Detrimental Effects of Boric-Acid-Treated Soil Against Foraging Subterranean Termites (Isoptera: Rhinotermitidae)

by

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ABSTRACT

In laboratory bioassays, boric acid (BA) mixed with soil caused significant subterranean termite mortality. In choice tests, eastern subterranean and Formosan subterranean termites were exposed to boric acid mixed with soil at concentrations of 0.05, 0.25, 0.50, 1.00, 2.00, and 4.00 percent AI (wt:wt). Termites could choose to remain in their main nests with non-treated substrate and adequate food, or to tunnel through BA-treated soil in an attached foraging tube to reach a satellite nest containing non-treated substrate and additional food. Termite survival, feeding, and tunneling, and gut protozoa populations were determined after 12 weeks. Exposure to BA-treated soil caused significant, steadily increasing mortality in both species, concomitant with a decrease in feeding as BA concentration increased. Boric acid was non-repellent, and termites removed BA-treated soil from foraging tubes and deposited it in main and satellite nests. Generally, at BA concentrations of 1.00-2.00% or less in soil, termite gut protozoa populations were not dose dependent and did not significantly decrease for either termite species as BA concentrations increased, except at the 4.00% concentration where termites appeared weakest. Overall, BA mixed in soil caused significant detrimental effects to both termite species.

Key words: Boric acid, *Coptotermes formosanus*, *Reticulitermes flavipes*, protozoa, termites.

INTRODUCTION

Boric acid (BA) is widely used for controlling household pests, and compounds containing boron and oxygen (borates) are gaining prominence as wood preservative treatments to inhibit termites, beetles, and some decay fungi. Termite attack and activity in wood has been reduced or eliminated using borate treatments. Once inside an insect, borates can cause death in a few hours or days (Grace 1990, 1991a; Rust &

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Knight 1990; Jones 1991; Klotz 1996; Mauldin & Kard 1996). Borate compounds vary widely in their water solubility depending on their chemical structure, and low–solubility borates may become candidates for use as treatments to soil for control of subterranean termites.

Termiticide treatment to soil is the most commonly used method for controlling subterranean termites in the United States, and several termiticides are currently registered by the Environmental Protection Agency (EPA) for use under and around buildings (Johnston *et al.* 1971; Smith *et al.* 1972; Mauldin *et al.* 1987; Kard 1998a,b, 1999). Chlorinated hydrocarbon, organophosphate (OP), and pyrethroid (PR) termiticides have been employed as treatments to soil, often providing 10 to 20 or more years of termite control (Beal 1986; Kard *et al.* 1989; Kard 1999). Currently, OPs and PRs dominate the United States termiticide market. However, the dynamic nature of the pest control industry in the United States necessitates evaluation of potentially successful new insecticides and control methods to identify the most effective new compounds for control of subterranean termites.

Bioassays with treated soil have demonstrated that borates mixed with soil are toxic to termites (Kard 1990). Borates are also used to treat wood to protect it from wood–destroying beetles and termites as well as to provide resistance to some decay fungi (Williams & Amburgey 1987). The insecticidal activity of boron compounds and the availability of low water solubility borates indicates potential for use as treatments to soil to create barriers against subterranean termites.

Borate treatments are used to protect lumber from wood–destroying beetles and termites (Thornton 1964; McQuire 1974; Clamp 1983; Williams 1985a,b; Miles 1994). Ortiz and Manuel (1965) and Gay *et al.* (1958) noted that boron–treated wood was moderately protected from termite attack. Lumber sprayed with water and then surface–treated with borax dust, or lumber dipped in a borax solution, was moderately toxic to foraging termites (Williams 1934). Borate treatments of South American wood being imported into the U. S. provides protection of the wood against subterranean termites (Williams & Amburgey 1987).

Randall *et al.* (1934) and Randall & Doody (1934) killed termites with BA or borax treatments in laboratory and field tests. In laboratory experiments, termites exposed to dry filter paper previously treated with a 5% solution of BA, all died within four days (Randall *et al.* 1934). Borax or BA dusts placed in direct contact with termites or injected into galleries of termite–infested wood, killed 80% of contacted termites in 1–3 wk (Kofoid & Williams 1934). Aqueous solutions of borax placed under a termite–infested house successfully prevented termite penetration of treated soil for a 1 yr test, and borax was recommended for soil

treatment under existing dwellings. The volume of solution applied was found to be a critical factor in these soil treatments, and 4 liters/m^2 was recommended (Brown *et al.* 1934; Randall & Doody 1934). Disodium octaborate tetrahydrate (DOT) and zinc borate (ZB) mixed with sand caused significant mortality to eastern subterranean and Formosan subterranean termites in laboratory studies (Grace 1991b). Boric acid and boron salts have been shown to be lethal to subterranean termites (Rierson 1966; Williams *et al.* 1990; Grace 1990; Grace & Abdallay 1990; Grace *et al.* 1992).

