Native Boron Levels and the Effect of Boron Treatment on *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), *Coptotermes acinaciformis* (Froggatt) (Isoptera: Rhinotermitidae), and *Mastotermes darwiniensis* Froggatt (Isoptera: Mastotermitidae)[1]

Margaret C. Gentz[2] and J. Kenneth Grace

University of Hawaii at Manoa, Department of Plant and Environmental Protection Sciences, 3050 Maile Way, Gilmore Hall 310, Honolulu, Hawaii 96822 USA

J. Entomol. Sci. 43(2): 217-224 (April 2008)

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 3 species yields a broader view of the toxicity of boron compounds in termites. *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae) were collected from colonies maintained in at the University of Hawaii at Manoa campus in Honolulu, HI (United States); *C. acinaciformis* (Froggart) from Darwin, North Territory (Australia); and *Mastotermes darwiniensis* Froggart (Isoptera: Mastotermitidae) from Darwin, North Territory (Australia). Termites were exposed to untreated composite board or board containing zinc borate and anhydrous boric acid (ZB/B_2O_3 in a 60/40 ratio, 0.75% BAE) in a no-choice test for 5 d, either in Honolulu (*C. formosanus*) or Australia (*C. acinaciformis* and *M. darwiniensis*); survival rates, wet weight, and boron content of the termites were determined. Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) was used to determine boron content in field-caught and experimental termites. There was a significant ($P < 0.01$) decrease in survival of the boron-treated *Coptotermes* in comparison with the untreated termites, although no mortality was observed in *M. darwiniensis*. All 3 species showed a significant ($P < 0.01$) increase in boron content in boron-treated individuals, and there were no significant differences observed between the field-caught and untreated termites.

Key Words Isoptera, Rhinotermitidae, Mastotermitidae, boron content, boron toxicity

In Hawaii, termites in the Rhinotermitidae and Kalotermitidae families cause significant structural damage, and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Rhinotermitidae), is the most important urban pest in the state. In addition to causing approx. US$100 million annually in structural damage (Osbrink et al. 2001), termites also can damage crops and trees (Tamashiro et al. 1996). All the termites found in Hawaii are invasive, and *C. formosanus* can be found on the 6 major islands of Hawaii (HTAC 1999). Presently there are 8 species of termites representing 3 families statewide (Grace et al. 2002). Australia, by comparison, has several native

[1]Received 24 September 2007; accepted for publication 4 December 2007.
[2]Address inquiries (email: m.gentz@imb.uq.edu.au; Current address: University of Queensland, Institute for Molecular Bioscience, Queensland Bioscience Precinct, Bldg. #80, 306 Carmody Road, St. Lucia, QLD 4072, Australia).

families of termites. *Mastotermes darwiniensis* Froggatt (Mastotermitidae) and *Coptotermes acinaciformis* (Froggatt) (Rhinotermitidae) are widely distributed, where they are recognized as both urban pests and important members of natural ecosystems (Gay and Calaby 1970).

The objectives of this project were to determine the native boron levels in these 3 termite species, and to determine whether they respond similarly to short-term exposure to boron compounds. To quantify their responses to boron ingestion, termites were exposed to wood composite boards containing anhydrous boric acid for a 5-d period. Termite weight change, survival rates, wood consumption, and boron content (assayed using Inductively Coupled Plasma-Atomic Emission Spectrometry, or ICP-AES) were measured and are reported.

Materials and Methods

Termites. 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 Manoa campus of the University of Hawaii in Honolulu (Husseneder and Grace 2001a, b). Within 24 h of collection, workers were aspirated from the collection container in groups of 200 and placed into plastic jars (8.5 cm wide × 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 approx. 2.5 × 2.5 × 1 cm on an aluminum 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 incubator maintained at 28°C and 68% RH in a covered plastic box. The bottom of the box was lined with damp paper towels to maintain humidity.

