caught termites

(approximately 7 µg/g B per 50 workers, Gentz and Grace 2007) although the difference was not significant ($P > 0.01$), possibly due to high variance.

After a 5-day recovery period, at day 10 of the experiment, the mean boron content of the termites decreased across boron formulations from those values obtained during a separate 5-day trial under the same experimental conditions (Gentz and Grace 2007) (Table 2). The boron content of the untreated termites, as well as those exposed to boron-treated timber, decreased between days 5 and 10. For termites exposed to either ZB/DOT or ZB/B₂O₃ the mean amount of boron that the termites were able to eliminate between days 5 and 10 was almost identical, approximately 227 µg/g.

4. Discussion

Current remedial management strategies for subterranean termites focus primarily on bait systems employing chitin synthesis inhibitors or slow-acting insecticides. With the deregistration of organochlorine soil treatments, e.g. chlordane and heptachlor, soil treatments in the USA are limited to synthetic pyrethroids, pyrroles (e.g. fipronil), and chloronicotynyls (e.g. imidacloprid). Topical liquids, dusts, foams, gasses, and extreme temperatures have also been explored as control options, but field results have not yet proved these methods to be reliable for treatment or termite eradication (Lewis 1997).

Table 1. Effect of boron exposure on mean weight, survival, and wood consumption of 100 *C. formosanus* after 5 days of exposure to boron-treated lumber and 5 days subsequent exposure to untreated composite boards.

Treatment	Mean individual weight at 10 days (mg)	Mean 10 days survival (no.)	0–5 days mean wood consumption (mg/termite)	5–10 days mean wood consumption (mg/termite)
Untreated MDI	3.55a (0.4)	91a [5]	0.530a (0.09)	0.808a (0.13)
ZB	3.09b (0.1)	89a [3]	0.234b (0.05)	0.324b (0.04)
ZB/DOT 60/40	3.17b (0.2)	79a,b [11]	0.195b (0.03)	0.577a,b (0.32)
ZB/B ₂ O ₃ 60/40	3.01b (0.2)	70b [9]	0.247b (0.03)	0.305b (0.26)
Field collected, unexposed	3.40 (0.05)	–	–	–

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 REGWQ ANOVA within a column; standard deviations follow in brackets.

Table 2. Observed boron content in treated worker termites and comparison of boron content after 5 days of continuous exposure and an additional 5-day recovery period on untreated composite board.

Treatment	Mean boron content at 10 days (µg/g)	Mean individual boron content at 10 days (µg/termite)	Mean boron content at 5 days (µg/g)*	Difference of mean boron content (5–10 days) (µg/g)
Untreated MDI	17.4a (15.4)	0.0618	30.2a (6.2)	12.8
ZB	55.5b (17.1)	0.172	170.0b (36.7)	114.5
ZB/DOT 60/40	79.0b,c (20.2)	0.250	306.3c (90.3)	227.3
ZB/B ₂ O ₃ 60/40	97.0c (15.6)	0.292	324.2c (12.4)	227.2

The observed boron content per termite was calculated from the mean actual boron content/g termites assayed using ICP–AES, an assay that used 50 workers from each independent replicate. Different letters after the numeric value indicate a significant difference ($P < 0.05$) from the REGWQ ANOVA test within a column; standard deviations follow in parentheses. See Section 2 for more details on the calculations.

*Indicates data in that column from Gentz and Grace (2007).

Integrated pest management (Su and Scheffrahn 1998) and biocontrol options (Culliney and Grace 2000; Lax and Osbrink 2003) for the management of subterranean termites have been explored, but have yet to become widely commercially available. Non-chemical preventive barriers, e.g. particle or metal barriers, and wood treatments, like boron-treated timber, provide a means of deterring termites from feeding on a protected structure, but are unlikely to be effective for colony elimination once an infestation has occurred.

Boron compounds have been well established as causing significant mortality at high concentrations in *C. formosanus*, with an estimated lethal dose of 721.29 $\mu\text{g/g}$ (Su et al. 1994). Our results demonstrate several effects of borates on termites, including a decrease in individual weight and an increase in boron content. Of particular interest is the finding that after 5 days of recovery time, the termites showed a decrease in boron content, due either to excretion or metabolism. Since termites appeared to eliminate high levels of boron in the ZB/DOT and ZB/B₂O₃ treatments at approximately the same rate, which was significantly higher than the rate of elimination in the ZB treatment alone, it is unclear whether the concentration of boron in a given treatment (which was the same for all three treatments), rather than the associated ion in the formulation, is likely to be responsible for toxic effects. It is particularly interesting that the difference in boron content (days 5–10) for ZB/DOT and ZB/B₂O₃ were the same, while the difference was significantly lower for ZB alone and again for the untreated control, suggesting the possibility of a threshold level that must be reached in order to maximize boron excretion.

Many organisms have mechanisms for processing excess nutrients in their diets and termites may have a similar excretory ability. If the termites are able to process excess amounts of boron then the reported time to mortality of around 15 days (Grace 1990; Grace et al. 1992; Toyoshima et al. 1997) might suggest that a gradual build-up to a certain threshold, rather than a single acute dose, of a boron compound would prove fatal in termites. All three samples of treated lumber had the same BAE (0.75%), but both the ZB/B₂O₃ and ZB treatments yielded significantly different mortality, weight loss, and boron content from the untreated control. There was variability in the amount of boron-treated wood that was consumed by termites exposed to borate-treated wood, and the trend suggested that the particular boron formulation might affect post-exposure 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; Campora and Grace 2007) 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. Our documentation of termite recovery from short-term feeding on a low dose of boron suggests that the delayed avoidance noted by Campora and Grace (2007) may result in recovery of at least some of the exposed termites, and limit any redistribution of boron through the colony due to shared feeding behaviors. Thus, boron treatments protect wood from sustained attack, but are very likely poor candidates as bait toxicants for subterranean termite control.

Future physiological and behavioral studies may be able to determine whether there is an internal boron content threshold that termites reach before 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 greater knowledge in this area may help to improve future management technologies. As control measures incorporating IPM become more popular with the general public, increased emphasis on preventing infestations may become more widespread. Boron-treated timber is a strong candidate for inclusion in termite IPM programs because of its low degree of interference with other management strategies, its low mammalian and vertebrate toxicity, and its high specificity to wood-eating organisms.

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