The eastern subterranean termite, *Reticulitermes flavipes* (Kollar), and the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, are important economic pests that annually cause hundreds of millions to billions of dollars in damage to wooden structures in the United States (Mauldin 1982; Beal *et al.* 1986; Sharma 1993). *Reticulitermes flavipes* is indigenous to the United States, but *C. formosanus* is an exotic pest thought to originate in China (Kistner 1985). *Coptotermes formosanus* is widely established on Taiwan, Japan, Guam, and Hawaii, and is found in several other locations including the southwestern, southern, and southeastern boundaries of the continental United States (Gay 1969; Bess 1970). Infestations have been found in Alabama, southern California, Florida, Georgia, Louisiana, Mississippi, North and South Carolina, Tennessee, Texas, and Virginia (Weesner 1970; Su & Scheffrahn 1986; Chambers *et al.*. 1988; Atkinson *et al.* 1993).

The objective of the laboratory study described herein was to evaluate the effects of several concentrations of BA mixed in soil on mortality, feeding, tunneling, and gut protozoa populations for both eastern subterranean and Formosan subterranean termites.

MATERIALS AND METHODS

Termites.

Termites collected from three field colonies each of *R. flavipes* and *C. formosanus* were used. Five groups of 0.50g of late–instar worker caste termites from each colony were counted to determine mean worker termite weight per colony. Then, thirty–five groups of 2,000 worker termites (by weight) were drawn from each colony. Therefore, 70,000 worker termites from each of six colonies, for a total of 420,000 termites were required. Five groups of 2,000 termites from each colony were allocated to each of the seven BA concentrations tested, and were placed in their individually assigned experimental unit "main nest" and allowed to acclimatize for 1 wk.

After 1 wk, a flexible plastic tube filled with soil mixed with BA at one of six concentrations, or containing non–treated control soil, was

connected to each main nest and an adjoining "satellite" nest. Each BA concentration in soil was replicated five times, requiring a total of 210 experimental units. After 12 wk, experimental units were dismantled and termites collected, counted, and weighed to determine mortality. Previously dried and weighed wooden blocks that provided food in each experimental unit were cleaned of soil and nest debris, dried, and reweighed to determine wood consumption.

Pine Blocks.

A southern yellow pine (*Pinus* sp.) sapwood block (7.5 x 4.0 x 1.7cm) previously dried for 24 h at 105°C and then weighed, was immersed in distilled, deionized water for 3 min to restore moisture, and then removed from the water and placed in the main nest on top of the substrate. A smaller, previously dried and weighed pine sapwood block (2.5 x 2.0 x 1.7cm) also was immersed in water and then removed and placed on the substrate in the satellite nest. All experimental units contained these pine blocks.

Substrate and Soil Preparation.

Substrate for the main and satellite nests was prepared by combining clean, sterile sand and vermiculite (Verlite®) in a 10:1 ratio (wt:wt) near-homogeneous mixture. Sterile distilled, deionized water was then added at a volume of 450ml/1000g of dry mixture to yield a moisture content of 31% by weight. This substrate was used in the main and satellite nests exclusively.

To prepare soil for BA treatment and subsequent placement into foraging tubes, Rumford sandy loam topsoil from the Harrison Experimental Forest near Gulfport, Mississippi, USA, was sifted through a number 10 mesh soil–sieve to remove pebbles and most organic matter, and then heat sterilized. An aqueous solution of BA was then mixed with the soil to achieve 10% moisture and a specific percentage of BA by weight. BA treatment concentrations were 0.00 (water only controls), 0.05, 0.25, 0.50, 1.00, 2.00, and 4.00 % (wt:wt).

Experimental Unit.