Coptotermes acinaciformis and *M. darwiniensis* workers were collected from field colonies identified by the Australian Commonwealth Scientific and Research Organization (CSIRO). The workers were used within 2 wks of collection under experimental conditions recommended by L. Cookson and J. Creffield (CSIRO/ensis, Clayton, Victoria, Australia). *Coptotermes acinaciformis* was collected from a mound on a stringy bark tree, halfway between the Elizabeth River and Channel Island (approx. 30 km from Darwin, Northern Territory, Australia). These termites were maintained in jars approx. 9 cm wide × 10 cm deep on moist carton material and kept in a dark incubator maintained at 28°C and 72% RH. *Mastotermes darwiniensis* was collected from a dead mango tree in Howard-Springs, approx. 30 km from Darwin (Northern Territory, Australia). These termites were maintained in plastic trays approx. 20 × 20 × 6 cm, filled with grade 4 vermiculite, *Eucalyptus raglans* (Victorian ash) sawdust, and 138 mL distilled water in an unlighted incubator at 32°C and 76% RH.

Boron ingestion. All wood samples were aspen composite particleboard prepared with methylene diphenyl diisocyanate (MDI) resin and were provided by Rio Tinto Minerals (US Borax). Zinc borate (ZB), zinc borate plus disodium octaborate tetrahydrate (ZB/DOT), or anhydrous boric acid (ZB/B_2O_3) was incorporated into the boards during manufacture at various ratios to equal a concentration of 0.75% BAE (boric acid equivalents). This is equivalent to a ZB concentration of 0.88%.

In Hawaii, the results presented here were part of a larger test (Gentz and Grace 2007) in which 3 replicates of each of the 4 wood samples were prepared in a 5-day no-choice test with 200 *C. formosanus* termites (90% workers, 10% soldiers) per replicate. The boron-treated samples were ZB (0.88%), ZB/DOT (60/40 ratio), and ZB/B_2O_3 (60/40 ratio), plus an untreated MDI control. Before and after exposure to termites, the wood blocks were oven-dried at 90°C for 24 h, placed into a desiccator for 1 h, and weighed to determine wood consumption. At the completion of both experiments, experimental (untreated or boron-treated) termite samples were dried at 50°C for 3 h 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 manner.

In Australia, 5 replicates of each treatment (zinc borate/anhydrous boric acid [ZB/B_2O_3] in a 60/40 ratio or a control composite board) were assembled for *C. acinaciformis* and *M. darwiniensis*. Only B_2O_3 was used because Gentz and Grace (2007) determined that this formulation yielded the highest boron content within the termite at the end of 5 d using *C. formosanus*. The 9 × 10 cm glass jars used in the experiments were filled with 150 g washed river sand, 30 mL deionized water, and 1 wood block (either untreated or boron-treated composite board) placed on 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 used in each replicate. Before and after the experiment, the wood blocks were desiccated at 40°C and weighed. After the experiment, survival and termite wet weight were recorded; both experimental (untreated or boron-treated) and unexposed (wild-caught) termite samples were dried at 50°C for 3 h, separated into groups of 200 for *Coptotermes* and 20 for *Mastotermes*, and retained in 1.5 mL polypropylene microcentrifuge tubes. The samples were sent to Rio Tinto Minerals (US Borax) for boron content analysis using ICP-AES.

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 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 standards in a 25% nitric acid matrix. These analyses were performed at Rio Tinto Minerals (Mark Mankowski, pers. comm.).

Boron content per termite. The boron content of each termite exposed to 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 average amount of boron in individual termites.

Statistical analyses. The mean wood consumption per termite, survival rate, and boron content were analyzed for significant effects. ANOVA with the Ryan-Einot-Gabriel-Welsch Multiple Q-test (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 was observed for the boron-treated *Coptotermes* species (no mortality in *M. darwiniensis* after 5 d), as well as a significant decrease in wood consumption and individual weight for treated *M. darwiniensis* (Table 1).