A "main nest" consisted of a cylindrical plastic container (15.2cm diameter, 6.3cm height) with a removable lid. A single circular hole, 1.27cm diameter, was drilled in the side 0.8cm above the bottom of the container. A second, smaller, cylindrical plastic satellite nest container (5.3cm diameter, 4.0cm height) with a removable lid was drilled in the same manner, and the holes in both nests were plugged with rubber stoppers. The main and satellite nests were partially filled with 307g and 35g of sand–vermiculite–water substrate, respectively, and tamped

down to a level surface while covering the drilled holes. Initially, only the main nest was partially filled with substrate for the 1 wk acclimatization period. After this period, substrate was placed in the satellite nest, and opposite ends of a flexible plastic foraging tube (Tygon®; 1.27cm outside diameter 30.5cm long) were inserted 1cm deep into the holes, connecting the nests. Prior to connecting the foraging tube, it was filled with 30.0g of soil containing BA at one of the concentrations designated for testing, or with non-treated soil.

Each main nest received 2,000 worker caste termites by weight; termites were not placed in satellite nests. A double layer of clean filter paper (2 X 4cm) was folded over a 2cm length along the lip of the main nest to break the lid seal and allow air exchange, and a lid was placed on both nests. Therefore, an experimental unit consisted of capped main (with termites) and satellite nests, each containing moist substrate and a food source, connected by a plastic tube containing BA-treated soil.

Tunneling and Removal of BA Treated Soil.

After placing termites into each main nest where they foraged freely, they were monitored daily (Monday–Saturday) to determine rapidity and depth of penetration into the BA treated soil in the foraging tube over time. Once termites reached the satellite nest, they continued to be observed for behavioral changes and mortality. The amount of BA treated soil that termites removed from foraging tubes was compared among treatments by removing, drying (24 h; 105°C), and weighing the treated soil remaining in each foraging tube after 12 wk .

Protozoa Counts.

Counts of live protozoa extracted from termites were determined immediately before and after the 12 wk test period. The guts of five worker termites from each replicate were excised, dissected, and examined microscopically in 0.8% saline solution. Protozoa of the primary genera found in each termite species were sub-sampled and counted on a grid haemocytometer microscope slide to determine mean counts (Mauldin and Rich 1980; Carter *et al.* 1981; Mauldin *et al.* 1981).

Protozoa genera counted were *Trichonympha, Dinenympha, Pyrsonympha*, and *Spirotrichonympha* for *R. flavipes*, and *Pseudotrichonympha, Spirotrichonympha*, and *Holomastigotoides* for *C. formosanus*. Although *R. flavipes* contains at least twelve species of protozoa, only the four most abundant primary species were studied (Yamin 1979). Mean live protozoa counts at the start of the test were compared with corresponding counts afterwards to determine population reductions. Protozoan population reductions attributable to each

treatment were determined by genera as well as for combined protozoan populations.

Data Analysis.

Termite mortality and soil removal data were evaluated by analysis of variance (ANOVA). Feeding and protozoa data were evaluated by analysis of covariance (ANCOVA; pretreatment wood weights and protozoa counts, respectively, were the covariates). Treatments were compared by contrast analysis (Steel & Torrie 1980). Final mean wood weights and live protozoa counts after treatments were statistically adjusted and are reported as least–square means (LSMEANS, SAS Institute 1982).

RESULTS

Survival.

Generally, termite survival decreased as percentage BA increased (Table 1). Survival of *R. flavipes* was significantly lower in the 0.50, 1.00, 2.00, and 4.00% BA replicates compared with 0.05 and 0.25% BA replicates. Survival of *C. formosanus* was significantly reduced in the 2.00 and 4.00% BA replicates compared with 0.05–1.00% BA replicates. At comparable BA percentages, *C. formosanus* survival was always significantly greater than *R. flavipes*, except at the 4.00% BA concentration where both species sustained ≥96.0% mortality.

Table 1. Survival of *R. flavipes* and *C. formosanus* after 12–wk access to soil treated with boric acid in a choice foraging test.

	% Survival, Mean ± SEM[a]	
BA concentration % wt:wt	*R. flavipes*	*C. formosanus*
0.00	86.3 ± 1.5a, x	91.9 ± 2.0a, x
0.05	62.0 ± 1.9b, x	94.1 ± 1.5a, y
0.25	29.3 ± 6.9c, x	72.3 ± 4.2b, y
0.50	17.7 ± 6.6d, x	73.2 ± 3.1b, y
1.00	13.7 ± 5.1de, x	63.0 ± 5.6b, y
2.00	9.1 ± 4.2de, x	40.2 ± 9.7c, y
4.00	4.0 ± 3.8e, x	1.0 ± 0.9d, x

[a]Means followed by the same letter are not significantly different, P > 0.05 (contrast analysis; Steel and Torrie 1980); a, b, c, d, and e down columns; x and y across rows.

Table 2. Weight loss of pine blocks from *R. flavipes* or *C. formosanus* feeding during 12-wk access to soil treated with boric acid in a choice foraging test.

BA concentration % wt:wt	Wood weight loss, g, Mean ± SEM[a] main nest	satellite nest
	R. flavipes	
0.00	9.17 ± 0.57a	2.34 ± 0.32a
0.05	8.22 ± 0.65ab	1.33 ± 0.31b
0.25	7.15 ± 1.00ab	0.21 ± 0.05c
0.50	5.95 ± 1.10c	0.16 ± 0.05c
1.00	6.88 ± 1.31bc	0.12 ± 0.02c
2.00	5.70 ± 0.98c	0.13 ± 0.03c
4.00	4.65 ± 1.05c	0.13 ± 0.07c
	C. formosanus	
0.00	9.42 ± 0.24ab	2.05 ± 0.10a
0.05	10.21 ± 0.20a	2.14 ± 0.10a
0.25	8.89 ± 0.30bc	1.45 ± 0.13b
0.50	8.41 ± 0.23bc	0.95 ± 0.08c
1.00	8.05 ± 0.49c	0.87 ± 0.12c
2.00	6.81 ± 0.72d	0.58 ± 0.07d
4.00	5.14 ± 0.47e	0.61 ± 0.07d

[a] In each column, means followed by the same letter are not significantly different, P> 0.05 (contrast analysis; Steel and Torrie 1980); means are adjusted least-squares.

Feeding.

Generally, wood weight loss due to termite feeding decreased as BA percentage increased, although at higher BA concentrations differences narrowed (Table 2). Differences in wood weight loss were more apparent in main nests compared with satellite nests as termites consumed more wood in the main nests. These similarities in feeding were more noticeable for *R. flavipes* than for *C. formosanus*. *Coptotermes formosanus* consumed more wood than *R. flavipes*, although the greater survival rate of *C. formosanus* probably attributed to differences in wood consumption between the two species.

Tunneling and Removal of BA Treated Soil.

Termites foraged through the BA treated soil in the foraging tubes, excavating and removing some soil into both the main nest and the satellite nest (Table 3). *Coptotermes formosanus* removed more soil than *R. flavipes* at all BA concentrations, and tunneled through the treated

Table 3. Weight of boric-acid-treated soil removed from foraging tubes after 12-wk tunneling by *R. flavipes* or *C. formosanus* in a choice foraging test.

	Soil removed, g, Mean ± SEM[a], (%)	
BA concentration % wt:wt	*R. flavipes*	*C. formosanus*
0.00	16.06 ± 0.83a, x (53.5)	20.30 ± 0.52a, y (67.7)
0.05	6.12 ± 0.38b, x (20.4)	11.52 ± 0.19b, y (38.4)
0.25	5.14 ± 0.31c, x (17.1)	10.25 ± 0.28c, y (34.2)
0.50	5.66 ± 0.39c, x (18.9)	7.39 ± 0.29d, y (24.6)
1.00	5.67 ± 0.35c, x (18.9)	6.94 ± 0.34d, y (23.1)
2.00	5.76 ± 0.39c, x (19.2)	7.00 ± 0.41d, y (23.3)
4.00	5.62 ± 0.34c, x (18.7)	6.86 ± 0.36d, y (22.9)

[a] Means followed by the same letter are not significantly different, $P>0.05$ (contrast analysis; Steel and Torrie 1980); a, b, c, and d, down columns; x and y across rows.

soil to reach the satellite nests 1–2 d earlier than *R. flavipes*. Within seven days, all satellite nests had been reached by both species with no noticeable mortality, demonstrating the lack of repellency of BA mixed in soil.

Protozoa Counts.

Generally, dramatic decreases in protozoa populations did not occur as BA concentrations increased, except at 4.00% BA. Protozoan densities did not appear to be dose dependent at BA concentrations of 1.00–2.00% or less, but significant loss to defaunation of protozoa occurred in termites exposed to 4.00% BA (Tables 4 & 5). The lack of dose dependency relative to BA concentrations was apparent in both *R. flavipes* and *C. formosanus*.