Table 1. Mean (± SD) weight, wood consumption, and survival rate of worker termites after 5 days of continuous exposure to treated and untreated wood*

Species	Treatment**	Replicate/ Number of individuals	Mean weight [mg/termite]	Mean wood consumption [mg/termite]	Mean Survival [%]
*C. formosanus****	Untreated	3/200	0.379a (0.02)	0.311a (0.48)	99a (3)
*C. formosanus****	ZB/B_2O_3	2/200	0.167a (0.03)	0.134a (0.06)	92b (9)
C. acinaciformis	Untreated	5/200	0.216a (0.26)	0.201a (0.03)	99a (1)
C. acinaciformis	ZB/B_2O_3	5/200	0.368a (0.11)	0.124a (0.02)	89b (7)
M. darwiniensis	Untreated	5/20	3.2a,b (4.2)	3.94c (0.65)	100a (0)
M. darwiniensis	ZB/B_2O_3	5/20	6.4b (3.3)	1.59b (0.337)	100a (0)

* Treatment means followed by the same lower-case letter within each column were not significantly different ($P < 0.01$).

** Composite wood samples were prepared with MDI resin, and either no preservative or ZB/B_2O_3 (60/40 ratio, 0.75% BAE).

*** Data from Gentz and Grace (2007).

Species-level comparison of boron exposure. In *C. formosanus* workers exposed to boron-treated boards, the boron concentration was an order of magnitude greater than in untreated worker termites, and the general toxicity trend based on weight loss and survival placed ZB/DOT approx. intermediate to ZB alone and ZB/B_2O_3 (Table 1). ZB and ZB/DOT had the lowest variability in mean weight loss per termite of the treated wood blocks, but the variability in termite boron content was lowest with ZB/DOT exposure. Although the variability of the boron content, weight, and survival within species and caste was small, the weight difference between *Coptotermes* and *Mastotermes* was over an order of magnitude (Table 2).

Table 2. Mean (± SD) weight of field-collected termites*

Species, caste	Number of replicates/ Number of individuals	Mean weight [mg/termite]
C. formosanus, worker	5/50	3.90 (0.13)
C. formosanus, soldier	3/50	3.97 (0.12)
C. acinaciformis, worker	5/50	3.61 (0.098)
C. acinaciformis, soldier	1/50	3.73
M. darwiniensis, worker	5/10	46.7 (2.0)
M. darwiniensis, soldier	1/10	56.7

* *Coptotermes formosanus* was collected in Honolulu, HI, and both *C. acinaciformis* and *M. darwiniensis* were collected near Darwin, Northern Territory, Australia. For *C. acinaciformis* and *M. darwiniensis*, $n = 1$ due to low soldier ratios in collected termites. Standard deviations, where available, are in parentheses.

There was a significant increase ($P < 0.01$) in boron content of about an order of magnitude in termites exposed to borate-treated wood (Table 3). Additionally, treated workers of each species displayed significant differences ($P < 0.01$) in boron content, increasing in this order: *M. darwiniensis* < *C. acinaciformis* < *C. formosanus*. Although all 3 species showed a significant increase in boron content after exposure to treated wood, there were no significant differences observed between the unexposed field-caught termites and termites exposed to untreated boards within each species. With each termite species, the boron-treated lumber was fed on less than the untreated composite board, but the only significant mass decrease was seen in *M. darwiniensis* exposed to boron-treated rather than untreated composite boards.

Discussion

We identified several effects of short-term boron exposure on termites that appear to remain constant across species, including a decrease in weight and an increase in boron content immediately after feeding, amounting to approx. an order of magnitude in comparison with those termites exposed to untreated boards. *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. darwiniensis* raises ques-

Table 3. Mean (± SD) boron content of unexposed termites and of termites exposed for five days to either untreated or boron-treated MDI composite boards*

Exposure	Species, caste	Replicate/ Number of individuals	Mean boron content [µg/g]
None	*C. formosanus,* worker	5/50	7.1a (0.6)
None	*C. formosanus,* soldier	5/50	7.3a (2.65)
None	*C. acinaciformis,* worker	5/50	2.6a (1.1)
None	*C. acinaciformis,* soldier	5/50	4.0a (1.1)
None	*M. darwiniensis,* worker	5/10	0.6a (0.1)
None	*M. darwiniensis,* soldier	1/10	1.1a (0.3)
Untreated MDI	*C. formosanus,* worker**	5/50	30.2a (6.2)
ZB/B_2O_3	*C. formosanus,* worker**	5/50	306.3d (90.3)
Untreated MDI	*C. acinaciformis,* worker	5/50	20.5a (4.4)
ZB/B_2O_3	*C. acinaciformis,* worker	5/50	122.1b (17.8)
Untreated MDI	*M. darwiniensis,* worker	5/10	32.7a (14.2)
ZB/B_2O_3	*M. darwiniensis,* worker	5/10	181.1c (43.6)

* Treated boards contained ZB/B_2O_3 in a 60/40 ratio (0.75% BAE). Means with the same letter after the numeric value are not significantly different within columns ($P < 0.01$); standard deviations of mean boron content are in parentheses.