DISCUSSION

The lethal effects of BA became apparent as the study progressed, as significant mortality occurred in most BA replicates. Overall, after 12 wk, *C. formosanus* survival was 40.2–94.1%, whereas survival of *R. flavipes* was significantly less at 9.1–62.0%, for replicates of 2.00% BA concentration. Thus, many termites in these foraging groups did not receive a lethal dose of BA, even at 20,000ppm in soil. This is in contrast to another study where sand was treated with disodium octaborate tetrahydrate (DOT) or zinc borate (ZB) at 5,000, 10,000, or 15,000ppm (Grace 1991a). After 10 days, *R. flavipes* mortality was 85.3% and 52.3% in the 5,000ppm DOT and ZB replicates, respectively. Survival

Table 4. Protozoa survival in *Reticulitermes flavipes* after 12-wk access to soil treated with boric acid in a choice foraging test

BA concentration % wt:wt	Protozoa survival (X 1000), Mean ± SEM[a]				
	P[b]	*D*	*T*	*S*	Total
			Colony 1		
0.00	5.02 ± 1.56a	8.98 ± 3.24	1.44 ± 0.27a	3.74 ± 0.98a	19.18 ± 5.88a
0.05	2.88 ± 0.36a	5.64 ± 1.38a	2.38 ± 0.24b	2.14 ± 0.47a	13.04 ± 1.21a
0.25-4.00[c]	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00b	0.00 ± 0.00b
			Colony 2		
0.00	1.68 ± 0.79ab	46.12 ± 3.23a	2.36 ± 0.68a	1.28 ± 0.33b	51.44 ± 5.92a
0.05	1.16 ± 0.46bc	28.36 ± 7.86b	2.32 ± 0.50a	1.88 ± 0.74ab	34.12 ± 9.08b
0.25	2.56 ± 0.85ab	29.48 ± 6.49b	2.72 ± 0.34a	3.24 ± 0.78a	38.00 ± 7.71b
0.50	3.16 ± 0.77a	31.64 ± 7.03b	2.12 ± 0.83a	2.88 ± 0.54a	39.80 ± 8.03b
1.00	2.04 ± 0.72ab	24.72 ±11.52b	1.36 ± 0.62ab	1.24 ± 0.56bc	29.36 ± 9.81b
2.00	1.04 ± 0.38bc	26.52 ± 8.25b	1.80 ± 0.57ab	1.32 ± 0.48bc	30.68 ± 4.52b
4.00[d]	0.64 ± 0.64c	5.24 ± 5.24c	0.32 ± 0.32b	0.52 ± 0.52c	6.72 ± 6.52c
			Colony 3		
0.00	1.20 ± 0.30a	29.14 ± 3.64a	3.46 ± 0.32a	3.01 ± 0.62a	36.80 ± 3.50a
0.05	1.04 ± 0.53a	26.52 ± 8.40a	2.16 ± 0.58ab	2.93 ± 0.79a	32.64 ± 9.58a
0.25	0.52 ± 0.24ab	27.36 ± 7.65a	2.00 ± 0.52b	1.60 ± 0.65ab	30.08 ± 8.24a
0.50[d]	0.20 ± 0.20bc	5.76 ± 5.76bc	0.44 ± 0.44c	1.04 ± 1.04b	7.44 ± 7.44bc
1.00	1.12 ± 0.30a	17.24 ± 6.31ab	2.48 ± 0.81ab	1.48 ± 0.40b	22.32 ± 7.20ab
2.00	0.20 ± 0.13bc	5.28 ± 4.39bc	0.08 ± 0.05c	0.08 ± 0.08c	5.64 ± 4.54bc
4.00	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00c

[a] In each column, means followed by the same letter are not significantly different, $P > 0.05$ (contrast analysis; Steel and Torrie 1980); means are adjusted least-squares.
[b] *P*, *Personympha* sp.; *D*, *Dynenympha* sp.; *T*, *Trichonympha* sp.; *S*, *Spirotrichonympha* sp.
[c] Termites from Colony 1 all died at BA concentrations of 0.25% or greater.
[d] Means and SEMs based on termites drawn from the one out of five replicates with surviving termites.

of *C. formosanus* was always greater than *R. flavipes*, with 80.3% and 77.7% surviving in the 5,000ppm DOT and ZB replicates, respectively. Even at the highest concentration tested, 15,000ppm, DOT and ZB were not repellent and did not inhibit tunneling in the treated sand (Grace 1991a).

This lack of repellency was similar to the BA treated soil in this study, as *R. flavipes* and *C. formosanus* tunneled extensively and removed soil

Table 5. Protozoa survival in *Coptotermes formosanus* after 12-wk access to soil treated with boric acid in a choice foraging test

	Protozoa survival (X 1000), Mean ± SEM[a]			
BA concentration % wt:wt	*P*[b]	*H*	*S*	Total
		Colony 1		
0.00	1.02 ± 0.23b	0.54 ± 0.21ab	0.54 ± 0.13bc	2.10 ± 0.50a
0.05	0.86 ± 0.22b	0.76 ± 0.09a	1.02 ± 0.27ab	2.64 ± 0.23a
0.25	2.22 ± 0.67a	0.88 ± 0.28a	1.00 ± 0.27ab	4.10 ± 0.82a
0.50	1.56 ± 0.69ab	0.78 ± 0.21a	1.36 ± 0.25a	3.70 ± 0.98a
1.00	1.30 ± 0.28ab	0.66 ± 0.19ab	1.14 ± 0.47ab	3.10 ± 0.84a
2.00	0.30 ± 0.17c	0.16 ± 0.06c	0.34 ± 0.18cd	0.80 ± 0.36b
4.00	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00b
		Colony 2		
0.00	1.34 ± 0.26a	0.68 ± 0.08ab	0.78 ± 0.12ab	2.80 ± 0.39a
0.05	1.04 ± 0.25a	1.12 ± 0.28a	1.04 ± 0.24a	3.20 ± 0.62a
0.25	1.32 ± 0.35a	1.12 ± 0.23a	0.72 ± 0.13ab	3.16 ± 0.54a
0.50	1.30 ± 0.33a	0.96 ± 0.30ab	0.82 ± 0.14ab	3.08 ± 0.66a
1.00	1.42 ± 0.47a	1.02 ± 0.26ab	0.72 ± 0.17ab	3.16 ± 0.88a
2.00	0.86 ± 0.21ab	0.40 ± 0.19bc	0.36 ± 0.14bc	162 ± 0.42ab
4.00	0.00 ± 0.00b	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00b
		Colony 3		
0.00	1.40 ± 0.41a	0.94 ± 0.22ab	1.12 ± 0.26ab	3.46 ± 0.79ab
0.05	0.84 ± 0.17ab	1.50 ± 0.27a	1.62 ± 0.36a	3.96 ± 0.48a
0.25	1.34 ± 0.33a	0.48 ± 0.15bc	0.38 ± 0.13cd	2.20 ± 0.38bc
0.50	1.02 ± 0.20ab	0.32 ± 0.11c	0.84 ± 0.29bc	2.18 ± 0.50bc
1.00	0.58 ± 0.18bc	0.40 ± 0.11bc	0.38 ± 0.15cd	1.36 ± 0.40c
2.00	0.34 ± 0.21c	0.44 ± 0.33bc	0.18 ± 0.16d	0.96 ± 0.68c
4.00	0.00 ± 0.00c	0.00 ± 0.00c	0.00 ± 0.00d	0.00 ± 0.00d

[a] In each column, means followed by the same letter are not significantly different, $P > 0.05$ (contrast analysis; Steel and Torrie 1980); means are adjusted least-squares.
[b] *P*, *Pseudotrichonympha* sp.; *H*, *Holomastigotoides* sp.; *S*, *Spirotrichonympha* sp.

from the foraging tube, although they removed significantly more non-treated soil from the foraging tubes in the control test units. Termites began tunneling in the BA treated soil in foraging tubes within 24–48 hours from the time the tubes were connected to the main nests, and continued to travel back and forth through the tube between the main and satellite nests for the 12 wk duration of the study. *Coptotermes*

formosanus always excavated more BA treated soil from foraging tubes than *R. flavipes* (*C. f.*, 22.9-38.4%; *R. f.*, 18.7-20.4%), and both species deposited BA treated soil into their main an satellite nests. Due to its water solubility, this may have resulted in BA diffusing into the substrate in both nests, spreading the toxicant, but also diluting its concentration. Some of the prepared sand–vermiculite substrate was moved from the main and satellite nests and deposited into the foraging tube, possibly reducing exposure of the termites to the BA treated soil by creating foraging tunnels lined with the non–treated substrate. Subterranean termites are known to line their galleries with excrement and externally gathered substrates (Lee & Wood 1971; McDaniel & Kard 1994).