** Data from Gentz and Grace (2007).

tions about the toxicity of boron among different termite species. This difference might be attributable to the larger size of *M. darwiniensis.*

The termites used in ICP-AES analysis were not washed or degutted. However, the boron was incorporated into the composite boards, so external contamination of the termites was unlikely. Although boron compounds are not repellent to termites, it has been shown that they will cease feeding on borate-treated woods after several days (Grace and Campora 2005), minimizing the amount of residual wood that may have remained in the gut.

Along with morphological and behavioral differences among termite species, there are relevant physiological distinctions as well. Termites have a range of reported gut pH values (Brune et al. 1995, Brune and Kuhl 1996), which may complicate our understanding of the mechanism of an oral toxicant. This is further complicated by the presence of different symbiotic protist and bacterial communities, although these populations are often highly reproducible within field and laboratory colonies (Yoshimura et al. 1993). It has been established that death of the gut protists assisting in cellulose digestion is not the primary mode of action for boron compounds in that mortality occurs more rapidly than can be accounted for by starvation alone (Grace et al. 1992, Yoshimura et al. 1994a). Seasonal fluctuations in protist populations 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 defining how boron compounds are metabolized by termites.

The much greater mass of *M. darwiniensis* suggests that this species may require larger boron concentrations and/or longer feeding periods for mortality than *Coptotermes* species. *Coptotermes formosanus* workers and soldiers had the highest native boron levels of the 3 species, which could be due in part to boron content of the volcanic soil in which they reside (Hue et al. 1988). In a no-choice laboratory test with boron-treated wood, Ahmed (2000) found that Australian termites from areas with soils with high boron content evidenced a slightly longer time to mortality than those from soil with low boron content. This dose-dependent response may be further complicated by species differences and gross differences in mass or physiology.

Nation (2002) reviewed the existing literature on the ability of insects to self-select nutritional elements from both natural foods and prepared diets. The possibility that termites may selectively feed on sections of the composite board, thereby modulating boron intake, cannot be overlooked. *Mastotermes darwiniensis* is known to have an undiscriminating appetite, able to feed on live plants including eucalyptus, sugarcane, and mango—not to mention leather, ivory, lead-sheathed cables—and even able to corrode glass and some metal surfaces (Gay and Calaby 1970). The 2 *Coptotermes* species may also attack live wood, although not with the same voracity as *M. darwiniensis.*

Differences in gut pH, as well as in bacterial symbionts, could mediate the effect of boron on these 3 species. More derived termites in the Family Termitidae have gut pH's that are 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 that the borate-NAD^+ complex will be the most biologically relevant. Further work with a variety of termite species, including those in the Family Termitidae, could help to determine the relevant parameters for understanding boron toxicity in insects.

Acknowledgments

The authors are grateful to Laurie Cookson and Jim Creffield of CSIRO/ensis in Clayton, Victoria, Australia, for assistance in collecting Australian termites and for access to the CSIRO/ensis facilities. We also thank Mark Mankowski and Mark Manning (Rio Tinto Minerals) for wood samples and chemical analyses, and Josef Seifert and Julian Yates (University of Hawaii) for assistance in interpreting the results and helpful reviews of the manuscript. This research was supported by USDA-ARS Specific Cooperative Agreement 58-6615-4-237, and McIntire-Stennis funds for forestry research administered by the College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

References Cited

Ahmed, B. M. 2000. The effects of boron-treated timbers against *Coptotermes* species in Australia. Ph.D. Diss., Univ. Melbourne, Australia. 335 pp.

[AWPA] American Wood-Preservers' Association Standard. 2005. E1-97: Standard method for laboratory evaluation to determine resistance to subterranean termites. Am. Wood-Preservers' Assoc. 3 pp.