Boric acid elicited significantly negative effects on the gut protozoa in both termite species. However, complete protozoa defaunation occurred only at the 4.00% (40,000ppm) BA concentration. The significant losses to defaunation of protozoa in termites exposed to 4.00% BA may have been partially due to reduced numbers and weakened condition of termites in these replicates after 12 wk. Where no live protozoa were found in surviving termites, these termites would have died in a few days or weeks. Generally, 0.05–1.00 or 2.0% BA concentrations caused similar reductions in protozoa numbers, and loss of protozoa did not appear BA dose dependent at these lower concentrations.

Aggregate protozoan densities for the species counted were variable, ranging from about 19,000 to more than 51,000 per single *R. flavipes*, and 2,100 to 3,400 per single *C. formosanus*, in the non–treated (control) replicates. Also, in the non–treated replicates, individual termites contained an average of ca. 30,000 protozoa for *R. flavipes* and ca. 2,800 protozoa for *C. formosanus*, for the protozoan genera counted. Thus, an individual *R. flavipes* contained ca. 10.8 times more protozoa than a single *C. formosanus* in the non–treated replicates. This may have implications for termite control using borates, as small losses from the much fewer protozoa in *C. formosanus* may cause relatively more stress due to the comparatively greater loss of cellulose digestive ability compared with a small protozoa loss from *R. flavipes*. Such losses would constitute a smaller percentage loss in *R. flavipes* and may not severely degrade cellulose digestion.

Antibiotics have been used to defaunate subterranean termites (Khoo & Sherman 1979; Mauldin & Rich 1980; Mauldin *et al.* 1994), but the effect of BA on termite gut protozoa has not been extensively studied. Loss of protozoan populations leads to starvation, and the resulting reduction in termite numbers may lead to weakening and

decline in colony vigor, decreasing the ability of the colony to attack wood (Yoshimura *et al.* 1992, 1993). Generally, it requires about 3–4 wk for defaunated termites to die of starvation, thus complete loss of protozoa in living termites in the 4.00% replicates would have had to occur in the 1–3 weeks before the end of this 12 wk study. Because many defaunated termites were alive and active at the end of study, defaunation was a recent occurrence before final protozoa counts were determined.

Tunneling of termites through the BA treated soil can be expected to reduce termite numbers, but the significant percentage survival of termites in this study indicates colony elimination is not likely. If defaunation continued over a longer period than in this study, increased starvation and progressive loss of termites may have been observed. However, if termites coat their foraging galleries with non-treated soil, wood debris, and excrement as observed in this study, as well as removing BA treated soil out of their foraging areas, they may recover from the initial effects of BA and continue to forage indefinitely.

Boric acid is relatively more water soluble compared with other boron compounds such as ZB or calcium borate, and may not be practical as a treatment to soil. More insoluble borates may better lend themselves to soil treatment, and as they diffuse more slowly through treated and adjacent soil they may create a long term toxic effect on tunneling termites. Through mutual grooming and trophallaxis, boron can be transferred among colony members (Jones 1991).

Boron has been investigated for use as a bait with moderate success (Grace *et al.* 1990; Jones 1991). Cellulose treated with DOT or other boron salts and then fed upon by termites would provide a more direct method of introducing a boron containing toxicant into a termite colony. The concentration of boron in a cellulose bait or treated wood has been effective at 0.15–1.4% in studies by Grace *et al.* (1990), Jones (1991), Forschler (1996), and Mauldin and Kard (1996).

This study demonstrates that BA is a non-repellent toxicant to *R. flavipes* and *C. formosanus*, but when used as a treatment to soil in this study it did not deter excavation and tunneling by these termites. Compared with conventional organophosphate or pyrethroid liquid termiticides currently used for treatments to soil, BA does not cause rapid knockdown and death. At similar concentrations, BA was more effective against *R. flavipes* compared with *C. formosanus*. Boric acid also demonstrated toxicity against symbiotic gut protozoa, significantly reducing their numbers. Further laboratory and field studies with borates of lower water solubility than BA may identify more effective, slow diffusing boron compounds that may be more effective in termite

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