Brune, A., D. Emerson and J. A. Breznak. 1995. The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. Appl. Environ. Microbiol. 61: 2681-2687.

Brune, A. and M. Kuhl. 1996. pH profiles of the extremely alkaline hindguts of soil-feeding termites (Isoptera: Termitdiae) determined with microelectrodes. J. Ins. Physiol. 42(11-12): 1121-1127.

Gay, F. J. and J. H. Calaby. 1970. Termites of the Australian Region, Pp. 393-448. *In* K. Krishna and F.M. Weesner (eds.). Biology of Termites. Academic Press, Inc., NY.

Gentz, M. C. and J. K. Grace. 2007. Different boron compounds elicit similar responses in *Coptotermes formosanus* (Isoptera: Rhinotermitidae). Sociobiol. 50(2): 633-641.

Grace, J. K., R. T. Yamamoto and M. Tamashiro. 1992. Resistance of borate-treated Douglas-fir to the Formosan subterranean termite. Forest Prod. J. 42(2): 61-65.

Grace, J. K., R. J. Woodrow and J. R. Yates. 2002. Distribution and management of termites in Hawaii. Sociobiol. 40: 87-93.

Grace, J. K. and C. E. Campora. 2005. Food location and discrimination by subterranean termites (Isoptera: Rhinotermitidae), Pp. 437-441. *In* C.-Y. Lee and W.H. Robinson (eds.). Proc. Fifth Intern. Conf. on Urban Pests.

Hall, D. G. (ed.). 2005. Boronic acids: preparation and applications in organic synthesis and medicine. Wiley-VCH: Weinheim, Germany. 549 pp.

[HTAC] Hawaii Terrestrial Arthropod Checklist. 1999. Bishop Museum: The State Museum of Cultural and Natural History, Honolulu, HI. Available online at: <http://hbs.bishopmuseum.org/arthrosearch.html>.

Hue, N. V., N. Hirunburana and R. L. Fox. 1988. Boron status of Hawaiian soils as measured by B sorption and plant uptake. Commun. Soil Sci. Plant Anal. 19: 517-528.

Husseneder, C. and J. K. Grace. 2001a. Evaluation of DNA fingerprinting, aggression tests, and morphometry as tools for colony delineation of the Formosan subterranean termite. J. Ins. Behav. 14(2): 173-186.

2001b. Similarity is relative: hierarchy of genetic similarities in the Formosan subterranean termite (Isoptera: Rhinotermitidae) in Hawaii. Environ. Ecol. 30(2): 262-266.

Kim, D. H., K. F. Faull, A. J. Norris and C. D. Eckhert. 2004. Borate-nucleotide complex formation depends on charge and phosphorylation state. J. Mass Spectro. 39: 743-751.

Nation, J. L. 2002. Nutrition, Pp. 65-87. *In* Insect Physiology and Biochemistry. CRC Press, NY.

Osbrink, W. L., A. R. Lax and R. J. Brenner. 2001. Insecticide susceptibility in *Coptotermes formosanus* and *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). J. Econ. Entomol. 94(5): 1217-1228.

SAS Institute. 2004. SAS user's guide: statistics. SAS Institute, Cary, NC.

Tamashiro, M., J. K. Grace and R. T. Yamamoto. 1996. Insecticidal and behavioral activity of etofenprox against the Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiol. 28(1): 53-66.

Tamashiro, M., J. K. Fujii and P.-Y. Lai. 1973. A simple method to observe, trap, and prepare large numbers of subterranean termites for laboratory and field experiments. Environ. Entomol. 2: 721-722.

Yoshimura, T., K. Tsunoda and M. Takahashi. 1993. Distribution of the three symbiotic protozoa in Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). International Research Group on Wood Protection. IRG/WP Document 93 – 10010. 5 pp.

1994a. Effects of starvation on the protozoan fauna in the hindgut of *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Japanese J. Environ. Entomol. Zool. 6(1): 31-35.

1994b. Cellulose metabolism of the symbiotic protozoa in termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Mokuzai Gakkaishi 40(8): 853-859.